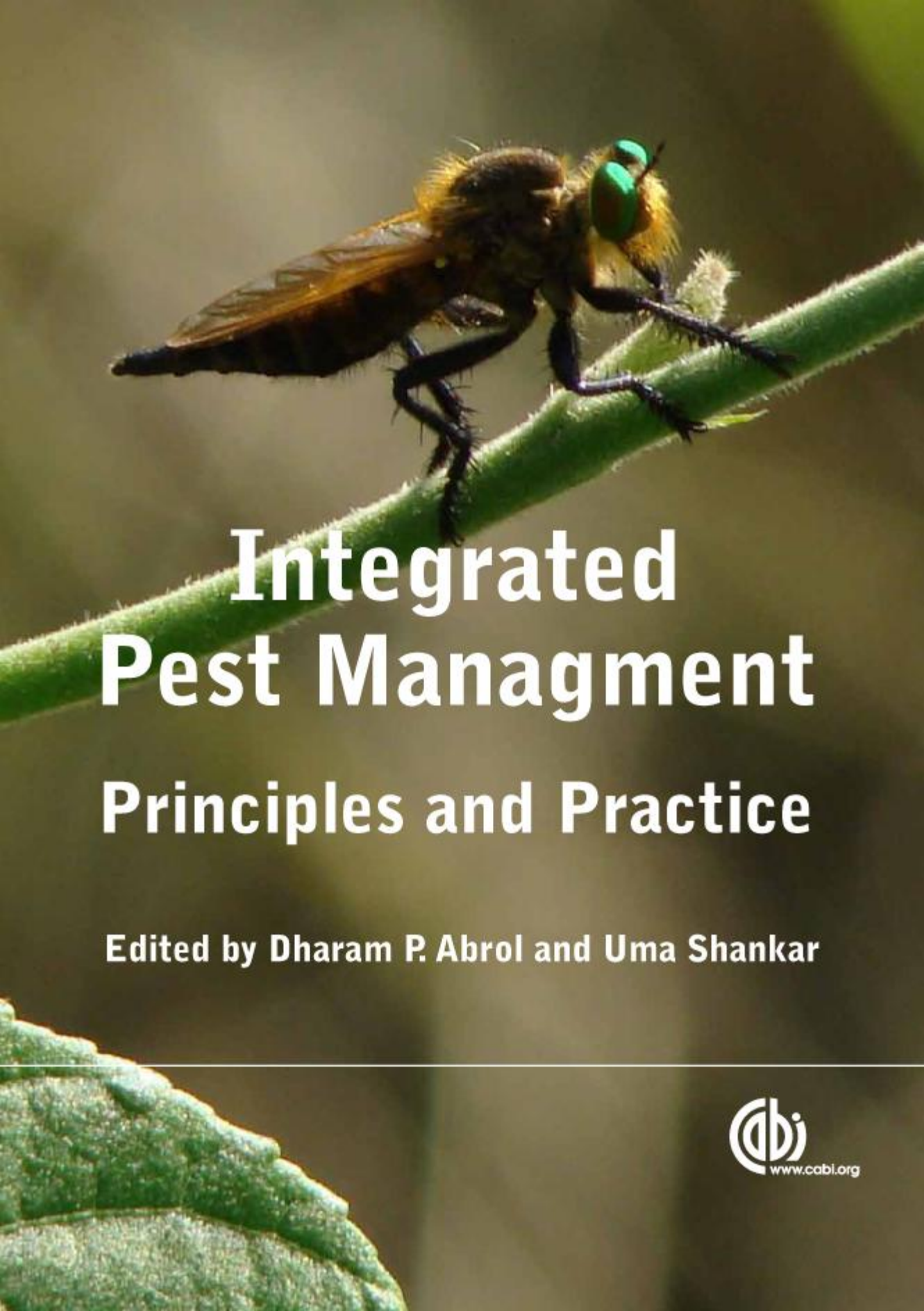

Integrated Pest Managment

Principles and Practice

Edited by Dharam P. Abrol and Uma Shankar

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Integrated Pest Management

Principles and Practice

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Edited by

Dharam P. Abrol

and

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Preface

In the 21st century, mankind is confronted with the Herculean task of providing food and environmental security to a burgeoning population, particularly in developing countries. In India, the population is growing at an alarming rate of around 2.5%/year. This makes it necessary that food grain production should also increase at the same rate or even faster. This has necessitated accelerated efforts on the part of agricultural scientists to develop high-yielding production technology and intensification in crop production practices. Their efforts have resulted in the development of high-yielding cultivars coupled with other components of crop production technology. Consequently, a boost in food grain as well as vegetables, fruit and fibre crop production has occurred. A significant rise in food grain production alone was recorded from 50 million t in the 1950s to more than 230 million t in 2008–2009. Even with this increase in food grain production, farmers were not able to realise the full potential of crop yield, one of the reasons being the heavy losses caused by insect and other arthropod pests. Traditional methods of crop production and protection became untenable with the introduction of new cultivars and the development of organic synthetic insecticides. The use of these insecticides became increasingly popular with farmers because of the spectacular results and easy method of application under different sets of conditions. The widespread use of insecticides, for longer periods and over a larger acreage, however, was not free from limitations. The development of insecticide resistant, resurgence, elevation of secondary pests to a status of primary importance, deleterious effects on non-target organisms, pollution of the environment and rising costs of application are all associated with the use of synthetic organic insecticides. This has necessitated a change in the concept and practice of pest control, if it is to contribute positively. During the last four decades, substantial information has accumulated to suggest that pest control must be extended beyond any single method to a system based on the principles of applied ecology. The emphasis at present is to promote a new paradigm to be: (i) safe for growers, farm workers and consumers; (ii) cost-effective and easy to adopt and integrate with other production practices; (iii) sustainable in the long term and without adverse environmental, economic and social consequences; and (iv) with ecosystems as the ecological focus. The availability of modern tools and transgenic crop protection technology has opened new vistas in the vast field of pest management. All these issues form the focus of this book, where they have been discussed by eminent scientists who are authorities in their respective fields. This book is an endeavour to cover integrated pest management (IPM) from multidisciplinary, multicountry and multifaceted components in

terms of holistic and unified IPM systems and its implementation in various fields concerned with pest management. This book is aimed to serve as a reference book for students, teachers, researchers, extension functionaries and policy planners associated with IPM. It can also be used as excellent reading material for graduate and post-graduate courses. The book deals not only with integration of pest management tactics but also with integration of different disciplines to provide a holistic view of IPM. Beyond IPM, pest management without pesticides in the tropics with empirical evidence is discussed. The book is an inter-disciplinary endeavour to document the content and process areas of IPM in industrialized, Green Revolution and subsistence agriculture systems and is the first of its kind where IPM has been discussed from different perspectives, documenting divergent thoughts to integrate their inferences to cater for the needs of scientists, graduate students, extension education specialists and policy makers associated with IPM research and development, implementation, evaluation and planning.

We hope that the book will prove useful to all those interested in promoting the cause of IPM in formal and informal applications in both developed and developing countries, so that sustainability in agricultural systems and environmental protection for future generations are achieved. All the contributors deserve special appreciation for writing chapters in their respective fields in great depth with dedication. Last but not least, thanks are also due to Rachel Cutts and Dr Nigel Farrar of CABI Publishing for taking great pains in publishing this book in a very impressive manner.

Dharam P. Abrol
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16 May 2011

1 History, Overview and Principles of Ecologically-based Pest Management

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1.1 Introduction

The human population is growing rapidly, disproportionately to the production of food supplies, which will necessitate production of more food in the near future (Thomas, 1999). The human population has already crossed the 7 billion mark (Smith, 2011) and is expected to rise over 8 billion by 2025 (Hinrichsen and Robey, 2000), thereby necessitating the need for more food production (Dhaliwal and Arora, 2001; Nwilene *et al.*, 2008). When agriculture came into existence around 10,000 years ago, the need for food for the increasing population was met by increasing the cultivatable area, but during the last century, the emphasis had been on increasing productivity per unit of land, as the available land required is decreasing day by day due to the increasing human population (FAO, 2001). The food productivity per unit of land is limited by several factors, which include the use of fertilizers, water availability, the genetic potential of the crop and the organisms such as arthropods (insects and mites), nematodes, bacteria, fungi, viruses, viroids, birds, rodents and other vertebrates associated with the crop that compete for food. According to Hall (1995), more than 10,000 species of insects, 30,000 species of weeds, 100,000 plant diseases caused by fungi, viruses, bacteria and other microorganisms, and 1000 species of nematodes damage food plants in the world. The yield losses from different categories of pests have been estimated to be US$500 billion worldwide (Oerke *et al.*, 1994; Liebhold and Tobin, 2008).

With the beginning of agriculture, ancient human beings lived with the crop pests without any effort to control them, but as the competition for food increased, humans learned to safeguard their crops against pests by using management tactics such as cultural mechanical, physical and biological control. It was during the mid-20th century that synthetic organic insecticides were developed, which provided spectacular results against pests (Metcalf, 1980; Dhaliwal *et al.*, 1998). However, dependence on chemicals has resulted in severe problems of insecticide resistance in over 500 insect pests (Georghiou, 1990), environmental pollution, pest resurgence and health problems. Publication of the book *Silent Spring* by Rachel Carson in 1962 was a turning point, which resulted in the development of the concept of integrated pest management (IPM) to reduce the harmful effects of pesticide and to reduce the use of chemicals for controlling crop pests (Table 1.1).

 Integrated Pest Management (eds D.P. Abrol and U. Shankar)

Table 1.1. The history of pest management.

Period	Landmark
8000 BC	Beginning of agriculture
4700 BC	Silkworm culture in China
2500 BC	First records of insecticides, e.g. sulfur compounds to control insects and mites
1500 BC	First descriptions of cultural controls, especially manipulation of planting dates
1200 BC	Botanical insecticides used for seed treatments and as fungicides in China
950 BC	First descriptions of burning as a cultural control method
200 BC	Oil spray for pest control
AD 300	Biological control on citrus orchard in China by predatory mites
AD 400	Root application of arsenic in rice to protect against insect pests
1750–1880	Discovery of the botanical insecticides pyrethrum and derris
Early 1800s	Appearance of books and papers devoted entirely to pest control covering cultural control, biological, varietal, mechanical and chemical control
1848–1878	Introduction of *Viteus vitifoliae* from the Americas and release of the natural enemy, *Tyroglyphus phylloxera*, to France from North America in 1873
1880	First commercial spraying machine
1883	*Apanteles glomeratus* imported from the UK to the USA to control cabbage white butterfly
1888	First major success with imported biological control agents *Cryptochaetum iceryae* and the coccinellid beetle *Rodolia cardinalis* from Australia for the control of cottony cushion scale in US citrus fruits
	Federal Hatch Act forms land-grant agricultural universities
1890s	Lead arsenate for insect control
1891	Board of Health, New York City, USA, seizes grapes with visible presence of Bordeaux Mixture and throws them in the river
1892	Canada passes law prohibiting the spraying of trees while in bloom with chemicals harmful to bees
1893	Recognition of arthropods as vectors of human disease
1894	The State of Utah, USA, requires mandatory spraying of fruit tree pests
1901	First successful biological control of a weed (lantana in Hawaii, USA)
1903	British Royal Commission on Arsenical Poisoning sets arsenic residue limit
1920–1930	More than 30 cases of natural enemy establishment recorded throughout the world
1921	First aerial application of insecticide against catalpa sphinx moths in Ohio, USA
1925	British threaten ban on American fruit exports such as apples with high levels of lead arsenate
1929	First area-wide eradication of an insect pest – Mediterranean fruit fly in Florida, USA
	Pest resurgence after repeated pesticide applications documented in Texas, USA
1930	Introduction of synthetic organic compounds for plant pathogen control
1932	*History of the Development of Organophosphate Poisons* published
1933	*100,000,000 Guinea Pigs* published with a chapter focusing on the hazards of arsenic and lead arsenate and lack of government action to deal with the problem
1939	Recognition of the insecticide properties of dichlorodiphenyltrichloroethane (DDT)
1942	Release of a wheat variety resistant to the hessian fly
	Rediscovery of the insecticidal properties of benzene hexachloride and in particular its gamma isomer
1948	Minimizing of the adverse effects on beneficial organisms by proper selection of pesticides pioneered by A.D. Pickett in Canada (Ripper, 1956)
1950s	First applications of systems analysis to crop pest control
1952	Pesticide handbook lists 4400 trade-name pesticides (in 1991, California registered approximately 9500 trade-name pesticides)
1959	Introduction of the concepts of economic thresholds, economic levels and integrated control by V.M. Stern, R. F. Smith, R. van den Bosch and K.S. Hagen
	The cranberry scare – Washington and Oregon growers use aminotriazole before registration (the use of aminotriazole, which causes cancer of the thyroid of rats, was discontinued in 1987)

Table 1.1. Continued.

Period	Landmark
1960	First insect sex pheromone isolated, identified and synthesized (from the gypsy moth)
1962	Publication of *Silent Spring* by Rachel Carson, widely credited with helping to launch the environment movement
1965	Release of the carbamate insecticides pirimicarb and pirimiphos ethyl
1967	Introduction of the term 'integrated pest management' (IPM) by R.F. Smith and R. van den Bosch and the term 'life systems' by L.R. Clark, P.W. Geier, R.D. Hughes and R.F. Morris
	Association of Applied Insect Ecologists chartered
1969	US National Academy of Sciences formalized the term 'integrated pest management'
	National Environment Policy Act (NEPA) passed
	Federal phase out of DDT for all but essential use
1970s	Widespread banning of DDT
	First 'Earth Day' on 22 April
	US Environmental Protection Agency (EPA) officially formed
	California Environmental Quality Act (CEQA) passed
1972	Release of *Bacillus thuringiensis* insecticide based on isolate HD-1 for control of lepidopteran pests
	Federal Environmental Pesticides Control Act passed
	California Law passed to promote pest management systems and to license pest control advisers
	Huffaker Project – US Department of Agriculture (USDA) funds first major IPM research effort
1975	Development and release of the synthetic pyrethroid insecticides permethrin and cypermethrin
1977	IPM programme started in the California Department of Food and Agriculture
1978	*The Pesticide Conspiracy* by Robert van den Bosch published
	IPM programme started at University of California
1979	IPM mentioned in President Carter's environmental message
	President Carter's memorandum to federal agencies to adopt IPM strategies
1980	USDA and EPA fund 2nd National IPM Research Programme – 'Adkisson Project'
	National Parks Service adopts IPM policy and implements IPM programme
1984	Pesticide Resistance Management Conference
1986	Sustainable agriculture programme started at the University of California, USA
1988	Major IPM successes in rice systems in Indonesia
1990	International Organisation for Pesticide Resistance Management (IOPRM) formed
1992	Concept of 'environment injury level' put forward by by L.P. Pedigo and L.G. Higley
	World Food Prize awarded to E.F. Knipling and R.C. Bushland for developing the Sterile Insect Technique
	Pivotal role of IPM in agriculture and policy in Rio de Janerio, Brazil (part of Agenda 21)
1993	EPA starts work on policy to register 'safer' pesticides
1994	EPA office of Pesticides Programme forms Biopesticides and Pollution Prevention Division
1996	Commercialization of first transgenic crop – cotton
2004	Resistance of *Helicoverpa zea* to *Bacillus thuringiensis*-transgenic (*Bt*) cotton
2006	World Health Organization suggests a resumption of the limited use of DDT to fight malaria
2006–2010	Societal concerns about engineered transgenic crops (genetically modified organisms or GMOs) increases in several regions of the world, especially Europe; public pressure dramatically slows down adoption of the technology of IPM systems
	Recognition and application of ecological and evolutionary principles become an increasingly important part of the overall development and management of agriculture, particularly in the context of climate change

Modified after Dhaliwal *et al.* (2004) and Dhawan and Peshin (2009).

1.2 The History of Pest Management

Over the centuries, farmers have experimented with and developed pest-management practices to minimize the damage caused by pests. In 1939, Paul Muller discovered the insecticidal properties of dichlorodiphenyltrichloroethane (DDT), which ushered in the pesticides era. Prior to the 1950s, arsenic-based pesticides were dominant. The discovery of the insecticidal properties of DDT led to complacency on the part of scientists, policy makers, extension workers, industry and farmers to abandon traditional practices. An overview of the history of pest management since 8000 BC is given in Table 1.1. Prior to the Second World War, pest control consisted of cultural control and mechanical control practices (Dhawan and Peshin, 2009). These practices evolved as a result of farmers' experimentation over time (Smith *et al.*, 1976). After the Second World War, DDT became the solution for all pest problems. There was a surge in research developing new types of pesticide. The period from the late 1940s to the 1960s has been called the 'Dark Ages' of pest control (Newsom, 1980). Calendar-based pesticide schedules were developed by the scientists and disseminated to farmers by extension workers. During this period, other control measures were not even considered for further research by the agricultural scientists. Farmers not adopting the calendar-based preventative pesticide schedules were termed traditional and laggards (Rogers, 1995). However, wide adoption of preventative pesticide use resulted in resistance and the resurgence of pests and pesticide residue problems. This led farmers into a 'pesticide treadmill' as farmers sprayed more and more pesticides without achieving the desired results. Some examples include cotton growers in Peru, Egypt, Texas (USA) and Punjab (India). In her book, *Silent Spring*, Rachael Carson (1962) criticized the scientists for propagating insecticides and called these insecticides biocides. These negative problems caused by pesticides led to the development of alternatives to reduce pesticide use. The theory and principles supporting IPM have evolved over the last 50 years since Stern *et al.* (1959) introduced the concept of integrated control based on ecological balance in ecosystems. The basic tactics of IPM were developed and applied to reduce crop losses against the ravages of pests before the term IPM was coined (Jones, 1973; Smith *et al.*, 1973). Entomologists at the University of California (USA) played a pivotal role in developing IPM tactics (Perkins, 1982). The development of resistance to insecticides and the elimination of natural enemies by insecticides led to the development of 'supervised control'. The publication of *Silent Spring* in 1962 was a defining milestone, for it brought the problems caused by pesticides to the attention of the public and scientists. The term 'integrated pest management' was used for the first time by Smith and van den Bosch (1967). In the five decades since integrated control, many synthetic pesticides have been banned. In 1972, 'integrated pest management' and its synonyms were incorporated into English dictionaries and accepted by the scientific community (Kogan, 1998). IPM is the main strategy accepted for pest management under Agenda 21 of the United Nations Conference on Environment and Development (UNCED, 1992). Kenmore *et al.* (1985) defined IPM as the 'farmers' best use of a mix of control tactics that are biologically, environmentally, economically, socially and culturally acceptable'.

Many initiatives have been taken throughout the world to implement IPM programmes to reduce the dependence on toxic pesticides (Beyer and Marek, 2008; Peshin *et al.*, 2009a). The initiatives for dissemination and uptake of IPM vary in developed and developing countries. In this chapter, we have attempted to give an overview of the history of pest management, pesticide use and the history of IPM in developed and developing countries.

1.3 Pesticide Use Throughout the World

The use of DDT in agriculture was banned under the Stockholm Convention on Persistent Organic Pollutants (POPs) but is still used in some developing nations to prevent malaria and other tropical diseases by spraying on interior walls to kill or repel mosquitoes. All organochlorinated pesticides have since been banned worldwide, except for endosulfan. Endosulfan is readily absorbed by the body, causing numerous cases of occupational poisoning and is highly toxic to wildlife. However, it remains legal and widely used in the USA, China and India. Countries that have banned endosulfan include Bahrain, Belize, Cambodia, Colombia, Cote d'Ivoire, Jordan, Kuwait, Malaysia, New Zealand, Norway, Oman, parts of the Philippines, Qatar, Saudi Arabia, Singapore, St Lucia, Sri Lanka, Syria and United Arab Emirates. India is the largest producer of endosulfan. In India, it is banned in the state of Kerala as a result of severe adverse effects arising from aerial spraying of endosulfan on cashew plantations in 2002. Sri Lanka banned endosulfan in 1998 (and all World Health Organization (WHO) category labelled pesticides in 1995) because they were the cause of frequent poisoning incidents. In Africa, endosulfan is used widely, especially in cotton cultivation. Nine West African countries have recently banned the use of endosulfan in cotton – Senegal, Mauritania, Mali, Guinea Bissau, Burkina Faso, Tchad, Cap-Vert, Gambia and Niger. (PAN North America, http://www.panna.org/node/1686), and the UK Co-operative has banned the use of endosulfan on its farms. More recently on 13 May 2011, the Supreme Court of India ordered a countrywide ban on the manufacture, sale and use of endosulfan, citing its toxic effects on humans and the environment. In June last year, the US Environmental Protection Agency (EPA) moved to ban endosulfan, with all uses of endosulfan to be phased out by 2016. The European Commission applauded the global consensus to add endosulfan to a list of substances to be banned, which was agreed at the Fifth Meeting of the Conference of Parties to the Stockholm Convention on POPs held on 25–29 April 2011 in Geneva. Endosulfan joins a list of 21 other POPs that were banned after the Stockholm Convention in 2004. However, exemptions will be available for 5 years, extendable for another 5 years. This would require 173 countries, which are parties to the Convention, to take steps for a ban on production and use of endosulfan.

Despite the banning of a number of pesticides, newer chemical pesticides continued to be developed that are toxic to target organisms at very low doses. In Europe, recent European Union (EU) legislation has been approved banning the use of highly toxic pesticides. Pesticide use as per 2007 estimates was 1.7 billion kg (technical-grade material). The total sales in 2007 were to the tune of US$35.85 billion (Agranova, 2008; Weddle *et al.*, 2009). The breakdown of sales of different groups of pesticides is given in Fig. 1.1.

The global average annual growth of pesticide consumption was negative at –1.3% (after inflation) between 1998 and 2007 (Agranova, 2008). However, in 2007, it was positive at 8.1% (after inflation). This 8.1% growth rate is the largest for the last 10 years. The proportionate consumption of insecticides in developing countries is much higher than in developed countries. In developed countries, proportionate consumption of herbicides is higher than that

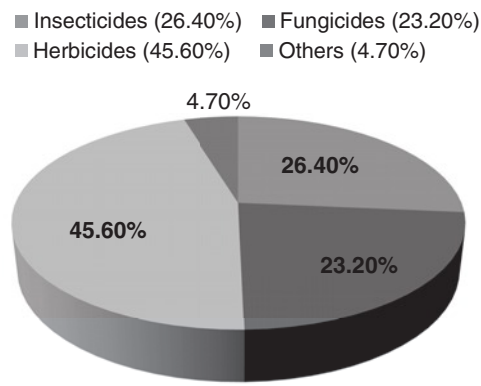

Fig. 1.1. Market for different groups of pesticides in 2007. (From Agranova, 2008.)

of insecticides (Fig. 1.2). In China and India, the insecticide consumption is 45 and 64%, respectively.

1.4 IPM: an Identity Crisis

IPM is experiencing an identity crisis. After its introduction, IPM became a 'buzzword' in conferences, journals and academic discussions to garner research grants. There are more than 77 definitions for IPM listed on the Database of IPM Resources website. A panel of experts on integrated pest control from the Food and Agriculture Organization of the United Nations (FAO), Rome, defined IPM as 'A pest management system that in the context of the associated environmental and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest population at levels below those causing economic injury' (FAO, 1968). Kenmore *et al.* (1985) defined IPM as the 'farmers' best use of a mix of control tactics that are biologically, environmentally, economically, socially and culturally acceptable'.

1.5 IPM Initiatives Around the World

IPM initiatives that have taken place around the world are described briefly in the following sections: first, IPM initiatives in

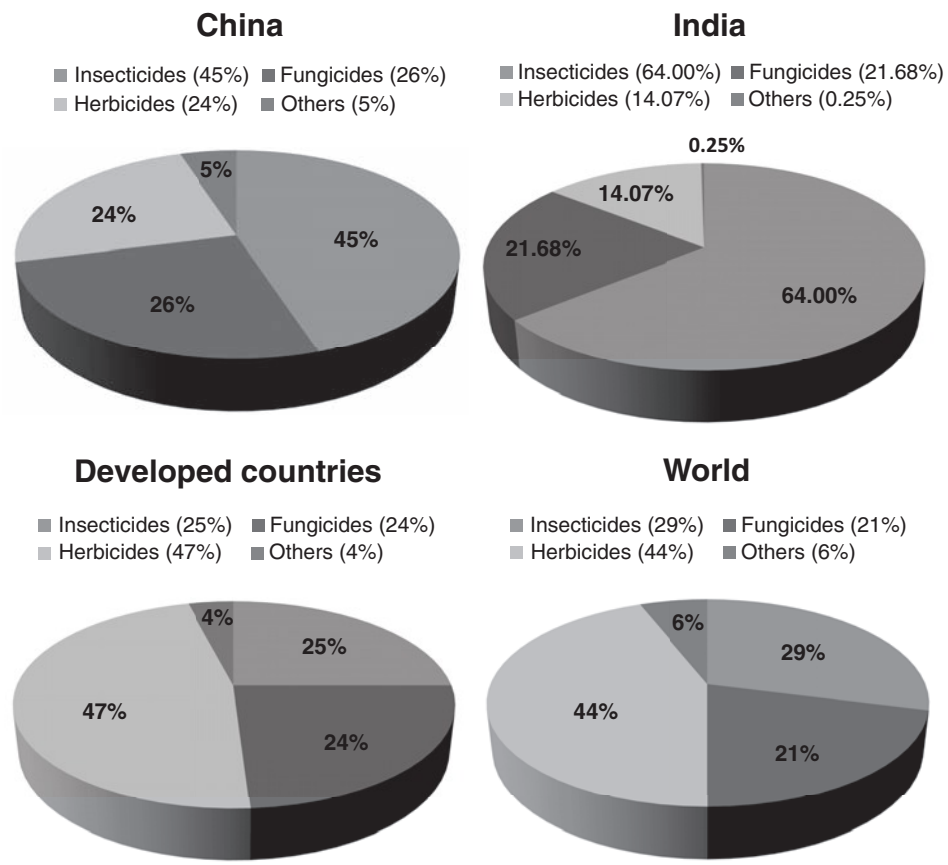

Fig. 1.2. Consumption of pesticides in China, India, developed countries and the world. (From Peshin *et al.*, 2009a,b.)

industrialized agriculture; secondly with Green Revolution lands and lastly with subsistence agriculture.

1.5.1 IPM initiatives in industrialized agriculture

USA

The development of IPM initiatives in the USA is documented in Table 1.2. Rachel Carson's book *Silent Spring* (1962) brought wide recognition to the fact that insecticides had become pervasive environmental pollutants. Political leaders and the public understood the pollution problem better than they did the problems of resistance and destruction of natural enemies (Perkins, 1982). Thus, pollution caused by pesticides helped entomologists gather political strength to get grants for research on IPM. The US Congress changed its regulatory scheme for pesticides so that no pesticide could be sold or used unless it had undergone extensive tests for environmental damage (Bosso, 1987). In 1971, Senate Bill 1794 approved funding for IPM research. The Huffaker Project for IPM in lucerne, citrus, cotton, pines, pome and stone fruits, and soybean with a coverage of 1.6 million ha (Huffaker and Smith, 1972) was the first large-scale initiative taken by the USA in implementing IPM. It was followed by a Consortium for IPM projects between 1979 and 1985, covering 5.76 million ha (Frisbie and Adkisson, 1985). In 1993, national IPM initiatives for implementing IPM practices on 75% of the USA's crop area by 2000 were started, and the progress made in implementing IPM programmes was evaluated by the US General Accounting Office (USGAO, 2001).

Economic evaluation of 61 IPM programmes conducted by Norton and Mullen (1994) reported that adoption of IPM methods resulted in lower pesticide use. The adoption of IPM strategies saved US agriculture US$500 million/year as a result of reductions in pesticide use (Rajotte *et al.*, 1987). In 2000, the adoption of IPM for field crops, vegetables, fruits and nuts in selected states covering most of the area under the surveyed crops was 86% for cotton, 62% for fruit and nuts, 86% for vegetables, 78% for soybean, 76% for

Table 1.2. IPM initiatives in the USA.

Period	Landmark
1950s	Sterile-male release against screw worm fly (*Cochliomyia hominivorax*)
	Integrated control by an entomologist at the University of California on lucerne (Smith and Allen, 1954)
1959	Economic threshold concept (Stern *et al.*, 1959)
1971	Senate Bill 1794 approving special funding for IPM pilot field research programme
1972	Overhauling Regulatory Scheme for pesticides: no pesticide sold unless tested for its environmental damage/risk
	IPM report published by the Council for Environmental Quality (Bosso, 1987)
1972–1978	The Huffaker Project for IPM in lucerne, citrus, cotton, pines, pome and stone fruits, and soybean with coverage of 1.6 million ha (Huffaker and Smith, 1972)
1978	Extension funding for all states to implement IPM programme (Olsen *et al.*, 2003)
1979–1985	The Consortium for IPM projects (Frisbie and Adkisson, 1985) with coverage of 5.76 million ha
1982	42 US states develop extension IPM education programmes
1985	Regional IPM programme launched with Consortium for IPM
1993	National initiative for implementing IPM practices on 75% of the US crop area by 2000
2000	The USA Government establishes four Regional Pest Management Centers
2004	National Roadmap for IPM programmes under which states receive a grant of US$10.75 million annually for IPM extension programmes

Source: Peshin *et al.* (2009a).

maize, 71% for barley, 65% for wheat, 40% for lucerne and 63% for other crops and pasture (USGAO, 2001).

Europe

In Europe, IPM and integrated plant production (IPP) were developed by orchard entomologists. Synthetic pesticides were introduced in apple orchards in the 1940s. Cases of resistance to pesticides were first noted around 1949 (Freier and Boller, 2009).

There have been a number of policy initiatives to promote IPM in Europe (Table 1.3). In Denmark and Switzerland, growers who adopt IPM are given incentives, while taxes are levied on pesticides in Denmark, Norway and Sweden (Cannell, 2007). Market support to promote the adoption of IPM is also seen in Italy, the Netherlands, Switzerland and the UK (Braun *et al.*, 2006; IP SUISSE, 2006; Cannell, 2007; see also www.co-op.co.uk).

EU research and development developed two indicators to access the outcome of IPM. These are the treatment index and environmental risk indicators. The treatment index is defined as the index representing the number of pesticide treatments in an area, considering reduced dosage smaller than the average. Elimination of 'unnecessary' treatments helps to reduce the risk to consumers, operators and the environment.

Since 2006, new directives and initiatives have been implemented to reduce pesticide use. In 2007, a new directive for sustainable use of pesticides and new regulations was adopted by the European

Table 1.3. History of IPM in Europe.

Period	Landmark
1951	European and Mediterranean Plant Protection Organization (UPPO) founded by 15 European countries; it has now 48 member states
1956	Establishment of the International Organization for Biological and integrated Control of Noxious Animals and Plants (IOBC)
1958	Establishment of the Commission on Integrated Control by the IOBC
1959	Establishment of a Working Group on Integrated Pest Control in Fruits Crops by the IOBC
1974	Adoption of the term 'integrated plant protection'
1976	The term 'integrated production' coined to integrate it with other disciplines
1977	Establishment of the 'Integrated Production' Commission
1989	Integrated production (IP) guide lines and endorsement to define a conceptual frame for IP
1992	*Integrated Production: Principles and Technical Guidelines* published for crop-specific IP guidelines
1993	IPM Europe established to promote the implementation of IP
1998	Concerted European Policy on IPM in International Cooperation, a framework towards a strategy for establishing common European IPM guidelines Strengthening of partnership with key stakeholders Promotion of effective utilization of European resources Establishment of a national IPM development committee
2000	IPM European Secretariat publishes *Guidelines for IPM Planning*
2001	IOBC postulate a total quality approach in the pre-harvest sector of agricultural production
2004	New *IOBC Standard 2004 for IP* for product, ethical production and social quality
2005–2006	*IOBC Standard 2004 for IP* field tested and implemented by IOBC, endorsed by farmers' organizations in France, Spain and Oregon (USA) (www.iobc.ch)
2006	PAN (Pesticide Action Network) worldwide network established to replace the use of toxic pesticides, with a PAN-Europe group meeting to analyse the status quo of IPM in Europe

Source: Freier and Boller (2009).

Commission. The council of members in 2008 approved the directives to include training activities to raise public awareness, compulsory checks on spraying equipment, a ban with restrictions for aerial crop spraying, the establishment of 'reduced' or 'pesticide-free' areas, measures to protect water resources and compulsory implementation of IPM from 2014 (Jiggins and Mancini, 2009).

Before compulsory implementation of IPM in 2014, there have been successful IPM strategies that need a brief mention here. A six-country case study (PAN Europe, 2007) highlighted the achievements of IPM in Belgium, Denmark, Switzerland, the Netherlands, Italy and the UK. In Belgium, the pesticides on the Red List are totally prohibited as per International Organization for Biological Control of Noxious Animals and Plants (IOBC) norms. In Denmark, Norway and Sweden a pesticide tax is levied, while farmers are provided with a direct subsidy for adopting IPM in Switzerland. The UK Co-operative group, one of the largest consumers' cooperatives in the world, has prohibited the use of 23 pesticides and restricted the use of 32 pesticides to reduce pesticide use by 50%.

Australia

In Australia, IPM programmes have been implemented in pome and stone fruits (Williams, 2000a), cotton (Fitt, 1994), wine grapes (Madge *et al.*, 1993), citrus (Smith *et al.*, 1997) and vegetable crops (McDougall, 2007). In citrus, the introduction of biocontrol agents for scale and mite pests, careful cultural control and limited use of selective insecticides has led to dramatic reductions in pesticide use (Smith *et al.*, 1997). Similarly, the conservation of native predatory mites in grapes has significantly reduced problems with mite pests (James and Whitney, 1993). In apples, IPM strategies include the use of introduced predatory mites, mating disruption and selective insecticides (Thwaite, 1997). In 2002, the number of sprays in apple orchards was reduced by 30% by adopting IPM (Williams, 2000b). In lettuce crops, the problem of lettuce aphid has been managed through an overall IPM strategy that emphasizes sampling, identification, management using non-chemical means (e.g. weed control, cultivation of crop residues, use of current lettuce aphid-resistant varieties) and selective insecticides (McDougall, 2007; McDougall and Creek, 2007). The problem of sugarcane soldier flies was managed by depriving them of food (Samson, 2006). The National Invertebrate Pest Initiative project, an initiative by the Grains Research and Development Corporation developed the PestFAX/PestFacts, is a free e-mail information service alerting growers and farm advisers across southern Australia to invertebrate pest issues and IPM-compatible solutions (Peshin *et al.*, 2009a). A similar approach is being used with a blog known as the Beatsheet developed by the Queensland Department of Primary Industries. To improve yield, rice production has been implemented through the Ricecheck system, developed in the 1980s by the New South Wales Department of Primary Industries, which provides rice growers with checks for production at critical phases of crop growth (Singh *et al.*, 2005).

A strong incentive for growers in eastern Australia to manage resistance and adopt more IPM-compatible strategies was provided as a result of the failure of cotton production in the Ord River irrigation area in north-western Australian in the mid-1970s due to insecticide resistance (Fitt, 2000). In the late 1990s, the emphasis shifted from insecticide resistance management (which was based mainly on reliance on chemical control) to sustainable and effective IPM, which incorporated insecticide resistance management. The availability of *Bacillus thuringiensis* (*Bt*)-transgenic cotton (expressing the Cry1Ac toxic protein) in the mid-1990s, initially capped to 30% of the area, and the registration of more selective control options for *Helicoverpa* control (e.g. the insecticides spinosad, indoxacarb and emamectin) greatly helped the uptake of IPM as growers could manage this pest with less effect on beneficials (Wilson *et al.*,

2004). At the same time, a set of guidelines for IPM was developed, which provided growers with a practical year-round strategy to manage pests, conserve beneficials and communicate with each other to coordinate efforts (Deutscher *et al.*, 2005). This was supported by a well-coordinated and highly focused effort from state and federal extension staff, including IPM field days, regular fact sheets and a well-supported website. The dramatic uptake of two-gene *Bt* cotton, which now accounts for more than 85% of the industry, saw a further reduction in insecticide use by about 85% (Pyke and Doyle, 2006).

1.5.2 IPM in Green Revolution agriculture

The increase in agricultural production around the world, beginning most markedly in the late 1960s, as a result of technological developments, is known as the 'Green Revolution'. Implementation of the Green Revolution paradigm of input-intensive agriculture, high-yielding varieties, chemical fertilizers, irrigation water and pesticides was disseminated through a top-down extension model, where farmers were passive adopters. The package for the technology was developed at the research stations and disseminated through extension agencies. The developed countries' model of diffusion of technologies was adopted for disseminating the Green Revolution technologies to developing countries. However, in the case of IPM, the developed countries' model of highly sophisticated IPM and extension and information systems is lacking in developing countries (Shepard *et al.*, 2009). Thus, a different approach is needed.

Widespread outbreaks of the rice brown plant hopper, *Nilaparvata lugens*, in the 1970s and 1980s was caused by the insecticides meant to control it and triggered the development of IPM strategies for pest management. The FAO Inter-Country Programme for the development and application of integrated pest control in rice in South and South-east Asia started in 1980. From 1977 to 1987, IPM moved from research towards extension. By 1988, the training and visit extension system in the Philippines, Indonesia, Sri Lanka, Bangladesh, India, Thailand and Malaysia attempted to introduce IPM to rice farmers through their system of 'impact points' or through strategic extension campaigns (Kenmore, 1997; Ofuoku *et al.*, 2008). An overview of IPM initiatives in Asia is given in Table 1.4.

The rice IPM programme in South-east Asia through farmer field schools (FFSs) is the foundation for implementation of IPM. FFSs were created by the FAO in Indonesia in the late 1980s to educate Indonesian farmers to avoid the needless use of insecticides in the rice crop, especially for stem borer and brown plant hopper. After observing the success of the FFS model in educating farmers about rice IPM, it was replicated in other crops. The FFS model is currently adopted in 75 countries in Asia, Africa and Latin America (Table 1.4).

India

With the advent of the Green Revolution in the mid-1960s, a new technological paradigm for the use of pesticides (in addition to high-yielding varieties and fertilizers) was adopted by India, largely imported from the USA. The surprising aspect of this paradigm shift is that insecticide-based insect pest management as the sole pest control strategy was advocated by the agriculture policy planners, entomologists and extension agencies when the world had taken note of the negative impact of pesticide use brought forward by Rachel Carson in her book, *Silent Spring*, in 1962 and entomologists were developing integrated control tactics (Peshin *et al.*, 2009a). In India, the first IPM programme was the 'Operational Research Project 1974–75' (Swaminathan, 1975) in cotton and rice. Since then, many IPM programmes have been initiated by different public-sector extension agencies, namely: (i) the Directorate of Plant Protection, Quarantine and Storage, Faridabad, India; (ii) state agricultural departments; (iii) state agricultural universities; and (iv) non-governmental organizations with national

Table 1.4. IPM initiatives in Asia.

Period	Landmark
1970s–1980s	Heavy reliance of insecticides resulting in widespread outbreaks of rice brown plant hopper, *Nilaparvata lugens*, which triggered the development of IPM strategies
1980	FAO Inter-Country Programme launched for the development and application of integrated pest control in rice in South and South-east Asia
1986	Indonesia bans 57 broad-spectrum pesticides
1977–1987	IPM moves from research towards extension
By 1988	Training and visit extension system in the Philippines, Indonesia, Sri Lanka, Bangladesh, India, Thailand and Malaysia attempts to introduce IPM to rice farmers through their system of 'impact points' or through strategic extension campaigns (Kenmore, 1997)
1988 onward	IPM moves towards education rather than training
1989	IPM FFS system first started in Indonesia, after the banning of 57 broad-spectrum pesticides in 1986
1989–2004	Coverage of IPM FFSs just 1–5% of all households in Asia Approximately US$100 million in grants allocated to IPM projects in Asia (Bartlett, 2005)
1993	'Global IPM Field Exchange and Meeting' held, where participants from Africa, the near East, Latin America and Europe observed the success of Asian IPM farmers in South-east Asia (Kenmore, 1997)
1995	Establishment of a global IPM facility with co-sponsorship by the FAO, UN Development Programme (UNDP), UN Environment Programme (UNEP) and World Bank (Kogan, 1998)
Late 1999	Establishment of the FAO/EU IPM programme for cotton in Asia, implemented in six countries: Bangladesh, China, India, Pakistan, the Philippines and Vietnam (Ooi, 2003)

Source: Peshin and Bandral (2010).

and international funding (Table 1.5). India abolished insecticide subsidies in the 1990s and banned pesticides that were extremely toxic. The pesticides banned in India are as follows (http://cibrc.nic.in/list_pest_bann.htm):

- *Banned*: aldrin, benzene hexachloride, calcium cyanide, chlordane, copper cetoarsenite, cibromochloropropane, endrin, ethyl mercury chloride, ethyl parathion, heptachlor, menazone, nitrofen, paraquat dimethyl sulfate, pentachloronitrobenzene, pentachlorophenol, phenyl mercury acetate, sodium methane arsonate, tetradifon, toxafen, aldicarb, chlorobenzilate, dieldrine, maleic hydrazide, ethylene dibromide, trichloroacetic acid, metoxuron chlorfenvinphos.
- *Banned for use but manufacture allowed for export*: nicotin sulfate, captafol 80% powder.
- *Formulations banned for import, manufacture and use*: methomyl 24% L, methomyl 12.5% L, phosphamidon 85% SL, carbofuron 50% SP.
- *Withdrawn*: Dalapon, ferbam, formothion, nickel chloride, paradichlorobenzene (PDCB), simazine, warfarin.
- *Refused registration*: calcium arsonate EPM, azinphos methyl, lead arsonate, mevinphos (Phosdrin), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), carbophenothion, vamidothion, mephosfolan, azinphos ethyl, binapacryl, dicrotophos, thiodemeton/disulfoton, fentin acetate, fentin hydroxide, chinomethionate (Morestan), ammonium sulfamate, leptophos (Phosvel).
- *Restricted for use in India*: aluminium phosphide, DDT, lindane, methyl bromide, methyl , sodium cyanide, methoxy ethyl mercuric chloride (MEMC), monocrotophos, endosulfan, fenitrothion, diazinon, fenthion, dazomet.

Table 1.5. IPM programmes/initiatives in India.

Period	Landmark
Mid-1960s	A new technological paradigm in the use of pesticides (in addition to high-yielding varieties and fertilizers) as adopted by India, largely imported from the USA. The surprising aspect of this paradigm shift is that insecticide-based insect pest management as the sole pest control strategy was advocated by the agriculture policy planners, entomologists and extension agencies when the world had taken note of the negative impact of pesticide use brought forward by Rachel Carson in her book *Silent Spring* in 1962, and entomologists were developing integrated control tactics (Stern *et al.*, 1959)
1974–1975	Research on IPM started on rice and cotton, under operational research projects (Swaminathan, 1975)
1980	India becomes a member country of the FAO initiated Inter-Country Programme
Mid-1980s	Government of India re-orients its plant protection strategy
1990s onwards	Central Integrated Pest Management Centres (CIPMCs) set up in 26 states to promote the concept of IPM in cotton and rice
1993 onwards	Intensification of IPM activities
Mid-1990s	Various state departments of agriculture implement IPM India abolishes its insecticide subsidy resulting in a saving of US$30 million annually and imposes a 10% excise tax, resulting in a US$60 million annual revenue to the government
1993	FAO Inter-Country Programme for IPM in rice crops Regional programme on cotton IPM by Commonwealth Agricultural Bureau International (CABI)
1994	The Directorate of Plant Protection, Quarantine and Storage, Government of India, the nodal agency for implementing IPM programmes, intensifies its efforts and adopts the FFS model for educating farmers through its 26 CIPMCs (currently, there are 31 CIPMCs)
2000	FAO/EU IPM programme for cotton adopted National Agricultural Technology Project (NATP) for IPM Government of India launches the Technology Mission on Cotton (Barik *et al.*, 2002)
2000 onwards	FAO/EU launch an IPM programme for cotton in India for 5 years
2002	Insecticide resistance management-based IPM programme by the Central Institute for Cotton Research (CICR), Nagpur (Peshin *et al.*, 2007) Introduction of *Bt* cotton
2002 onwards	Sir Ratan Tata Trust project (a private sector-funded project) supports the Department of Entomology at Punjab Agricultural University, Ludhiana, India, towards further developing, validating and disseminating cotton IPM technology in cotton-growing districts of Punjab
2008–2012 (XI Plan Period)	Targets for conducting 3250 IPM FFSs. An outlay of US$2.8 million earmarked for state-level training programmes and FFSs out of a total outlay of US$266.7 million for 'strengthening and modernizing of pest management approaches in India'
2008	India is the world's fifth largest grower of GM crops with an estimated 6.9 million ha (*Bt* cotton) sown in 2008

Modified after Peshin *et al.* (2009a); Peshin and Bandral (2010).

The IPM initiatives taken in India since 1974/1975 are outlined in Table 1.5. Although there are multiple public-sector extension agencies, the extensional personnel are superficially trained about IPM in developing countries (Shepard *et al.*, 2009). The professionals associated with IPM are not comfortable with the IPM principles nor the extension methodology of FFSs (Peshin *et al.*, 2009b). The 'National Agriculture Policy 2001' envisages IPM as the main strategy of plant protection, but the research entomologists and extension entomologists are mainly propagating integrated insecticide pest management.

China

In China, rice and cotton IPM programmes were initiated in 1988. These programmes were funded by the FAO Inter-Country Rice IPM programme and the Asian Development Bank Cotton IPM Programme (Peshin *et al.*, 2009a). In 2000, the EU cotton IPM programme was started. Under these programmes, training was imparted to develop qualified trainers and standardize FFSs (Zhang *et al.*, 2001). As early as the start of the 1950s, China put forward the concept of 'integrated control' (Jing, 1997). In 1975, plant-protection scientists formulated the principle of plant protection with a 'focus on prevention and implementing integrated control', namely the IPM framework (Peshin *et al.*, 2009a). The first-generation IPM (1981–1985) based on economic threshold and biological and physical methods was a pest-specific approach. The second-generation IPM (1986–1990) was crop-centred, while between 1991 and 1995, a large number of IPM systems were developed incorporating natural control. The third-generation IPM – ecosystem-centred IPM – is currently being implemented (Peshin *et al.*, 2009a).

1.5.3 IPM in subsistence agriculture

Push–pull IPM strategies, based on the manipulation of insect pests and their natural enemies by means of stimuli that act to make the protected crop resources unattractive or unsuitable to the pests (push) while attracting them towards another source (pull) where the pest can be removed, is in fact the most successful application in subsistence farmers in Africa (Jiggins and Mancini, 2009). In 2006, over 160,000 smallholder farmers were using push–pull strategies to protect their maize and sorghum crops (Khan *et al.*, 2006). The FFS model of extension, which is spreading rapidly throughout eastern and southern Africa, is helping to carry the strategies to increasing numbers of farmers (www.farmerfieldschool.info/). The FFS model has been abused in many Green Revolution and African countries. Instead of using the farm as an experimentation laboratory and curriculum being evolved in the field, the scientists propagate their ideas about pesticide use. Nederlof and Odonkor (2006) and Nederlof and Dangbegnon (2007) reported the situation for cowpea FFSs in Ghana where scientists pushed improved varieties and pesticide use. According to Isubilalu (2007), the FFS method is turned into a platform where researchers promote their mandate and interest rather than addressing farmers' interests.

1.6 Ecologically-based IPM

As insect pests, pathogens and weeds pose a continuous threat to the yield and quality of production of agricultural commodities, the development of effective management strategies is essential for sustaining productivity and maintaining long-term profitability. Ever-increasing problems in containing pests in recent years is probably the result of dependence on single control tactics employing chemical controls. It was during 1962 when Rachel Carson attracted the attention of the world through her book *Silent Spring* on the adverse effects of chemicals in the ecosystem. This clearly highlighted the fact that chemical controls alone will not provide long-term control of pests (Cuperus *et al.*, 1990; Zettler and Cuperus, 1990; Reed *et al.*, 1993; Abrol, 2009a,b; Aluja *et al.*, 2009; Saenz-de-Cabezon *et al.*, 2010). Reliance on single control tactics have resulted in environmental degradation, contamination of food products, problems of residues and resistance in target species, thereby seriously impairing the sustainability (Vega *et al.*, 2009; Miller *et al.*, 2010). It is therefore essential to devise a sound management system that is based on ecological principles resulting in sustainable agricultural production without disturbing the balance of nature (Overton, 1996; Lewis *et al.*, 1997; Kennedy and Sutton, 2000). The aim of this new approach is to shift management strategies so that they have less reliance on chemicals and more on the biology of pests

and their interactions with crops. Thus, ecologically-based IPM combining all approaches – physical, cultural, chemical and biological – is the only option for sustaining productivity and maintaining the health of ecosystems (Kennedy and Sutton, 2000). IPM programmes need to be designed in a way to manage pests on the one hand and ensure the build-up of beneficial organisms on the other (Altieri, 1994; Barnett *et al.*, 1996; Boland and Kuykendall, 1998; Kennedy and Sutton, 2000; Altieri and Nicholls, 2003; Carriere *et al.*, 2010). Some of the key issues that need attention are as follows:

1. Emphasis should be on understanding the ecological relationships between the host plant and the management practices, such as cultural, biological and host-plant resistance.
2. All the components – biological, chemical, cultural and physical – need to be integrated.
3. Such programmes should minimize economic, environmental and health risks and provide sustainability over time.

1.6.1 Safety concern of the consumers

Consumers want food that is free from contamination with hazardous chemicals or toxins. They also express concern about the genetic origin of the food. It has been reported that more than 75% of consumers are very concerned regarding pesticides residues in food. All these issues have a direct bearing on the marketing of the products (Hallman *et al.*, 2003).

1.6.2 Food safety and ecologically-based IPM

It has been observed that the public responds to genetically modified crops in the same way as they are projected, as bias can occur as a result of ineffective communication (Hallman *et al.*, 2003). Although major concerns are for hortcultural crops, other crops that are fed to dairy cattle are considered equally harmful (Ward *et al.*, 1995).

1.6.3 The influence of markets

Insect pests, diseases and weeds inflict enormous losses on potential agricultural production. Anecdotal evidence also indicates rises in these losses, despite the increasing use of chemical pesticides. At the same time, there is a rising public concern about the potential adverse effects of chemical pesticides on human health, environment and biodiversity. These negative factors cannot be eliminated altogether; however, their intensity can be minimized through the development, dissemination and promotion of alternative technologies such as biopesticides and bioagents as well as good agronomic practices, rather than relying solely on chemical pesticides. India has a vast flora and fauna that have the potential to be developed into commercial technologies.

The use of chemical pesticides such as DDT, benzene hexachloride and organophosphate pesticides for insect pest management has resulted in the decline of biocontrol agents and other beneficial insects, secondary pest outbreaks and the emergence of insect strains resistant to pesticides. There is growing concern among the public over the accumulation of pesticides in the food chain. These problems have prompted the scientific community to find non-toxic and environment friendly alternatives to chemicals. From time to time, researchers have found many ecofriendly alternative methods such as botanical pesticides, attractants and repellents, insect growth inhibitors and biological control. IPM strategies have gained increased attention in recent years as a potential means of reducing commodity losses to pests.

Ecologically-based IPM focuses on an understanding of how ecosystem processes, such as predation, parasitism, herbivory, competition and mutualism, can be manipulated effectively for the management of pests. It emphasizes the management of

weeds, insects, mites, fungi, bacteria, viruses and nematodes that interfere with crop production. The development of ecologically-based IPM systems that take a broader view of all pests within an agro-ecosystem context is currently emphasized. This emphasis is being laid more on the ecological principles and their applications in pest-management strategies in the context of whole farming systems representing a sustainable approach to managing pests combining biological, physical, chemical and cultural tools to ensure favourable economic, ecological and sociological consequences. Thus, new pest-management systems must be developed that are effective in the long term, cost-effective and not detrimental to human and environmental health.

IPM has had many successes, but the magnitude of the problem that farmers currently face forces us to look once again at pests and diseases and at their management. Pests and diseases are not an isolated part of agriculture but rather a symptom of a broader problem, and need thus to be seen and managed accordingly (Levins and Wilson, 1980). There is a need for ecologically-based IPM when addressing the presence of pests and diseases in agriculture (Turner, 1992; Higley and Pedigo, 1996). Building on the wide range of experience gained with IPM during the last two decades, an ecological pest-management approach will be one that focuses on managing pests as one component of a larger ecosystem (National Research Council, 1996). As such, ecological pest management is based on minimizing disturbances to the environment caused by agriculture, decreasing the vulnerability of plants to pests and understanding the life cycle of pests. These aspects are discussed in the following sections.

1.6.4 Minimizing the disturbances caused by agriculture

A successful pest-management strategy is based on mimicking nature, for example redesigning a farm so that it resembles a complex ecosystem. This will mean maximizing a farm's positive ecological processes (such as nitrogen fixation and nutrient mineralization), while at the same time minimizing undesirable processes such as nutrient loss or erosion. In some cases, it may be convenient to reduce tillage and thus achieve minimal soil disturbance; in others, it will be necessary to include perennial species and enhance a farm's overall diversity. The interaction of different species can have interesting results, contributing to the system's overall resilience. Undisturbed ecosystems include a host of plants, birds and other animals, ensuring a balance among crop–pest–predator relationships. An abundance of natural predators minimizes the need for specific plant-protection measures.

1.6.5 Decreasing the vulnerability of plants

Field observations show that pests prefer to attack plants under stress. The best way to prevent the attack of pests and diseases is thus by providing a healthy and balanced environment and food supply. There are many factors that can affect a plant's internal balance and thus increase or decrease its susceptibility to pest and disease attacks. These are related to the plant itself (e.g. adaptation to the local climate or its age) or to the environment (e.g. climate, light, temperature, humidity, wind). Plant vulnerability is also related to the different management practices that regularly take place on a farm, such as spacing, tilling, pruning and time of planting.

1.6.6 Understanding pests and acting accordingly

In pest and disease management, one of the main considerations is the way the pest species behave – some have the ability to reproduce often and disperse widely, while others are able to withstand competition or adverse conditions. 'Know your enemy' is

thus a key strategy in every pest-management approach. This knowledge needs to be translated into action, considering, for example, the release of beneficial insects at a particular moment, adding bird nesting sites to a farm or changing the sowing time of certain crops.

These principles are clearly visible in many traditional low-input agricultural systems, where ecological principles form the basis for all pest-management strategies. Examples of methods used to keep pests away are raising companion crops emitting strong odours and the use of botanical insect repellents. Thus, these methods work with nature, not against it. In agricultural terms, this means growing plants in the right soil at the right time, nourishing the soil and relying on the system's biodiversity as a natural means of safeguarding the health of the whole system. Traditional wisdom is being maintained by many societies, and is also being recreated in many 'modern' farms. Managing a farm, however, and relying on its ecological processes, requires a thorough understanding of how these work. Therefore, ecological pest management is, above all, based on farmers' skills, abilities and knowledge.

1.7 Building Knowledge

If ecological pest management is based on farmers' understanding of their ecosystem and of the processes taking place in it, then training, education and knowledge-building processes are essential. Many different participatory learning approaches for promoting sustainable agriculture have been developed, most of which work towards improving farmers' decision-making capacities and stimulating local innovations. Through FFSs, farmers are trained to make an analysis of their agro-ecosystem. In this way, they become aware of the pest–predator balance and of the damaging effect of pesticides on this balance. FFSs are being popularized by non-governmental organizations and governments on a small as well as a large scale. They are learner-centred, field-based, experiential learning methodologies. They involve observation, analysis, assessment and experimentation over a time period sufficient to understand the key agro-ecological and socio-ecological relationships. These group learning processes empower farmers in decision making and taking up leadership roles, ultimately resulting in building social capital. FFS methodology is being used extensively by incorporating changes as necessary to suit the local conditions and needs, enlarging its scope from IPM to holistic farm management, and from a training tool to a scaling-up tool.

Classical tools of ecological genetics and population genetics, and new tools of molecular biology, are being used by researchers in attempts to slow the rate at which pests adapt to chemical and physical stresses and, at the same time, increase the rate at which beneficial predators adapt to these stresses and to the defences of their target prey. For example, Lewis and Martin (1990) found that pre-conditioned parasitoids are more effective than non-conditioned parasitoids when released into the environment. Some examples of the progress that has been made are outlined below.

1.7.1 Multispecies and multitrophic-level interactions and pest population dynamics

The knowledge base in this area has expanded in the past decade, but a complete understanding of such multitrophic-level interactions will be essential for designing effective and reliable biological control measures for ecologically-based IPM.

1.7.2 Behavioural, physiological and molecular mechanisms

Although there have been some major developments in studies of plant–host interactions (e.g. the finding that a specific plant host, by cellular signalling, indicates

recognition of a pathogen), issues such as arthropod signalling, antagonism/toxicity/antibiosis, host selection, target-host diseases, target-host parasitism and plant-disease resistance are subjects in need of understanding at behavioural, molecular and physiological levels.

1.7.3 Signalling between target hosts and biological control organisms

As an example, acetosyringone released from the wounds of certain plant species induces the transcription of the *vir* genes of Ti plasmid-containing strains of *Agrobacterium tumefaciens*, initiating the process of crown gall formation (Gelvin, 1992). It is reasonable to assume that similar signalling occurs between many of the co-evolved components of rhizosphere and phylloplane communities.

1.7.4 Using natural antagonism, toxicity and antibiosis to control pests

Over the past decade, compelling evidence of the ecological roles of natural antibiotics produced *in situ* by microorganisms has been reported (Weller and Thomashow, 1993). Whereas many scientists formerly believed that antibiotic compounds were produced as artefacts of laboratory culture conditions, it is now known that such compounds can be and are produced by microorganisms, such as *B. thuringiensis* and *Agrobacterium radiobacter*, inhabiting natural substrates and in concentrations adequate to inhibit pests. Microorganisms that produce antibiotic compounds are among the most successful biological control agents.

1.7.5 Improving strains of parasites, parasitoids, predators and pathogens

To date, the major emphasis in genetic improvement of arthropod predators, parasitoids and pathogens has been developing natural control agents that are resistant to pesticides so that they can function within a system dominated by broad-spectrum pesticides. Genetic engineering could be used to increase the efficiency of biological control agents in specific agricultural systems. Characteristics that could be altered include heat and cold tolerance, host range and migratory cues.

1.7.6 Pest-resistance mechanisms of plants

Host-plant resistance is the most important component of many plant-disease management systems. The development of molecular biology research tools has resulted in identification and analysis of genes that control the interactions of plants and pathogens and is contributing to efforts to produce transgenic crop plants resistant to a variety of plant pests.

1.7.7 Identification and conservation of natural resources needed for ecologically-based IPM

A founding principle of classical biological control of exotic pests is that natural enemies of the pest can be found in the geographical region where the pest evolved. Likewise, plant geneticists have found that the best place to find pest-resistant plant varieties is in the geographical region where the plant and pest co-evolved. Collection and identification of germplasm from such regions has been a high priority for plant breeders. It is probable that every pest has at least one biological control agent that could be identified by this approach. Systematic exploration for biological control agents, however, has been limited to a relatively small number of arthropod and weed pests. For fungi, bacteria and nematodes, even less exploration has occurred. Research to identify, collect and document the natural enemies of pests in their native habitat should continue to be an important component of efforts to discover useful biological control agents.

1.7.8 Grower-friendly diagnostic and monitoring methods

New methods to study, monitor and evaluate agricultural and forest system processes as well as the effectiveness of pest-management tools need to be devised. Providing researchers and farmers with improved diagnostic techniques is key to both developing and increasing the use of biological organisms, products and resistant plants.

1.7.9 Production, stabilization and delivery of biological control organisms and products

An important challenge to the implementation of ecologically-based IPM will be to provide reliable sources of living biological control organisms. Another obstacle to the implementation of biological control is the large amount of inoculum required for the efficacy of certain microbial agents. Research to optimize the placement of biological control agents in close proximity to target pests and production of propagules at the most effective physiological state will do much to remedy this problem.

1.7.10 Implementation and evaluation of ecologically-based IPM

The research needs for ecologically-based IPM discussed in the sections above have emphasized the need for new knowledge about biological processes, interactions or organisms useful for biological control. Emphasis should be placed on implementation research to increase the adoption of ecologically-based IPM. There is a need for applied implementation research, as well as farm-scale and area-wide evaluation of the biological and socio-economic impacts of new management tactics.

1.7.11 Implementation research

Moving from the discovery phase to the implementation phase requires information on scale-up, compatibility of new and established technologies and other factors that affect the viability of approaches in commercial agriculture.

1.7.12 Evaluation of the impact of new technologies

Implementation of ecologically-based IPM strategies must be tied to studies of the effect that these new methods may have on non-target species, agricultural practices and human health. Social and economic factors will also play significant roles in determining whether ecologically-based IPM will be widely adopted.

1.7.13 Development of interdisciplinary cooperation

Every crop faces multiple assaults from numerous arthropods, diseases and weeds simultaneously; thus, management decisions become complex. Many of the chemical and cultural tactics (e.g. tillage and crop rotation) available to agricultural and forest producers have broad impacts on the system. In suppressing one pest, it is likely that other components of the system will be disrupted. Interactions among organisms in managed systems will enable scientists to design tools and methodologies to reduce populations of problematic organisms without negatively affecting the balance of the system. Thus, the complexity of ecosystems necessitates coordinated multidisciplinary and interdisciplinary research to develop and implement ecologically-based IPM.

1.7.14 Multidisciplinary research

Despite the generally recognized need for a systems approach to solving pest problems, the operating philosophy and organizational infrastructure in pest management is largely fragmented among different disciplines, agencies and research institutions. This fragmentation is partly a result of the

tendency for science to solve a problem by breaking it into parts, i.e. disciplines. Science education and research experiences are largely clustered around subject disciplines. For example, a biologist may specialize in molecular and cellular processes, or in whole organisms and organism–organism interactions, or in analysing patterns that govern whole ecosystems. It is rare that a single researcher is accomplished in research across all these levels of biological organization. Research expertise can be further segmented by types of organisms and functions such as virology, entomology, plant pathology, microbiology, plant physiology, plant breeding, taxonomy, genetics and epidemiology, to name but a few. Other disciplines are likewise divided into distinct groups that often consider each other rivals in the pursuit of resources and recognition.

Barriers have also been erected between the various pest-science disciplines, despite the fact that these groups are, in essence, interdisciplinary. Entomology, plant pathology, nematology and weed science study the interactions of different groups of organisms and thus require broad backgrounds in the plant sciences, microbiology or invertebrate biology, and environmental sciences. The barriers are best exemplified by these disciplines' development of parallel nomenclatures that describe similar processes, effectively further insulating the disciplines from each other.

Institutional structures, including professional societies and academic departments, and consequently funding patterns for research, are largely responsible for the development of barriers that then become hardened through competition for limited research and extension funds and other types of institutional support and recognition. Events over the past few decades that are unique to the pest sciences are challenging efforts to encourage interdisciplinary cooperation among these groups.

Many programmes that were conducted under the umbrella of IPM can be criticized for becoming '*insect* pest management' programmes or 'integrated *pesticide* management' programmes. It is unfortunate that, because of the way IPM programmes were implemented, one of the legacies of IPM is more, rather than fewer, barriers between the pest-science disciplines.

The goals of ecologically-based IPM cannot be reached without collaboration across taxon-specific disciplines and across hierarchical levels of organization. Interdisciplinary research and other incentives for collaboration among groups must be crafted in ways that make researchers accountable for doing truly collaborative work. Interdisciplinary activity in the pest sciences must contend with historically based divisions at the research and administration levels or the problems that have plagued IPM research will be repeated.

1.8 Challenges

Ecological pest management is about bringing the balance back to disturbed ecosystems; it is also about learning to observe such balances (Geier and Clark, 1961). The enormous impact that pests and diseases have on today's agriculture forces us to pay special attention to this issue in terms of resource management, cost–benefit analysis, and the impact on health and the environment. Our main challenge is therefore to keep the collective learning spirit that has been built by approaches such as FFSs alive. There are no standard recipes or solutions available. We need to continue to strive to enrich this learning methodology building on our wide range of experience and wisdom. The challenge is to apply holistically the agro-ecological and socio-economic relationships through knowledge-empowering methodologies.

Of the several methods available to control these pests, conventional insecticides are still relied on by the vast majority of farmers (Kogan, 1986; Kogan and McGrath, 1993). However, an 'insecticide treadmill' situation is developing due to the use of poor application equipment, poor choice of insecticides and the development of resistance (Carson, 1962). In addition to huge direct economic costs, the indirect

costs such as deleterious effects of pesticides on the environment and human health are becoming increasingly severe all over the world. Therefore, there is a need to take a critical look at the adverse effects of pesticides in the environment, and to develop appropriate technologies and guidelines for judicious application of pesticides for crop production. Several natural plant products, biopesticides (such as entomopathogenic bacteria, fungi, protozoa, nucleopolyhedroviruses and nematodes), parasites and predators have been proposed as a safer alternative to the synthetic pesticides. However, there is little information on the biosafety of these products to humans and the environment. These products have not been subjected to the same rigorous biosafety testing as the synthetic chemicals. Therefore, there is a need to look at the biosafety of these biological methods in the context of environmental safety and sustainable crop production. Significant progress has been made over the past three decades in the introduction of exotic genes into genetically modified organisms (GMOs) for pest management. Recombinant DNA technology has helped to extend the range of conventional genetic manipulation to meet the increasing need for food and fibre. GMOs have been deregulated for use in pest management for increasing crop production in several countries. Genetic engineering to improve the effectiveness of crop protection technologies offers an environmentally friendly method of crop protection and has resulted in a drastic reduction in the use of pesticide sprays and in increased crop production. The products of genetic engineering are not conceptually different from those derived through conventional technologies. Crop cultivars derived through conventional breeding have not been subjected to biosafety assessments. However, the potential of recombinant technologies to allow a greater modification than is possible with conventional technologies has raised some concerns, and therefore GMOs have been subjected to rigorous biosafety assessments. Procedures employed for assessment of the biosafety of transgenic crops to non-target organisms, coupled with the interpretation and utilization of such information, are important components for the sustainable deployment of transgenic crops for food and environmental safety in the future.

Therefore, overall, there is a need to take a critical look at the procedures employed for assessing the biosafety of synthetic pesticides, biopesticides and transgenic crops and their impact on the environment, in order to frame appropriate procedures and guidelines for the testing and deployment of these technologies for sustainable crop production and food and environmental safety in the future.

1.9 Conclusions

The adoption of a more ecological approach in which the actions and interactions of the component technologies are fully understood within the context of the local agroecosystem could lead to the development of more effective and sustainable 'truly integrated' pest management. The need for this approach derives from the fact that the single-solution 'magic bullet' approach has undermined our basic understanding of how individual components actually work and has oriented IPM toward quick fixes, often underpinned by commercial incentives. Developing a truly integrated pest management system that addresses the problems of sustainable agriculture will require that more emphasis is placed on long-term solutions, even if the practical outputs are inherently simple. It must be remembered that the complexity of nature also makes it difficult to apply conventional area-wide prescriptions successfully across all systems. Equally, compensating for a lack of understanding with excessive reliance on single technologies and short-term solutions does not make sense; our experience of the last 30 years has shown that the greater the apparent success in achieving pest or disease control in the short term, the greater the likelihood of a serious breakdown in the long run (Conway, 1997). Many IPM tools are available now

(e.g. partially resistant germplasm, partially effective indigenous natural enemies, and potentially effective but unreliable microbial agents) that do not require further technological breakthroughs to be useful. The immediate challenge lies in the genuine application of ecological research to understand how these components interact and to identify more effective and sustainable integrated strategies for their use.

Agriculture is the source of diverse selective forces, and modern agriculture – its species and practices – is the outcome of a continuous process of change that has dramatically changed physically and genetically all components of agroecosystems (Thrall *et al.*, 2010). Further advances in the genetic potential of crops and livestock will continue to be dominated by conventional breeding strategies made increasingly efficient by advances in marker technologies. In addition, however, the introduction of genes from other species via molecular manipulation (GM technologies) will become increasingly common but generally restricted to introducing changes that are unattainable via conventional approaches. Overall, therefore, recognition and application of ecological and evolutionary principles will be an increasingly important part of the overall development and management of agriculture, particularly in the context of climate change (new plants and animals), and the imperative for greater sustainability (shifts in land management). As such, we suggest that biologists might do well to consider agroecosystems as useful models for the scientific investigation of evolutionary processes.

Finally, in any particular setting, the role of IPM needs to be considered in the context of the overall production system. To do this, it is necessary to determine the role of different constraints in the production chain and to define where the greatest gains per unit investment can be made. Thus, a critical step in future efforts to increase productivity will be to move away from generalist, area-wide management prescriptions towards local solutions developed in response to questions generated at the local level. Unfortunately, such an approach presents considerable challenges and may even conflict with existing research incentives and institutional structures (Waage, 1997, 1998). This conclusion indicates that, whatever technological or methodological advances to improve productivity are identified, effective implementation will be possible only with appropriate economic and political support. Rogers (2003) generalized five attributes that determine the rate of adoption of an innovation, namely, relative advantage, complexity, compatibility, observability and trialability. Therefore, IPM is the farmers' best use of a mix of control tactics that are biologically, environmentally, economically and socially compatible with the farming system and farmers. Farmers will base their decisions on their knowledge and perceptions of pests, compatibility with their farming system, and relative advantages in terms of control of pests and yield. The implementation of IPM around the world has been going on for the last four decades, but the uptake of IPM has been slow. In developed countries, the challenge is to reduce the high use of pesticides, while in the developing world, the challenge is to reduce or maintain the low levels of pesticide use. The methodologies and initiatives for implementing IPM vary from country to country, from developed countries to developing countries, and from crop to crop. All the actors involved with agriculture production systems, namely producers (farmers), consumers, researchers, extension agencies, and market and government agencies need to be the part of the IPM innovation system to increase the uptake of IPM.

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2 Integrated Pest Management for Sustainable Agriculture

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2.1 Introduction

Global agriculture is currently challenged to provide increasing supplies of food for a growing population, to reduce losses of greenhouses gases, which contribute to climate change, to increase efficiency in the use of inputs and to reduce environmental impacts from production. Achieving eco-efficient agriculture, where both the ecological and economic dimensions are considered, is the Holy Grail (Keating *et al.*, 2010). In the context of these demanding requirements, the efficient management of insect pests should have a high priority given that insects still take about 15% of potential global crop yields (out of a total of 42% lost to all pests) (Yudulmen *et al.*, 1998) and input for their control (in the form of insecticides) adds economic and environmental costs to the food production equation.

It is just over 50 years since the seminal work of Stern *et al.* (1959) articulated the integrated control concept that flourished into the framework of integrated pest management (IPM) as we know it today. In celebrating that paper, Naranjo and Ellsworth (2009) discussed the evolution of IPM concepts built on the original four components: (i) sampling systems; (ii) thresholds for determining the need for control; (iii) understanding and conserving the biological control capacity of the system; and (iv) the use of selective pesticides when needed. IPM has long been proposed as a more sustainable approach than reliance solely on pesticides and today has evolved well beyond these four components to accommodate a broader range of cultural controls, habitat manipulations and pest-resistant crops, which are increasingly genetically modified (GM) crops (Romeis *et al.*, 2008). Numerous successful case studies are available (Kogan, 1998; Matteson, 2000; Wilson *et al.*, 2004; Cook *et al.*, 2007) however, the adoption of a truly integrated pest management approach has been extremely patchy. In some systems, the adoption and impact of IPM has been spectacularly successful, such as Asian rice (Matteson, 2000) or the highly innovative push–pull systems in East African maize (Cook *et al.*, 2007). The reality is that, 50 years on from the publication of Stern and colleagues, the global use of pesticides continues to increase (Birch *et al.*, 2011). Often IPM relates to integrated pesticide management, and some practitioners despair about the future prospects (Zalucki *et al.*, 2009).

While Bajwa and Kogan (2004) have listed some 67 definitions of IPM, the current definition of the Food and

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Agriculture Organization of the United Nations (FAO) still captures the basic concepts of integration of multiple approaches. Broadly, IPM can be defined as 'the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms' (FAO, 2002).

Here, we describe the implementation of IPM in Australian cotton as an example of the broad strategic and tactical approach that could be applied to a range of field crops. The Australian cotton industry illustrates well the numerous challenges of integration and implementation of IPM and how these might be overcome. We then discuss some of the constraints and challenges to adoption of IPM principles in the extensive Australian grain industries (e.g. wheat, canola, legumes) where uptake has been minimal (Horne *et al.*, 2008).

2.2 IPM: Strategies and Tactics

In considering how IPM might be applied in any situation, it is important to consider both strategic and tactical approaches to managing a pest situation and note that IPM at its broadest is a whole of the landscape, year-round approach.

Strategic components of IPM essentially set the structure and context of the production system (crop and surrounding landscape) in which the IPM game will be played. Strategic components include decisions such as the choice of crop to be grown, choice of crop varieties, including those with pest resistance traits (conventional or GM), location of fields in relation to other crops or natural vegetation, the timing of planting and the rates of fertilizer to be applied. These are all components that could potentially moderate pest abundance or exposure of the crop to damaging pest densities. These strategic decisions might also include manipulation of the cropping landscape in ways that encourage the overall abundance and diversity of beneficial insects, which might then play a role in pest suppression within the crop (Schellhorn *et al.*, 2008). Decisions on these strategic IPM components may be made long before pests actually interact with the crop and, once made, these strategic decisions are essentially irreversible.

Tactical components of IPM are those that form the real-time management of the crop during the growing season. There are many different tactical components that could be applied and these will vary greatly across production systems and pests, but the following are probably common across many:

- Regular crop sampling using well-validated and pest-specific sampling systems.
- The use of action or economic thresholds that indicate the point at which pest-management interventions are necessary or will be economically relevant.
- Integration of the abundance and impacts of beneficial insects in the crop through pest:predator ratios and so on.
- Augmentative release of beneficial species – predator or parasitoids – as a direct pest-suppression measure.
- The use of biological pesticides or selective synthetic pesticides that do not disrupt species other than the target pest.
- In-season management of nutrients and water to modify crop attractiveness to pests.
- Various cultural practices and tillage that directly impact on pests.

Strategic IPM components tend to be one-off decision points and have relevance across a broader spatial scale than tactical components, which tend to be more crop-focused and repeatable.

2.3 The Australian Cotton Industry as an Example

Many IPM components have been applied in the cotton industry since the late 1970s when a computer-based decision support system, SIRATAC, was released to industry (Hearn and Bange, 2002). These systems were field-focused and included the early use of pest-tolerant cotton varieties and well-validated sampling systems linked to fixed or dynamic thresholds that triggered the use of pesticides. Today, IPM has evolved to include a strategic landscape dimension, driven in part by the extensive adoption of genetically modified *Bacillus thuringiensis* (*Bt*) transgenic cotton varieties in which the gene encoding the *Bt* toxin, a natural insecticide, has been inserted; these varieties provide wide-scale benefits in terms of minimizing disruption of beneficial insects in the system (Fitt, 2000).

Insect pests have been an enduring challenge for the Australian cotton industry (Hearn and Fitt, 1992; Fitt, 1994). Key pests include the noctuid moths (*Helicoverpa armigera* and *Helicoverpa punctigera*), spider mites (*Tetranychus urticae*) and a suite of sucking pests such as aphids (*Aphis gossypii*) and mirids (*Creontiades dilutus*). Other challenges linked to pests are insecticide resistance in the primary pest (*H. armigera*) and two secondary pests (mites and aphids); escalating costs of production and environmental concerns over off-farm movement of insecticides. More recently, the challenges relate to sustainable management of GM cotton varieties and concerns about resistance to *Bt* proteins in both *Helicoverpa* species (Downes *et al*., 2010a).

There is little doubt that the crises caused by dichloro-diphenyl-trichloroethane (DDT) resistance in the late 1970s and resistance to synthetic pyrethroids from 1983 onwards (Forrester *et al*., 1993) were key drivers for the major research, which aimed to reduce pesticide dependence and implement IPM systems (Wilson *et al*., 2004; Fitt, 2004). To be implemented by industry, an IPM system needed to reduce insecticide use and deliver environmental sustainability while maintaining yield and economic returns and maintaining the susceptibility of pests to new insecticides. At the same time, it needed to be practical and workable in the context of the whole farming system and, importantly, to be relevant to both conventional cotton and transgenic (*Bt*) cotton varieties, which both form important components of the current industry.

The Australian cotton IPM system addresses these goals through a number of key principles:

1. Integration of all farm management activities throughout the annual cycle of production, not just during the cotton season.
2. Applying best practice agronomy to ensure a healthy crop.
3. Rigorous sampling of pests, beneficial insects and damage, linked to well-validated thresholds.
4. Conservation and utilization of beneficial insects.
5. Preferential use of selective insecticides.
6. Pre-emptive management of resistance to insecticides and GM traits.
7. Extension support through communication and training.

These principles were captured in the *Integrated Pest Management Guidelines for Cotton Production Systems in Australia* (Deutscher *et al*., 2005) and underpinned by an emphasis on both profitability and sustainability, ensuring that both input costs and yield are considered, rather than a more traditional emphasis on maximizing yield alone.

This IPM framework partitions the annual crop cycle into five key periods: (i) planting to first flower; (ii) first flower to first open boll; (iii) first open boll to harvest; (iv) a post-harvest period; and (v) a pre-planting period. The first three periods deal with the growth cycle of the crop. The final two deal with the 'off' season or winter period. Inclusion of the winter period is essential, as many of the actions and strategic decisions taken through this period have important implications for the

success of IPM in the following growing season. In each of these phases, key non-insecticidal tools that can be used to manage pests or to enhance the abundance of beneficial species were identified. These included a range of agronomic, varietal and physiological factors that are part of the farming system but are not normally thought of as pest-control tools. These factors can all be manipulated in a strategic way to contribute toward the goals of IPM.

2.4 IPM Components Applied During the Cotton Season

The following sections list the key IPM components utilized in Australian cotton production (see Wilson *et al.*, 2004, for further details).

2.4.1 Pest-tolerant varieties: the foundation

The crop itself is the starting point for any IPM system. Cotton plants have a number of naturally occurring biochemical (e.g. tannins, terpenoids) and morphological (e.g. leaf shape) defences against arthropod pests (insects and mites) and a high capacity to compensate for pest damage, as the plant produces many more flower buds than it can mature as bolls (Sadras, 1995; Sadras and Fitt, 1997). Several conventional host-plant resistance traits have been incorporated into modern Australian-bred varieties. These include resistance to key diseases (e.g. bacterial blight, *Verticillium* wilt, *Fusarium* wilt) and morphological traits (e.g. okra leaf, smooth leaf) that provide incremental benefits for insect resistance by reducing the rate of population increase of pests such as mites (Wilson, 1994a). Since 1996, *Bt* cotton varieties (Ingard and then Bollgard II) have provided a quantum leap in pest tolerance achieved through expression of the delta-endotoxin genes from *B. thuringiensis* subsp. *kurstaki*. These insect-resistant transgenic cottons provide an excellent platform for IPM (Fitt, 2000; Fitt and Wilson, 2000) as they reduce the need to control *Helicoverpa* spp., the primary cotton pests in Australia, thereby reducing disruption to beneficial insects caused by insecticide use, and conserving and maximizing beneficial insect activity. *Bt* cotton varieties have also been an important tool in the implementation of IPM concepts with growers, as they are able to develop confidence in seeing 'living crops' filled with predators and some pests and so are able to realize the benefits of IPM more easily (Fitt, 2008).

2.4.2 Agronomic components

Agronomic components can be divided into three main factors:

1. Optimal planting time. Planting outside the optimal time period (early October in most established production regions) adversely affects yield potential and is counterproductive to IPM. Very early planting (mid-September) in cool districts increases the risk of damage due to severe cold, slow early growth and greater susceptibility to early pest damage from thrips. Late-planted conventional cotton has decreased yield potential and increased risk of late infestations of *H. armigera*, mites and aphids that are difficult and expensive to control. By contrast, the Bollgard II cotton varieties can potentially be planted later, as they are able to set a crop within a tighter boll-setting window (Bange *et al.*, 2008). Planting time is now constrained by the requirements of the *Bt* resistance management strategy (Fitt, 2003).

2. Optimal water management. For irrigated cotton, a critical decision is the timing of the final irrigation. Unnecessary late irrigation will extend the period of attractiveness to pests but may not enhance yield potential. Optimal irrigation management can avoid this problem.

3. Strategic use of plant growth regulators. Optimal irrigation scheduling and rates of nitrogen fertilizer will generally control vegetative growth. However, excessive

vegetative growth will produce crops that remain attractive to pests and mature late, thereby increasing the need for pest control. Appropriate use of growth regulators helps to reduce the severity of this problem.

2.4.3 Use of trap crops to concentrate pest populations

Trap cropping is a key example of habitat manipulation of a cropping system for the purposes of pest management (Shelton and Badenes-Perez, 2006). One Australian example is the use of spring chickpea crops to capture eggs from *H. armigera* moths that emerge from overwintering diapause (Sequeira, 2001). These moths are potential carriers of genes for pesticide resistance from one season to the next (Daly and Fitt, 1990). Trap crops are a means of concentrating *H. armigera* populations into a limited area where they can be destroyed using biopesticides or by cultivation of the trap crop, thereby reducing the carry-over of resistance genes and overall population size (Sequeira, 2001). This is now a mandatory component of the resistance management strategy for *Bt* cotton in tropical regions of Australia (central Queensland) where *Helicoverpa* populations do not enter a winter diapause.

A second example is the use of spring lucerne trap crops to capture adults of the green mirid, *C. dilutus*, and avoid infestation of adjoining cotton. Green mirids are important pests in cotton, often causing plants to shed squares (flower buds) or young bolls and damaging maturing bolls, causing yield loss. Green mirids prefer lucerne (new growths or shoots) to cotton. Lucerne crops adjacent to, or as strips within, cotton crops act as a sink for green mirids. By alternatively slashing half of the lucerne at 4-weekly intervals, new regrowth of lucerne can be maintained and the green mirids can be maintained in the lucerne without moving into the cotton (Mensah and Khan, 1997). Unfortunately, such practices are not widely used, in part because they are time-consuming to deploy effectively and disrupt the agronomic management of the whole farm.

2.4.4 Conservation, enhancement and integration of predators into pest management

Cotton fields may harbour as many as 450 different insect species, and a significant proportion of these are beneficial insects (beneficials). It is striking that the key beneficial groups in cotton are similar in many parts of the world (Hearn and Fitt, 1992), but their impacts and value are difficult to demonstrate.

While predators and parasites are important components of IPM systems, there are often severe limitations in the capacity of beneficials to control some pests, particularly the heliothines, which are highly mobile, fecund and well adapted to exploit diverse cropping systems (Fitt, 1989, 1994). Conservation of natural enemies requires considerable ecological understanding of their seasonal phenology, habitat and prey requirements. The majority of predators are generalists, able to sustain their populations on a diversity of prey types. Predator abundance can readily be monitored, and estimates of abundance are utilized in decision making through a predator:prey ratio that indicates when predators are sufficiently abundant to have an impact (Mensah, 2002a, b).

Mechanisms to enhance beneficial insects include the use of selective insecticides (discussed below), the use of explicit on-farm 'nursery crops' and the maintenance of on-farm native vegetation, which may provide refuge for beneficial populations. Such refuges for beneficials can offer a permanent on-farm habitat, providing some buffer against the unpredictability of natural populations. Increasingly, however, it is being realized that management of beneficial insects as part of IPM needs to be implemented at a landscape scale, beyond individual fields or even farms (Schellhorn *et al.*, 2008; Bianchi *et al.*, 2010), just as the dynamics of many pests reflect landscape-scale processes.

2.4.5 Rigorous and regular crop sampling

Regular crop sampling for pests, plant damage and beneficial insects is critical for effective IPM (Binns and Nyrop, 1992; Naranjo and Ellsworth, 2009). Sampling systems need to be tailored to the spatial distribution of pest stages within and between plants, and thus it may be challenging to find a single sampling method that provides reliable information on a suite of pests and beneficials (Deutscher *et al.*, 2005). Regular sampling (every 3 days during crop development) means that decisions to delay control can be monitored and action taken if the situation changes while the pest population can still be effectively controlled with selective insecticides.

2.4.6 Use of combined pest and damage thresholds

Thresholds are an essential tool to ensure that insecticides are only applied if economic loss is reasonably expected to occur (Deutscher *et al.*, 2005). However, thresholds based solely on pest numbers alone assume that all cotton crops respond to pest density in a similar way. Many other factors may ameliorate crop response to pests (e.g. vigour, disease, temperature and nutrition) such that the crop compensates for damage. Guidelines have been established for the amount of damage that plants can tolerate without loss of yield or delay (Wilson *et al.*, 2003). By integrating compensatory responses into thresholds, it is possible to identify those situations where pests may have exceeded a threshold but the crop will recover without loss, thereby avoiding use of an insecticide to prevent non-economic damage.

2.4.7 Preferential use of selective insecticides

With a much closer environmental focus on agriculture, recently developed insecticide groups are increasingly more selective than the older suite of organophosphate, carbamate and pyrethroid pesticides that characterized Australian cotton 20 years ago. New compounds and chemical groups appear regularly, and, together with biological pesticides (e.g. nuclear polyhedrosis virus and *Bt* sprays), they can provide powerful IPM tools as they are less disruptive to beneficial populations. Understanding the particular characteristics and spectrum of impacts of insecticides is important, and so independent information on the efficacy and non-target effects of all current insecticides has been obtained locally (Wilson *et al.*, 2002).

2.4.8 Effective resistance management strategies

Australia has a long history of problems with the evolution of pesticide resistance in key pest populations (Forrester *et al.*, 1993) and has managed these threats through an insecticide resistance management strategy (IRMS), which constrains use of pesticide groups and ensures rotation of chemicals. Today, with 90% of the Australian crop being *Bt* cotton (Bollgard II), the need for insecticide resistance management is reduced, but the critical importance of pre-emptive resistance management for *Bt* proteins remains (Gould, 1998; Fitt, 2008).

2.4.9 IPM components applied outside the cotton season

An example of this is the destruction of diapausing pupae of *H. armigera*, which are a potential reservoir of resistance genes. Fitt and Daly (1990) showed that cotton fields may harbour significant populations of overwintering pupae of *Helicoverpa* spp., mostly *H. armigera*. These populations provide a direct bridge for resistance genes from one season to the next (Daly and Fitt, 1990) and their removal through cultivation (Roush *et al.*, 1998) provides a core non-insecticidal component of both the resistance management and IPM strategies.

Prior to the advent of *Bt* cotton, growers were advised to sample cotton stubble for overwintering pupae, using published guidelines, and to cultivate those fields with overwintering pupae. With *Bt* cotton now dominant, cultivation of crop residues has become a mandatory component of the *Bt* resistance management strategy with the aim of minimizing the survival of any *Bt* resistant individuals and also to eliminate any regrowth of harvested cotton plants, which might support an autumn generation of the pest.

2.4.10 Selection of rotation crops to reduce pest carry-over

Rotation crops also have implications for pest management. Some rotation crops provide an overwintering host for pests and some cotton diseases (e.g. faba beans (mites, aphids), safflower (mites, mirids), chickpeas (*H. armigera*) or cereals (*H. armigera* and thrips)). At the same time, winter rotations provide a seasonal refuge for beneficial insect populations. Balancing of these issues needs to be taken into account in the choice of rotation crop and its management.

2.4.11 Management of weeds and cotton regrowth that are overwinter pest hosts

Weeds and cotton regrowth following harvest can provide overwinter hosts for a number of pests including *Helicoverpa*, mites (Wilson, 1994b), mirids, aphids, tipworm, cutworm, armyworm and whitefly. Poor in-field hygiene is a particular problem with spider mites, aphids and mirids, as these pests can move off the weeds and on to seedlings in the following season. Again, weeds also provide a refuge for beneficial species and the trade-off between pests and beneficials needs to be considered. At the scale of individual fields on farms, it is likely that the safest course is for strict management of in-field weeds and regrowth.

2.4.12 Optimization of fertilizer strategies to avoid excessive plant growth

Most nitrogen fertilizer for cotton is applied prior to sowing, often several months beforehand, and so it is a strategic decision that can impact on pest management during crop growth. Nitrogen availability can directly affect pest management by modulating tritrophic interactions of crop, pest and natural enemies (Chen *et al.*, 2010), as well as impacting on potential yield and maturity. Too little nitrogen will decrease yield. Excessive nitrogen can create excessive end-of-season plant growth, making the crop more attractive to *Helicoverpa*, requiring additional inputs of expensive insecticides for control, potentially delaying crop maturity by 1–2 weeks and making crops harder to defoliate (Rochester *et al.*, 2001).

2.4.13 Selection of varieties matched to region and pest complex

Australian growers have access to a wide range of varieties adapted to certain geographical regions or production situations. These days, all varieties express *Bt* proteins and may also carry herbicide tolerance traits. However, selection of the most appropriate varieties for the combination of season length, yield potential, pest complex and agronomic situation remains important for IPM.

2.4.14 Selection of appropriate seed insecticide treatments

By definition, the use of 'at-planting' insecticides, applied in the soil or applied directly to the seed as a seed treatment (e.g. imidacloprid, thiodicarb and fipronil) is 'prophylactic' and may not seem compatible with an IPM approach. However, they are reasonably selective (Wilson *et al.*, 2002), as they act systematically and are thus not accessible to most beneficial species. The main target for seed treatments is thrips, and where there is a reasonable expectation of an economic benefit from control of

thrips, the use of 'at-planting' insecticides will often be more IPM compatible than applying pesticides to seedlings based on a threshold, particularly as this is the time when many beneficial species will be colonizing cotton crops from surrounding vegetation.

2.5 The Impacts of IPM

So what have been the outcomes from the adoption of an IPM approach in Australian cotton? There is no doubt that over the last 10–15 years there has been a dramatic reduction in pesticide use (Fitt, 2008). However, the growth in awareness, research implementation, industry training and support for IPM has overlapped substantially with the introduction of GM technology in the form of *Bt* cottons expressing first one and now two *Bt* genes (discussed more fully below) and likely within a few years to contain a third insecticidal protein, VIP3A (Llewellyn *et al.*, 2007). This period also has also seen sustained drought, which reduced the abundance of many pests and also brought a suite of new selective pesticides that were active at much lower concentrations. Elsewhere, the argument has been presented that *Bt* cotton has been the foundation on which IPM has been built and has made a difference (Fitt, 2000; Fitt and Wilson, 2000; Wilson *et al.*, 2004; Fitt, 2008). Prior to *Bt* cotton and its capacity to effectively manage the most challenging pest in the system, IPM principles were applied but made little headway. Now, there is a much changed attitude across industry, and one important possibility is that the coincident release of *Bt* cotton and the industry-wide extension effort on IPM allowed many growers to gain confidence in the potential for IPM by managing their *Bt* cotton crops. They were able to become more comfortable with seeing a 'living' crop, filled with numerous and mostly innocuous or beneficial insects, became more attuned to the critical importance of managing agronomic inputs, and were more willing to work cooperatively with neighbours through coordinated IPM and area-wide management groups. In this sense, GM technology has been very compatible with IPM (Kos *et al.*, 2009).

2.6 GM Crops in IPM

GM crops expressing insecticidal proteins such as the delta-endotoxins from *B. thuringiensis* subsp. *kurstaki* (*Bt* crops) offer great potential to dramatically reduce pesticide dependence for control of the major crop pests and consequently offer real opportunities as a component of sustainable and environmentally acceptable IPM systems. Since their introduction in the mid-1990s, there has been considerable debate about the compatibility of GM technologies with biological control and hence with IPM. However, the developing consensus is that *Bt* crops do provide a valuable platform for IPM systems (Fitt, 2008; Romeis *et al.*, 2008; Kos *et al.*, 2009; Lundgren *et al.*, 2009).

Bt cotton technology was introduced in to Australia in a phased way with single-gene (*Cry1Ac*) cotton (known as Ingard) grown from 1996 until 2004, after which two-gene varieties (*Cry1Ac*/*Cry2Ab*, known as Bollgard II) were introduced and completely replaced the single-gene lines. Fitt (2008) provides a full account of the impacts of *Bt* cotton on pesticide use and IPM adoption. During the first 8 years of use of *Bt* cotton, there was an average reduction of 59% in insecticide applications and of 44% in active ingredients applied for the key pests, *Helicoverpa* spp. With the introduction of Bollgard II varieties, this reduction averaged 80–90% fewer insecticide applications compared with conventional cotton and a 65–75% reduction in active ingredients on a per-hectare basis. Bollgard II varieties essentially provide season-long control of *Helicoverpa* spp. (Pyke, 2007) with only an occasional crop requiring one spray at the end of the season. In addition, there has been little change in the total number of sprays for all other pests (mirids, mites, aphids and thrips) (Fitt, 2008), although, as Gregg and Wilson (2008) showed, most sprays are now directed at

mirid bugs. These dramatic reductions in pesticide use translate to much less disruption of the system and hence greater opportunity for other IPM approaches for the remaining suite of pests that are not controlled by *Bt* proteins. Despite this significant impact, Gregg and Wilson (2008) raised some timely concerns about whether the Australian industry, and by analogy those in other parts of the world, may be relying too much on *Bt* technology with the attendant risks that *Bt* resistance would bring (Downes *et al.*, 2010b). They expressed concern that the focus on IPM may be lost in the highly simplified pest management system now existing in *Bt* cottons. There is clearly a need for balance and ongoing emphasis on a suite of tools for IPM and on the research and extension support needed to mould them into a comprehensive and enduring IPM framework.

The major challenge to sustainable use of transgenic *Bt* crops, particularly high-value crops such as cotton, is the risk that target pests may evolve resistance to the *Bt* proteins. For this reason, a pre-emptive resistance management strategy was implemented to accompany the commercial release of *Bt* varieties in Australia (Roush *et al.*, 1998) and elsewhere. The development, implementation and progress of these strategies in Australia is well described by Fitt (2008), Baker *et al.* (2008) and Downes *et al.* (2010a). Similar strategies will be needed with *Bt* crops being increasingly deployed around the world, most recently *Bt* rice in China (Chen *et al.*, 2011).

2.7 IPM in Australian Grains

Australian grain industries face a changing mosaic of pest challenges, which is driven by changes in crop types, pesticide use patterns, climate change and farm management practices (Hoffmann *et al.*, 2008). As in cotton, there are elements of integrated management in response to key pests such as red-legged earth mites (Ridsdill-Smith *et al.*, 2008), where targeted chemical control, cultural controls, biological control and host-plant resistance can all be effectively utilized. Likewise, for mobile pests such as the diamondback moth, which attack not only broadacre crops of canola but also numerous horticultural crops, there are opportunities to integrate selective chemistry with biological control and host-plant resistance (Furlong *et al.*, 2008). None the less, the potential to implement IPM remains challenging, as the pest is migratory and sporadic with no forecasting system available, many of the selective chemicals are not registered in that crop and knowledge about the real impacts of beneficial species is limited.

An ongoing challenge for IPM in many grain systems is the reality that insecticides are inexpensive and still effective and are thus often used in a prophylactic manner, often combined with other farm management practices, such as weed control. Despite this, the risk of pesticide resistance in key pests of the establishment phase of grain crops (e.g. numerous earth mites; Umina, 2007) or of disease-vectoring aphids during the growing phase is very real (Edwards *et al.*, 2008) and is likely to maintain pressure to reduce reliance on pesticide inputs. Past episodes of resistance have been the real driver towards IPM adoption and enlightened deployment of transgenics in the cotton industry, and may well lead to similar changes in grains.

Perhaps a bigger distinction between these two sectors, and one that probably applies in many parts of the world, is the relative size of these industries in terms of numbers of farmers and advisors and the capacity of extension services to deliver new knowledge and management options effectively. Australian cotton has some 800 growers and is well serviced by research and extension, whereas Australian grains have some 40,000 growers widely dispersed across the country. This creates a challenging situation for achieving industry skill development and confidence building in the real opportunities for IPM.

This challenge of large numbers of farmers has been addressed directly in smallholder cropping systems in Asia, where the FAO has supported the ‘farmer

field school' and 'train the trainers' approaches to impart simple but well-designed messages that allow farmers to implement their own IPM (Matteson, 2000). Similar approaches are emerging in Africa (Abate *et al.*, 2000), although traditional agricultural practices already reflect a more biologically based approach to pest management. In Australian grain farming, the clear need is to develop an economically based value proposition for how IPM can benefit a whole-farm management approach and to develop alternative, well-validated and demonstrated IPM approaches that can be implemented in a practical way. Even then, the exceedingly low costs of generic pesticides will make IPM a difficult case to sell.

2.8 Extension and Implementation of IPM

Defining and formalizing an IPM system based on the best possible science is just the first step. Achieving effective implementation requires a consistent and coherent communication and extension effort. The FAO programmes in numerous countries have been just this – well-targeted extension based on simple IPM messages. In Australia, the mechanism for extension support of agricultural industries has evolved dramatically over time as government support extension services have been reduced and private consultants and advisors have started to play a more prominent role.

Historically, the national cotton extension team involved industry extension specialists in all regions who used a range of strategies to deliver the IPM system (Christiansen, 2002), which reflected the industry attitudes and need for education around IPM (Christiansen and Dalton, 2002). These included field days to discuss IPM issues, coordinated experiments and demonstrations across several regions, production of regular newsletters and published IPM guidelines (Deutscher *et al.*, 2005), together with other technical information presented in the form of information packs, such as ENTOpak, NUTRIpak, WEEDpak and DISEASEpak, all of which include issues or approaches relevant to IPM (Australian Cotton Cooperative Research Centre, https://www.cottoncrc.org.au).

The cotton industry drive for environmental management of cotton farms also lead to the adoption of a best management practice (BMP) approach (Williams and Williams, 2000), now delivered online as MyBMP (https://www.mybmp.com.au/home.aspx). This provides a framework for growers to evaluate their management performance against the best standards in the industry, to identify areas of improvement and to document this in an auditable fashion. The core principles of the IPM guidelines form one module in the *Australian Cotton Industry Best Management Practice Manual*. This provides growers with a means to assess how they are progressing in adopting IPM principles on their farm.

Effective extension also fostered the development of regional IPM groups, where groups of growers agreed on core goals and communicated throughout the season to achieve a local regional approach to pest management. In some instances, this participatory research approach grew to a truly area-wide management system where pest management efforts were coordinated across a region by using understanding of the pests' ecology and interactions with the farming system to reduce abundance (Ferguson and Miles, 2002). To a large degree, the current high reliance on Bollgard II cotton (>90% adoption) has seen some of these innovative area-wide approaches decline. None the less, the expertise and experience is there to allow this type of coordinated response to common problems to be resurrected if needed.

The final critical factor in gaining support for IPM systems has come from favourable economic analyses of IPM. Hoque *et al.* (2000) analysed grower information and showed that the 'soft', IPM approach generally had equal or higher gross margins than a more traditional approach using 'hard', more disruptive chemicals. The difference was attributed to higher beneficial insect populations in the

fields managed with more selective insecticides. This analysis was critical in providing 'economic' credibility for the IPM approach. Again, the advent of Bollgard II has dramatically changed the cost structure of cotton production such that nuances about IPM are overshadowed.

Similar economic analyses in support of the 'value proposition' for IPM are not yet available for Australian grain systems, where economic returns are lower than cotton and where the range of pest management options and supporting expertise are both lower.

2.9 Conclusions

IPM is often projected as a complex, highly technical response to a pest management dilemma and one driven by an ideological desire to reduce or eliminate pesticide usage. This is simply not the case, in that the basic principles of IPM – sampling, thresholds and consideration about environmental impacts in selecting how to respond to a pest situation – can be simply applied to many cropping situations given an appropriate extension and education support programme. However, it is true that, in high-input Western agricultural systems growing highly valuable crops, IPM systems will be more complex than pesticide-based systems, and will require greater training and effort on the part of crop managers, whether they be professional consultants or farmers themselves. In essence, IPM reflects a sound interaction of science and pragmatism to achieve productive, viable and sustainable production systems. A key ingredient will always be investment in education and extension to build on the values of sound science.

As farming systems change in response to climate change and global market pressure, the pest complex will also change. The fundamental role of IPM in reducing pest pressure and insecticide use means that it must continue to evolve. Insecticidal GM crops with activity against one or more key pests are likely to be increasingly integrated into IPM as a strategic component, particularly to maximize the role of naturally occurring beneficial organisms (predators and parasites), which are likely to rebound significantly in cropping systems where insecticide use is reduced. Achieving an integration of approaches that rivals the predictability and simplicity of relying on conventional insecticides will be an ongoing challenge (Way and van Emden, 2000).

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3 Pest Monitoring and Forecasting

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3.1 Introduction

Monitoring for pests is a fundamental first step in creating a proper integrated pest management (IPM) programme. Pests are monitored through a variety of monitoring tools such as pheromone traps, light traps, coloured sticky traps, pitfall traps and suction traps. The trap capture data serves several purposes: (i) ecological studies (Pathak, 1968; Crummay and Atkinson, 1997; Hirao *et al*., 2008); (ii) tracking insect migration (Drake *et al*., 2002); (iii) timing of pest arrivals into agroecosystems (Klueken *et al*., 2009); (iv) initiating field scouting and sampling procedures; (v) timing of pesticide applications (Lewis, 1981; Merril *et al*., 2010); (vi) starting date or biofix for phenology models (Knutson and Muegge, 2010); and (vii) prediction of later generations based on size of earlier generations (Zalucki and Furlong, 2005). Forecast for pests is an important component of the IPM strategy. Early warnings and forecasts based on biophysical methods provide lead time for managing impending pest attacks and can thus minimize crop loss, optimize pest control and reduce the cost of cultivation. Prevailing and anticipated weather information can help in crop planning and scheduling spray and farm operations to maximize crop yields and returns. Computer models have been developed to support various aspects of crop management in general and plant protection in particular and are widely in use in developed countries. A decision support system integrates a user-friendly front end to often complex models, knowledge bases, expert systems and database technologies. Decision support systems have emerged as essential tools to bridge the gap between science-based technology and end-users who make day-to-day management decisions. Web-based models and decision support systems are becoming popular and in future may become an abosolute requirement for local, regional/area-wide and international implementation of IPM systems (Waheed *et al*., 2003). This chapter undertakes a selective review of published work on insect pest monitoring and forecasting and therefore is neither comprehensive nor exhaustive in its coverage.

3.2 Pest Monitoring through Traps

Among the various methods and devices used in pest monitoring, the most popular and widely used are sex pheromone traps for selective monitoring of individual flying species and light traps for flying species that are attracted to light. While adult males

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are mostly caught in sex pheromone traps, adults of both sexes are trapped in light traps.

3.2.1 Sex pheromone traps

Pheromones are chemicals for species-specific communication. Most often, these sex pheromones are produced by females to attract a mate and are most well known for adult Lepidoptera. Commercially produced by synthesizing and blending the appropriate chemicals, the sex pheromones are loaded into dispensers, which can be placed in traps of various designs for deployment in agriculture, horticulture, forestry and storage. Pheromone traps are the most popular and widely used tools for pest detection and population monitoring. Pheromone traps have been exploited for three useful applications: (i) monitoring; (ii) mass trapping; and (iii) mating disruption. The most important and widespread practical applications of sex pheromones in pest management have been reviewed recently (Witzgall *et al.*, 2010). Population monitoring relates trap captures to the abundance of, or to the damage caused by, an insect species. The numbers caught over time have been used for initiating field scouting for egg laying, and assessing the need for timing of control measures based on action thresholds (Wall *et al.*, 1987; Gurrero and Reddy, 2001). However, traps do not always accurately indicate the overall pest pressure for use as thresholds for action, as trap catches are influenced by the efficacy of the lure, the dispenser (Arn *et al.*, 1997), the trap design (Fadamiro, 2004; Spear-O'Mara and Allen, 2007) and the trap location (Reardon *et al.*, 2006; Gallardo *et al.*, 2009). Pheromone traps are the most effective and sensitive enough to detect low-density populations. They are therefore handy tools for tracking invasive species in the establishment phase (El-Sayed *et al.*, 2006; Liebhold and Tobin, 2008) or for population monitoring to determine the extent of an outbreak area and the effectiveness of eradication campaigns (Cannon *et al.*, 2004).

The timing of adult male catches in the trap indicates the start of the pest flight activity in the area. This information is important for some pests, as it is used as the biofix date for accumulation of heat units above a base temperature in phenology models or sustained first flight for others (Knutson and Muegge, 2010).

Sex pheromone traps are useful for monitoring difficult pests that evade early detection of economic damage when a trap catch is used to calculate: (i) growing degree-days (GDD) for onset and completion of moth emergence (Spear-O'Mara and Allen, 2007; Knutson and Muegge, 2010); (ii) starting dates of egg hatch (Isaacs and van Timmeren, 2009); and (iii) onset of first larval damage (Knutson and Muegge, 2010). A linear relationship between male catches in sex pheromone traps and GDD is possible after appropriate transformation of variables (Gallardo *et al.*, 2009), and in some cases variability is better explained by including other variables related to density of host plants or suitable plant parts (Spear-O'Mara and Allen, 2007). Validation of the degree-day model is done by comparing the timing of predicted and observed phenological events through field scouting and damage assessments, and estimating the prediction accuracy and error (Knutson and Muegge, 2010).

Monitoring through a network of sites is most useful for studying spatial distributions of pests, early detection of infestations and identification of hot-spot locations to initiate appropriate management interventions on a spatial scale. Monitoring at the regional level improves the reliability of population monitoring for implementation of appropriate area-wide IPM systems (Ayalew *et al.*, 2008). Moth captures in a network of pheromone trap sites established across the Canadian prairies, when used in conjunction with backward trajectories provided by meteorological services, were helpful in providing early detection of diamondback moth infestations (Hopkinson and Soroka, 2010). Peak trap captures are often correlated with associated weather to identify positive or negative influences of weather parameters

on moth activity and pest build-up (Gwadi *et al.*, 2006; Reardon *et al.*, 2006; Monobrullah *et al.*, 2007, Prasad *et al.*, 2008). However, trap catches and weather may not necessarily serve as predictors of the future abundance of certain species in cropping regions (Baker *et al.*, 2010).

3.2.2 Light traps

Insect attraction to light has been exploited for monitoring insect populations with a view to providing early warning of the presence of pests, as well as for many other uses. Light traps have been widely used for monitoring the population dynamics of Lepidoptera and Coleoptera (Wolda, 1992; Watt and Woiwod, 1999; Kato *et al.*, 2000). When compared with other sampling methods, light-trap sampling was found to be more efficient for lepidopteran population dynamics (Raimondo *et al.*, 2004). However, many factors affect catches of insects in light traps (Bowden, 1982). Trap design, the light source and its energy, and the attraction efficiency under certain conditions all contribute to sampling errors. The effects of weather conditions and moonlight on light-trap catches are well documented. For example, trap efficiency for Lepidoptera is positively correlated with temperature and the thickness of cloud cover, and negatively correlated with wind speed, precipitation and the fullness of the moon on the trap night (Bowden, 1982; Dent and Pawar, 1988; Yela and Holyoak, 1997; Butler *et al.*, 1999). The effect of weather factors on the abundance or species richness of Coleoptera captured by light traps has been reported (Rodriguez-Del-Bosque, 1998).

Networks of light traps have been used for year-round monitoring of moth species and the data used to assess the magnitude and reasons for seasonal, annual and long-term faunal changes and their population dynamics in Britain (Lewis, 1980) and India (Anon., 2009), and for weekly larval forecasts on cereal crops in Africa (Odiyo, 1979). Light-trap captures have been used to predict the emergence date of adult beetles from overwintering using a degree-day model (Zou *et al.*, 2004) and for prediction of population sizes based on moth catches (Raimondo *et al.*, 2004). Long-term light-trap data is highly useful in studying the seasonal dynamics of pests. For example, regression analyses have indicated that the spring generation of two species of *Helicoverpa* in eastern cropping zones in Australia could be related to rainfall in putative inland source areas (Zalucki and Furlong, 2005). Light-trap catch data is also useful for validation of simulation model outputs (Reji and Chander, 2008).

3.2.3 Monitoring of migration

Pedgley (1993) discussed and illustrated the role of forecasting and preventive management strategies from a variety of taxa and geographical areas, emphasizing the need to understand the effects of weather on migration. Modelling migration patterns of pests is useful to know their arrival time, identify periods with migration potential in order to time field evaluations, and to provide information on the size of migrating populations. Modelling studies using multi-location long-term suction trap data have indicated that temperature, global radiation and wind speed have a major impact on the flight activity of cereal aphids immigrating on to winter cereal crops during the early autumn and spring seasons (Klueken *et al.*, 2009). A network of light traps along with radars was used for studying the seasonal migration of cotton bollworm (*Helicoverpa armigera* Hübner) (Feng *et al.*, 2009). Furthermore, with automatic systems for monitoring, retrieving and analysing data from remote insect monitoring radars and meteorological equipment, it has been possible to generate daily statistical summaries and graphical representations of the migration activity observed by the radar during the previous night in terms of intensity, altitude, speed and displacement direction of the migrations, as well as the orientation, size and wing-beat frequencies of the migrants,

together with the surface weather conditions, at each site. This data was then available over the Internet to users the next day. Such a network has been used in inland eastern Australia since 1999 in studies of the spatial ecology of mobile insect populations and of the utility of migration-monitoring information for operational pest forecasting (Drake *et al.*, 2002). Similarly, analysis of the migration waves of rice brown planthopper (*Nilaparvata lugens* Stål) during June to July into South Korea using the boundary layer atmospheric (BLAYER) model and geographical information system (GIS) explained the spurt in light-trap catch data during late July (Zhu *et al.*, 2000).

3.3 Pest Forecasting

In pest forecasting, several intrinsic attributes of the insects and the determining environmental and host factors need to be considered. Most pest forecast models take into account the phenology of the herbivore and its host. Near real-time pest incidence data coupled with remote sensing and GIS tools facilitate early warning of impending pest build-up in a temporal and spatial perspective. In addition, collection and analysis of weather data from pest-affected areas is an essential input for models. The practical application of model outputs is aided by decision support systems, which are discussed in the following sections.

3.3.1 Considerations in pest forecast research

Accurate forecasting of pest attacks before they actually take place is desired in pest control programmes, so that control measures can be planned with maximum efficiency. Pest dynamics display fluctuations in timing and intensity depending on location and season. Mostly, they tend to fluctuate over a mean level. This average population over time, when computed across several years, results from the sum of action of all positive and negative factors influencing pest populations. Pests of host plants in undisturbed habitats such as forestry have their natural cycles in response to their ecosystem interactions and are most likely to attain equilibrium points in their population levels. Pests of agroecosystems, however, experience rapidly changing environments due to changes in cropping systems and a host of management interventions. As a result, crop pests show a greater degree of instability in population levels. Pests vary in their biology and in their response to their environment. Pests in colder climates in general have discrete generations and resting phases in their life cycles, while in the warmer climates, most species exhibit polymodal patterns of occurrences, with several generations in a year, resulting from continuous breeding opportunities and food availability. On a global scale, seasonal temperatures and rainfall patterns constitute major factors that determine the distributions of organisms (Birch, 1957). Tropical insects generally have the same annual variability as insects from temperate zones, but insect populations from dry areas, such as temperate or tropical regions, tend to fluctuate more than those from wet areas (Wolda, 1978). The effect of environmental stresses such as weather on insect dynamics cannot be explained easily. While environmental stresses such as drought and temperature fluctuations have been recorded preceding insect outbreaks, the precise mode of action of these stresses is unknown (Wallner, 1987).

In nature, pests are regulated by their natural enemies: parasitoids, predators and pathogens, which are in turn influenced by biophysical factors (Hence *et al.*, 2007; Thomson *et al.*, 2010). Therefore, a precise understanding of population dynamics can result from comprehensive ecological studies. However, despite our best efforts, gaps in pest ecological databases remain as a result of the complexity of interactions among the ecosystem components.

Worldwide, one important outcome of understanding population dynamics is to aim for a forecasting capability for appropriate management decisions. Succes-

sful forecasting techniques are those that are as simple as possible and that are based on knowledge of the biology and ecology of the pests concerned. In temperate regions, these are basically emergence warnings as the first of the overwintering eggs hatch or the first adults emerge from the overwintering pupae (Collier *et al.*, 1991; Trnka *et al.*, 2007). Because of the climatic regulation, most emergence takes place over a relatively short period of time and is not too difficult to monitor. In the tropical parts of the world, where weather conditions permit continuous breeding of pests most of the time, the warning is generally for the first occurrence of the pest in the crop (Krishnaiah *et al.*, 1997), or sometimes the recording of immigrants from an adjoining area for serious pests with a recorded history of economic damage (Otuka *et al.*, 2005). GIS technology is useful for interpolation of the spatial distribution and spread of crop pests and diseases based on multiple factors including weather conditions (Wu *et al.*, 2008). Quantitative seasonal studies are required over several years to determine seasonal range, variability in numbers and geographical distribution (Hill, 2008). Such studies must use sampling methods appropriate to the pest and its abundance (Cullen *et al.*, 2000), and the seasonal counts should be related to climate and topographical data (Ferguson *et al.*, 2002). By sampling immature stages of insect pests, it is possible to monitor these pests and arrive at approximate estimations of the numbers expected in later stages (Finch, 1989).

Pests that survive on alternative hosts may be sampled so that an estimate of their probable pest density on the main crop can be made. This method has been applied to the peach-potato aphid and the black bean aphid, which are often sampled as overwintering eggs on spindle trees (Leather, 1993). The best spraying date for many Lepidoptera is determined by sampling eggs on the crop. For example, in many parts of Africa, the major cotton bollworms are examined in the field for immature stages (Javaid, 1990).

3.3.2 Insect phenology models

Insects are incapable of internal temperature regulation and hence their development depends on the temperature to which they are exposed. Studies of insect population dynamics often involve modelling growth as a function of ambient temperature. The rate summation methodology has perhaps proved to be the most viable approach to such modelling (Stinner *et al.*, 1974).

The most common development rate model, often called degree-day summation, assumes a linear relationship between development rate and temperature between lower and upper development thresholds (Allen, 1976). This method works well for optimum temperatures (Ikemoto, 2005). The linear model assumes that rates are proportional to temperature, and as amounts are integrals of rates, the amount of development is the integral of the temperature (or a linear function of it) along a time axis and has units of temperature and time (e.g. degree-days). Temperature-dependent development in insects can also be approached using developmental time. The rate of development is traditionally utilized because rate models were created from biochemical and biophysical properties (Sharpe and DeMichele, 1977), although some complications can arise when using rate instead of time (Kramer *et al.*, 1991). Most of the earlier models failed to take into consideration variation between individual insects in their rate of development, which is responsible for the spread of activity of a pest (Regniere, 1984; Phelps *et al.*, 1993). Significant models for modelling the effects of variable temperatures on the development of individual insects within a given population deal with mean rate versus temperature relationships (Wagner *et al.*, 1984a) and distribution of development times (Wagner *et al.*, 1984b, 1985). Instead of treating rate summation as a deterministic quantity, efforts have been made to consider rates as random variables (Stinner *et al.*, 1975). Stochastic approaches to modelling insect development vary in the choice of random variable to be

modelled and in the form of the frequency distribution applied to the random variable (Sharpe *et al.*, 1977; Curry *et al.*, 1978). The coefficient of variation of the rate distributions is relatively independent of temperature (Sharpe *et al.*, 1977), indicating that a single temperature-independent distribution of the normalized rate of development adequately describes the distribution at all temperature, which has been validated for 80% of 194 sets of published data on 113 species of insects and mites (Shaffer, 1983). Insect species that exhibit seasonality generally have resting phases – diapause or aestivation – in their life cycles, which can be accommodated in Monte Carlo simulation modelling (Phelps *et al.*, 1993).

As some temperatures are lethal to organisms, it is obvious that development must be a non-linear temperature function at the temperature extremes. Non-linear development rate functions based on enzyme kinetics were developed to describe high-temperature (Johnson and Lewin, 1946) and low-temperature (Hultin, 1955) inhibition, as well as both extremes (Sharpe and DeMichele, 1977). Another non-linear model of temperature-dependent development (Stinner *et al.*, 1974) utilized a function that is a simple sigmoid curve with an inverted relationship when the temperature is above the optimum. This model, as originally given, assumed symmetry about the optimum temperature but can be easily modified for asymmetry. The non-linear model by Logan *et al.* (1976) uses an equation that is asymmetric about the optimum but becomes negative for very high temperatures. Schoolfield *et al.* (1981) modified the model of Sharpe and DeMichele to enhance its overall utility and to simplify parameter estimation. As pointed out by Worner (1992), the interaction of cyclical temperatures with non-linear development can introduce significant deviations from the linear development rate model, especially in the low- and high-temperature regions of the development rate function. Stinner's model gave the best fit for Russian wheat aphid developmental rate data as judged by mean square error and successful convergence when 14 insect developmental models, both deterministic and distributed, were tested (Ma and Bechinski, 2008) using population model design system software developed by Logan and Weber (1989). Ma (2010) applied a survival analysis approach to model development of Russian wheat aphid in relation to temperature and plant growth stages.

Phenology models help predict the time of events in an insect's development and are important analytical tools for predicting, evaluating and understanding the dynamics of pest populations in agro-ecosystems under a variety of environmental conditions. Accurate predictions, however, require accurate recording of the temperatures experienced by the organisms (Morgan, 1991) as well as the duration of development (Danks, 2000).

Degree-day models (Higley *et al.*, 1986) have long been used as part of decision support systems to help growers predict spray timing or when to begin pest scouting (Welch *et al.*, 1978). Phenology models are also used as one component of risk analysis for predicting exotic pest establishment (Baker, 1991; Jarvis and Baker, 2001). A well-known example is the DYMEX modelling package (Su and Fa, 2002; Yonow *et al.*, 2004; Stephens and Dentener, 2005). CLIMEX, although not strictly a phenology model, uses some developmental requirements for risk assessment (Sutherst *et al.*, 1991, 1999, 2000). Another example is the web-based North Carolina State University APHIS Plant Pest Forecast (NAPPFAST) modelling system, which links daily climate and historical weather data with biological models to produce customized risk maps for phytosanitary risk assessments (Borchert and Magarey, 2005). Resources like the *Crop Protection Compendium* (CAB International, 2004) offer insect development summaries, while the University of California Statewide IPM programme lists development data for insects on their website (http://www.ipm.ucdavis.edu/MODELS) for use in degree-day models. An Insect Development Database containing the developmental

requirements for over 500 insect species has been created (Nietschke *et al.*, 2007). Insect Life Cycle Modeling (ILCYM) software, a generic open-source computer-aided tool, facilitates the development of phenology models and prediction of pest activity in specific agroecologies (Sporleder *et al.*, 2009).

3.3.3 Life tables and population models

Ecological life tables are one of the tools most useful in the study of population dynamics of insects having discrete generations. Such tables record a series of sequential measurements that reveal population changes throughout the life cycle of a species in its natural environment. Conventionally, a life table is a systematic tabular presentation of survival and mortality in a population for a known cohort of individuals (Morris and Miller, 1954). Long-term data from carefully planned population studies in which all the relevant factors have been measured accurately are important for constructing population models that adequately relate to biological reality. The goal of life-table analysis is to develop a population model that mimics reality. Apart from generating population estimates, this analysis is best done by careful identification and measurement of the independent factors causing mortality such as parasitoids, predators, pathogens and weather factors.

From the life-table studies, it is possible to identify the key factor responsible for increases and decreases in numbers from generation to generation (Morris, 1963; Varley and Gradwell, 1970). A multiple-regression approach involving all the survival components gives greater emphasis to the interaction between different age intervals (Mott, 1967). The equations for different mortalities are combined into a model to predict either the generation-to-generation changes in an insect population density or the average level around which these changes take place. The same analytical approaches used for insects having discrete generations are not applicable to insects with overlapping generations (Varley and Gradwell, 1970). Life table analysis was also utilized to model both the development and survival of the Russian wheat aphid (Ma and Bechinski, 2008). Ecological studies do not often lead to reliable forecasts of the time and size of population peaks because of gaps in the ecological databases such as short-range dispersal, overwintering behaviour, colonization patterns and age-specific mortality including inter- and intraspecific competition (Kogan and Turnipseed, 1987).

3.3.4 Pest simulation models and decision support systems

Simulation models based on mathematical descriptions of biological data as influenced by the environment are more easily applied across locations and environments. Computer programs or software to run these models facilitate the practical application of these models in understanding population dynamics and dissemination of pest forecasts for timely pest management decisions (Coulson and Saunders, 1987). Simulation approaches offer flexibility for testing, refinement, sensitivity analysis as well as field validation of developed models over a wide range of environmental conditions. Thorough descriptions of cropping systems being managed or studied are needed to explain the interactions among pests, plants and the environment (Colbach, 2010). Systems models or other prediction schemes can be used with appropriate biological, environmental, economic or other inputs to analyse the most effective management actions, based on acceptable control, sustainability and assessment of economic or other risks (Strand, 2000).

In an effort to improve *Helicoverpa* management in Australia, a comprehensive population dynamics model (HEAPS: HElicoverpa Armigera and Punctigera Simulation) has been developed, which incorporates the spatial structure of the habitat and pest population and explicitly

simulates the adult movement within a regional cropping system (Fitt *et al.*, 1995). This model incorporates modules based on adult movement, oviposition, development, survival and host phenology, and estimates the population in each unit of a grid (Dillon and Fitt, 1990). The EntomoLOGIC decision tool was derived from the SIRATAC decision support system deployed by the Australian cotton industry from 1976 to 1993 to reduce the risk associated with pest management using chemical pesticides. This was developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in collaboration with the University of Western Sydney, Australia (Hearn and Bange, 2002). Advances in hand-held computing have resulted in expanding the development of CottonLOGIC for use with Palm OS handhelds for widespread adoption by cotton growers in Australia (Bange *et al.*, 2004).

A suite of predictive computer models called MORPH has been developed at the Horticulture Research International, UK (Phelps *et al.*, 1999), for use in fruit and vegetable crops. Using a multi-generation phenology model, ECAMON, Trnka *et al.* (2007) could explain 70% of the variation in the timing of key developmental stages based on daily weather data. ECAMON simulations correctly predicted the presence/absence of the European corn borer over a study region in the Czech Republic during the 1961–1990 reference period. It helped to explain the sudden increase in maize infestation during the unusually warm periods of 1991–2000 and it also estimates that this potential niche will expand within the next 20–30 years. RICEPEST, a model simulating yield loss due to several rice pests under a range of specific production situations in tropical Asia was developed by the International Rice Research Institute (IRRI) in the Philippines.Validation of the model under field experiments yielded promising results (Willocquet *et al.*, 2002).

Web-based models and decision support systems are becoming popular and in future may become an absolute requirement for local, regional/area-wide and international implementation of IPM systems (Waheed *et al.*, 2003). In the USA and the Netherlands, commercial firms are applying mesoscale modelling techniques to forecast insect development and produce gridded products for regional and on-farm planning and pest management (Strand, 2000).

A decision support system has been developed for forecasting black bean aphid (*Aphis fabae*) outbreaks in fields of spring-sown beans. The system takes into account the regional forecast and additionally information provided by the user on individual characteristics of the field and crop such as field shape, size, plant density and sowing date, which are used to downscale the area forecast to the specific field. The system also contains a module for the aphicides that are cleared for use on spring beans and calculates the economics of application (Knight and Cammell, 1994).

SOPRA is applied as a decision support system for eight major insect pests of fruit orchards on a local and regional scale in Switzerland and southern Germany and has a wide range of possible applications in the alpine valleys and north of the Alps (Samietz *et al.*, 2008). Applying time-varying distributed delay approaches, phenology models were developed driven by solar radiation, air temperature and soil temperature on an hourly basis. On the basis of local weather data, the age structure of the pest populations is simulated and crucial events for management activities are predicted by the SOPRA system. Phenology is directly linked to a detailed decision support system and to extended information about the pest insects, as well as to the registered plant protection products. Through a web interface, the simulation results are made available to consultants and growers (www.sopra.info). SIMLEP is a regional forecasting model used in practice for Colorado potato beetle (*Leptinotarsa decemlineata*) in Germany and Austria on a large scale and in the western part of Poland. The SIMLEP decision support system contributed significantly to the improvement of farmers' control measures for

L. decemlineata (Jorg *et al*., 2007) and later its use expanded to Slovenia (Kos *et al*., 2009).

In the mid-1990s, CIPRA (Computer Centre for Agricultural Pest Forecasting) software was conceptualized, developed and implemented to access, in real-time, weather data from a network of automated stations. It allows the user to visualize forecasts for 13 insects, two diseases, two storage disorders in addition to the apple crop phenology. These bioclimatic models, which have been developed, implemented and improved over the last 13 years, vary from a simple degree-days approach based on air temperature to more detailed epidemiological models based on air temperature, relative humidity and duration of leaf wetness. Many field specialists are using these model forecasts along with field pest scouting to provide valuable additional information for decision making in pest management and in apple storage strategies (Bourgeois *et al*., 2008).

3.3.5 Integration of pest and crop simulation models

Crop system models can be used to generate information on the status of the crop as influenced by the growing environment and pests, and including different management options. In practice, there are few examples of these models that include all the necessary components for practical decision making. However, a more practical approach has been the development of individual crop and pest components that can be analysed at the same time to give information that can improve decisions.

The development of decision support systems for agrotechnology transfer (DSSAT 4 funded by the United States Agency for International Development (USAID)) has allowed the rapid assessment of several agricultural production systems around the world to facilitate decision making at farm and policy levels. The trend in development of crop system models is to go for the modular approach (http://www.icasa.net). The development of stand-alone decision support systems for pest components could lead to their practical use. In developed countries, dynamic websites that include interactive models, GIS-based decision systems, real-time weather and market information are rapidly being developed and made available on the Internet (http://www.effita.net) to give farmers real-time benefit in crop management.

The conventional approaches of using empirical models to quantify yield losses are limited in their scope and application, as these are data specific and insensitive to variable cropping and pest conditions. Crop growth models provide a physiologically based approach to simulate pest damage and crop interactions. There have been many efforts to use crop growth models to simulate the effect of pest damage on crop growth and yield by linking the damage effect of pest population levels to the physiological rates and state variables of these models. Insect pests and crop modelling has been discussed in detail by Boote *et al.* (1983) and Coulson and Saunders (1987). A distribution delay model including attrition was applied to simulate population changes in rice leaf-folders. Based on a metabolic pool approach, leaf-folder feeding and hence leaf mass losses to the rice plant were described with a generalized functional response model, which is 'source' and 'sink' driven (Graf *et al*., 1992). Furthermore, this model stresses the influence of adult migration and natural enemies on leaf-folder population dynamics, both of which are significant and poorly investigated aspects of the leaf-folder life cycle. Later, a generic approach to simulate the damage effects of single or multiple pests was attempted using crop growth models such as CERES-Rice (which is a part of the DSSAT) in the Philippines (Pinnschmidt *et al*., 1995) and InfoCrop in India (Chander *et al*., 2007; Reji *et al*., 2008; Yadav and Chander, 2010). Pest damage levels from field scouting reports can be entered and damage is applied to appropriate physiological coupling points within the crop growth model including leaf area index, stand density, intercepted light, photosynthesis, assimilate amount

and translocation rate, growth of different plant organs and leaf senescence. Equations and algorithms were developed to describe competition among multiple pests and to link the computed total damage to the corresponding variables in the crop models. These approaches provide a basis to explore dynamic pest and crop interactions in determining pest management strategies that minimize yield losses.

3.3.6 Remote sensing for pest monitoring and forecasting

Remote sensing techniques are useful in detecting crop stresses such as nutrient deficiency, pest infestation, disease development and to monitor drought. Plants may respond to pest and disease stress in a number of ways, including leaf curling, wilting, chlorosis or necrosis of photosynthetic plant parts, stunted growth and, in some cases, a reduction in leaf area due to severe defoliation. While many of these responses are difficult to quantify visually with acceptable levels of accuracy, precision and speed, these same plant responses will also affect the amount and quality of electromagnetic radiation reflected from plant canopies. The basic premise here is that healthy plants give a higher reflectance in the near-infrared region and a lower one in the visible region, while the opposite is the situation in the case of diseased plants (Teng and Close, 1977). Thus, remote sensing instruments that measure and record changes in electromagnetic radiation may provide a better means of objectively quantifying biotic stresses than visual assessment methods. Additionally, remote sensing can be used repeatedly to collect sample measurements non-destructively and non-invasively (Nilsson, 1995; Yang *et al.*, 2004).

Recent advancements in the field of remote sensing provide ample scope to use this technology for pest monitoring and detection (Prabhakar *et al.*, 2012). Riley (1989) provided an exhaustive review on the use of remote sensing in entomology. Pest damage was associated with spectral indices based on leaf pigments (Riedell and Blackmer, 1999; Yang and Cheng, 2001; Prabhakar *et al.*, 2006, 2011). Optical and video imaging in near-infrared and microwave regions were used to quantify the nocturnal flight behaviour of *H. armigera* (Riley *et al.*, 1992**)**. Fitzgerald (2000) demonstrated that multispectral remote sensing (MRS) would allow farmers to detect early infestation of mites in large-scale cotton fields due to colour shifts and changes in canopy appearance over time. Areas identified on the map could be located with the help of portable GPS equipment by field scouts to verify the mite populations in these areas and recommend regions in the field that require pesticide application.

Remote sensing improves spatial and temporal resolution compared with traditional methods for pest monitoring based on environmental changes (Bhattacharya *et al.*, 2007; Jiang *et al.*, 2008; Dutta *et al.*, 2008). However, the major limitation in use of satellite-borne data in pest forewarning is the timely availability of cloud-free data with the desired spatial and spectral resolution. Better standardization of aerial imagery and accounting for perturbing environmental factors will be necessary to make remote sensing techniques applicable to early pest detection (Luedeling *et al.*, 2009). In addition, the acquisition of airborne data is limited to few high-value crops because of the high costs involved.

3.3.7 Agromet networks for operational pest forecasting

Farmers are mainly interested in current disease and pest severity data, preferably for their localities to aid their decision making in crop protection. Pest monitoring data along with complementary weather data is crucial to run pest forecast models and provide forecasts for operational use. Weather measurements under field conditions from several geo-referenced sites in the crop-cultivated regions additionally provides spatial information that can be used for generating pest forecast maps

(Huang *et al*., 2008). In Bayern (Germany), a measuring network of 116 field weather stations is used to estimate the development of pests in relation to weather requirements based on forecast models and computer-based decision support systems for near real-time dissemination to farmers (Tischner, 2000). The results of crop- and horticulture-specific models and decision support systems are supplemented by field-monitoring data, which then serve as the main input for the warning services and are disseminated cost-effectively through the Internet (Bugiani *et al*., 1996; Jorg, 2000). A computerized national forecasting network in apple orchards transmits data from the field to system headquarters automatically. The national forecasting network in Turkey has been expanded and covered apple orchards in 34 provinces in 2006, using 115 electronic forecasting and warning stations (Atlamaz *et al*., 2007).

3.4 Conclusions

Pest monitoring is the foundation for the issue of early warnings, development and validation of pest forecast models and decision support systems, which are crucial for the design and implementation of successful IPM programmes. Models are potential tools for synthesizing the available information and knowledge on population dynamics of pests in agroecosystems and natural habitats. The development of long-term monitoring spatial data on crop–pest–weather relationships will narrow the gaps in knowledge required for reliable forecasts. Computer-based systems have increased the speed and accuracy of forecasting, and decreasing its costs. Recent developments in information and communication technology offer great scope for wide dissemination and use of pest forecasts. In the tropics, agroecosystems are characterized by greater crop diversity in small parcels of land with dynamically changing weather. Available generic simulation models need to be validated with location-specific inputs for greater accuracy. In developing countries, there is a strong need to establish agro-meteorological networks for specific crop sectors with the major objective of pest forecasting through models and decision support systems.

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4 Augmentation and Conservation of Natural Enemies

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4.1 Introduction

Although natural enemies of insects have been used in pest management for centuries, it was not until 1919 that the late Harry Smith of the University of California apparently came up with the term 'biological control' (Smith, 1919). Recently, there has been much debate regarding the scope and definition of biological control due to technological advances in the tools available for pest management as well as cross-disciplinary differences in terminology (Nordlund, 1996; Eilenberg *et al*., 2001). We have chosen to follow the historically entomological definition presented by DeBach (1964) as the 'study, importation, augmentation, and conservation of beneficial organisms to regulate population densities of other organisms'. This is in contrast to 'natural control', which is defined as 'the maintenance of a more or less fluctuating population density of an organism within certain definable upper and lower limits over a period of time by the actions of the abiotic and/or biotic environmental factors' (DeBach, 1964). Natural control occurs regardless of human involvement. Biological control is distinguished from other biologically based pest-control approaches such as those using bacteria, viruses, fungi, microbe-derived toxins, behaviour-modifying chemicals, botanicals or plant resistance (Ridgway and Inscoe, 1998). Biological control is a population-level process, involving the use of natural enemy populations to suppress target pest populations, and it can still be organized under the three general approaches of importation, augmentation and conservation (DeBach, 1964; Bellows and Fisher, 1999). This chapter presents an overview of augmentation and conservation biological control efforts conducted with predators and parasitoids targeted towards insect pests. It will try to highlight actual implementation of biological control by growers in pest-management programmes, and some of the barriers that prevent wider adoption.

4.2 Approaches to Biological Control

Importation biological control, not dealt with in this chapter, usually involves the reuniting of natural enemies with pests that have escaped them in a new geographic area, and is often referred to as classical biological control because of its historically predominant use (DeBach, 1964; Bellows and Fisher, 1999).

Conservation biological control can be defined as 'modification of the environment or existing practices to protect and enhance

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specific natural enemies or other organisms to reduce the effect of pests' (Eilenberg *et al.*, 2001). The primary method by which conservation biocontrol is implemented is through modification of pesticide-use practices (Ruberson *et al.*, 1998). However, modification of other farming practices and, in some cases, the agricultural environment is done to improve pest management.

Augmentative biological control involves the production and periodic release of indigenous or exotic biological control agents into specific crop situations for the management of native and non-native pests (DeBach, 1964; Bellows and Fisher, 1999). These releases are made using one of two methods. Inundative releases are made using large numbers of natural enemies with the expectation of immediate pest suppression and no expectation of suppression by the enemies' offspring (van Lenteren, 2003a). Seasonal inoculative releases, on the other hand, are made with the expectation that pest suppression will occur over multiple pest generations within a growing season or crop cycle (van Lenteren, 2003a). Augmentation has been recognized for some time by the general public, at least in the USA, because of the widespread availability of natural enemies of arthropods such as lady beetles (especially *Hippodamia convergens*) and mantids in garden catalogues and nurseries (Cranshaw *et al.*, 1996). This awareness has probably expanded due to the increasing availability of these organisms through Internet sales.

4.3 A Brief Historical Perspective

Predatory insects were the first natural enemies to be used as insect management tools. As early as AD 300, Chinese citrus growers reportedly placed paper nests of native Asian weaver ants (*Oecophylla smaragdina* F.) into trees to protect the citrus crop from herbivorous insects (van Lenteren, 2005). The sale of these ants was first reported as early as AD 304; it was first observed by Western scientists in 1915 and was practised up until recent times (Huang and Yang, 1987). This appears to be the first use of augmentation biological control and the most sustained use of any biological control practice.

The first recorded instance of conservation biological control implementation also apparently involved *O. smaragdina*. From approximately 1600 onwards, augmentation efforts with the ant were helped along by the practice of placing bamboo rods as runways or bridges between trees to aid inter-tree movement of the ants (DeBach, 1974; Huang and Yang, 1987).

These ants are still recognized for their value in suppressing populations of heteropteran, lepidopteran and coleopteran pests in cashew, citrus, cocao, coconut, coffee, eucalyptus, litchi, mango and oil palm (Way and Khoo, 1992; van Mele and Cuc, 2000). For example, where weaver ants were abundant in Mekong delta citrus production, pesticide expenses were halved while yield and farmer income remained the same (van Mele and Cuc, 2000). However, in this same area, use of weaver ants is considered an antiquated practice and is more likely to be employed by older farmers (van Mele and Cuc, 2000). Several studies have enhanced *O. smaragdina* populations either by using pesticides to reduce populations of other ant competitors, or by using interplanted shrubs and ground cover in coconut plantations. The aggressive behaviour of these ants has in some cases limited their use in pest management (Way and Khoo, 1992). Recent research has demonstrated that weaver ants can interfere with pollinators in rambutan plantations, although this did not have a significant economic impact on fruit production (Tsuji *et al.*, 2004).

Although predatory insects were observed and utilized long ago, it was not until much later that the less obvious parasitoids were recognized and their life histories understood. Parasitism by tachinid flies (probably the Uzi fly, *Exorista sorbillans*) was first correctly interpreted in China in the 11th century; however, the first scientific reports of these flies as pests of silkworms did not appear until 1925 (Cai *et al.*, 2005). It was not until 1669 that

hymenopteran parasitism (by braconid wasps) was correctly interpreted in Europe (van Lenteren and Godfray, 2005).

One of the first written proposals to use insect predators for pest control was given by Carl Linnaeus in 1772, who suggested that 'predatory insects should be caught and used for disinfecting crop plants' (Hörstadius, 1974). The European predatory stinkbug (*Picromerus bidens*) was reportedly recommended for the biological control of bedbugs (*Cimex lectularius*) as early as 1776 (Clausen, 1940; DeBach, 1974). By the early 1800s, others, such as Erasmus Darwin, were advocating the use of syrphid flies and coccinellid beetles to control aphids in greenhouses (Kirby and Spence, 1815; DeBach, 1974). Reports on the value of entomophagous insects for suppression of European agricultural and forest pests began appearing early in the 19th century (Kollar, 1837; Riley, 1931). During the 1800s, the practice of collecting and selling ladybugs for release in European hops, a practice that may have been conducted for centuries, became popular and widespread (Doutt, 1964). The concept of using insect parasitoids for pest control in Europe also developed during this period (van den Bosch *et al.*, 1982). Hartig, in 1826, proposed that parasitized caterpillars should be collected and stored in order to harvest adult wasps, which could then be released later to control cabbage butterflies (Sweetman, 1936). Actual efforts to experimentally manipulate populations of natural enemies (carabid and staphylinid predators) in agricultural settings began with Boisgiraud in 1840 and were continued by Antonio Villa in 1844 (Trotter, 1908). The first practical attempt towards augmentation of parasitoids in Western Europe appears to have been made in 1899 by Decaux who devised a complete management programme for apple orchards, including releases of field-collected ichneumonid wasps (Biliotti, 1977).

The primary focus of early efforts in biological control from the late 1800s was importation of natural enemies, especially after the highly successful use of the vedalia beetle for citrus pest management (DeBach, 1964). Although modest in comparison with modern use, early efforts in augmentation and conservation biological control were significant.

The first sustained, large-scale successful augmentation biological control project involved mass production of the ladybeetle *Cryptolaemus montrouzieri* targeting citrophilus mealybug (*Pseudococcus calceolariae* Fernald), a citrus pest in southern California (Luck and Forster, 2003). Large-scale releases began in the early 1920s, and continued for decades, with as many as 40 million beetles being produced annually. Although the fortunes of the insectaries involved in this effort waned in the 1950s with the advent of synthetic organic insecticides, they began a resurgence and diversification in the 1970s fuelled in part by public concerns over pesticide use (Luck and Forster, 2003). This beetle is still available through commercial insectaries in both the USA and Europe (van Lenteren, 2003b).

The first successful inoculative releases of a natural enemy involved the parasitoid *Encarsia formosa* for management of the greenhouse whitefly (*Trialeurodes vaporariorum*) in the UK (Pilkington *et al.*, 2010). This ultimately led to the development of the commercial augmentation industry in Europe in the 1960s (Pilkington *et al.*, 2010). From the 1970s onwards, the number of species offered by the industry grew at a rapid rate (van Lenteren, 2003a)

In the 1960s, the US Department of Agriculture began funding augmentation research, primarily focused on natural enemies of cotton pests. These initial efforts blossomed into large-scale field studies that evaluated the feasibility of augmentative beneficial arthropod releases through the 1970s (Vail *et al.*, 2001).

Conservation of natural enemies through modification of pesticide practices was recognized and developed in the 1950s and 1960s (Stern *et al.*, 1959; DeBach, 1964; Newsom and Brazzel, 1968). Other early research in conservation biological control was directed towards building artificial nesting structures, offering supplemental feeding (insectary plants or honeydew),

providing alternative hosts, controlling ants and modifying adverse crop production practices (van den Bosch and Telford, 1964). Although research continued in these areas, practices other than pesticide-use modification provided little practical value in integrated pest management (IPM). Ehler (1998) noted that, at least in the developed world, despite the ecological soundness of conservation biological control methods aimed at environmental modification, economics and integration with crop production practices precluded their implementation.

4.4 Augmentation Biological Control

Worldwide, the area under some form of augmentation was estimated by van Lenteren (2000a) to be over 17 million ha, 58.6% of which was in Russia. Europe and North America made up only 0.6 and 0.4% of this total, respectively. There are many organisms produced for augmentation and numerous examples of augmentation biological control used in greenhouses and other cropping systems (van Lenteren, 2003b, 2006; van Lenteren and Bueno, 2003; Luck and Forster, 2003; Shipp *et al.*, 2007). Insectaries that produce natural enemies are either commercial for-profit enterprises or centralized production units that are government or grower-industry owned (Cock *et al.*, 2010). Despite producing a fraction of the materials for release on a relatively small hectarage, commercial augmentation has garnered the most attention among scientists. The viability of augmentative biological control as a pest-management strategy is heavily influenced by the commercial natural enemy industry and the cost, availability and quality of natural enemies it produces (Bolckmans, 2003; Warner and Getz, 2008).

4.4.1 Commercial augmentation

Compared with the pesticide market, the commercial augmentation market is small. Global commercial sales of beneficial arthropods are valued between €150 and €200 million, which is less than 1% of global pesticide sales (Cock *et al.*, 2010) and approximately 2% of global insecticide sales (Keily *et al.*, 2004). Commercial augmentation products are focused on the greenhouse market, and only four pest groups (whiteflies, thrips, spider mites and aphids) account for 84% of expenditure (van Lenteren, 2003b). More than 75% of all activities in commercial augmentative biocontrol (expressed in monetary value) take place in North Europe and North America (van Lenteren and Bueno, 2003). This commercial market is concentrated in Europe, where the insectary industry is roughly three times the size of the North American industry (van Lenteren, 2003b; Warner and Getz, 2008).

The European commercial augmentation industry appears strong (van Lenteren, 2003b; Cock *et al.*, 2010), perhaps as a result of strong partnerships between government agencies and industry (van Lenteren, 2000b). However, the industry in North America faces a number of serious challenges (Warner and Getz, 2008). North American insectary industry annual sales are approximately US$25–30 million, which is 10% of the biologically based pest control product market and less than 1% of the USA insecticide market (Warner and Getz, 2008). The major challenges include difficulty obtaining investment capital, static or declining market and product prices, recruitment of cooperating researchers and problems with cross-border shipments (Warner and Getz, 2008). There also appear to be problems with acceptance of greenhouse biocontrol strategies in areas with larger potential markets in the USA (Wawrzynski *et al.*, 2001). Warner and Getz (2008) reported that the insectary industry in North America perceives no significant benefit from the factors often listed in scientific literature as being conducive to increased implementation of biocontrol. These include the introduction of narrow-spectrum insecticides, pesticide resistance and the expansion of the organic industry (Warner and Getz, 2008).

Over half of North American insectaries'

sales are for outdoor crops (Warner and Getz, 2008), and survey results indicate that urban and suburban residents in the USA are willing to pay more for biological control versus chemical controls for outdoor pest management (Jetter and Paine, 2004). The demand for biological control products in outdoor crops can be difficult to predict, which can lead to product shortages and price volatility (Warner and Getz, 2008). This contrasts with the controlled environments in greenhouses, which are more amenable to augmentation because of greater predictability of pest and natural enemy populations (van Lenteren, 2000b).

Augmentation biological control has been criticized (Collier and van Steenwyk, 2004) and debated (Collier and van Steenwyk, 2004; van Lenteren, 2006) in the literature over the scientific foundation, efficacy, ecological limitations and cost effectiveness of its use in pest management. Several authors have called for the development of predictive models to assist in implementation of augmentation biological control (Huffaker *et al.*, 1977; Stinner, 1977; King *et al.*, 1985; van Lenteren and Woets, 1988; Ehler, 1998), but this has only rarely been done (Parrella *et al.*, 1992). Because of the lack of supporting data for many augmentation approaches (Parrella *et al.*, 1992), recommendations cannot be made regarding rates and application methodologies that provide predictable results. Vorsino *et al.* (2009) demonstrated the value of molecular tools in developing an understanding of the population dynamics of field releases to help improve predictability.

Pesticides are routinely tested and compared for their efficacy in managing pest populations under field conditions, results are published (e.g. the journal *Arthropod Management Tests* published by the Entomological Society of America) and these tests form the basis for recommendations to growers. There are tremendous logistical difficulties involved in conducting the large-scale, statistically valid, detailed studies that are required to evaluate natural enemy augmentation effectively (Luck *et al.*, 1988). This means that many of the studies done to support augmentation do not have adequate experimental designs and do not include insecticides as a treatment comparison (Collier and van Steenwyck, 2004). Parrella *et al.* (1992) suggested that the perceived similarity between augmentative releases and insecticide applications has discouraged research interest in augmentation.

Although serious efforts to deal with quality control of mass-reared beneficial arthropods began in 1980, the issue is still a major concern (Bolckmans, 2003). The poor quality of released natural enemies or incorrect release rates can lead to unsatisfactory pest suppression and can contribute to the unpredictability of augmentation biological control (Hoy *et al.*, 1991). Great strides have been made recently to remedy quality and predictability concerns (see articles in van Lenteren, 2003c). However, augmentation will probably not become a mainstream pest-management tool until good-quality products are consistently available and end-user knowledge and support systems are sufficient (Bolckmans, 2003).

Typically, commercial augmentation is restricted to higher-value cropping systems such as greenhouses, orchards, nurseries, fruits and vegetables (Hale and Elliott, 2003). This is because higher-unit-value crops are able to support the increased management required to make augmentation successful. However, in some cases, commercial augmentation can be cost-effective in lower-value crops. For example, commercial releases of *Trichogramma* wasps comprise approximately 10% of the pesticide market for management of the European corn borer (*Ostrinia nubilalis*) in Western Europe (Orr and Suh, 1999). Even in predictable greenhouse environments, for small-scale North American growers augmentative releases may be prohibitively expensive for some cropping systems. Vásquez *et al.* (2006) examined the efficacy and cost of release of the parasitic wasp *Aphidius colemani* and imidacloprid applications for management of the cotton aphid (*Aphis gossypii*) on greenhouse-grown chrysanthemums. When pest-

management outcomes were the same, augmentative releases of parasitoids cost 4.7 times as much as pesticide use, primarily as a result of the shipping costs required for multiple releases. The use of banker plants presents a possibility for reducing this cost by sustaining reproducing populations of enemies on non-crop plants infested with non-pest alternative host insects. To date, most banker plant research and commercial sales have focused on aphid management in greenhouses, primarily using the aphid *Rhopalosiphum padi* on cereal plants hosting the parasitoid *A. colemani* (Frank, 2010). However, this method may have greatly expanded utility in the future (Frank, 2010).

Few countries regulate macroorganisms used for augmentation in a manner similar to that done for pesticides, or even other biologically based pest-management products. Where regulation does exist, there are advantages and disadvantages to the process. In Switzerland, for example, registration of biocontrol macroorganisms requires data on the organisms' efficacy and 'bioecology' (an assessment of risks to humans and the environment), as well as information on evaluation and registration in neighbouring countries (Bigler, 1997). The advantages of this approach are that quality-control protocols are followed, ineffective products are not marketed, and environmental and human hazards are assessed. The disadvantage is that higher costs associated with registration may delay or prevent implementation of products.

4.4.2 Non-commercial augmentation

The non-commercial state- and farmer-operated insectaries primarily rear egg parasitoid wasps in the genus *Trichogramma* for release against lepidopteran pests of agriculture and forestry (Smith, 1996; van Lenteren, 2003a; van Lenteren and Bueno, 2003). Li (1994) estimated that approximately 20 species of *Trichogramma* have been regularly released to manage primarily lepidopterous pests in at least 22 crops and trees on an estimated 32 million ha annually. Most of these releases are inundative, in order to rapidly suppress target pests. The primary reasons for this widespread use of *Trichogramma* are the relative ease of rearing and the fact that they kill their insect host in the egg stage, preventing feeding injuries (Wajnberg and Hassan, 1994). The use of non-commercial augmentation appears to be expanding in less-developed countries worldwide (van Lenteren and Bueno, 2003).

4.4.3 Genetically modified natural enemies

Genetic modifications in several natural enemies over the last few decades have been focused on the development of pesticide-resistant strains. The goal of these efforts has been the better integration of chemical and biological control (Beckendorf and Hoy, 1985; Croft, 1990). Genetically manipulated arthropod natural enemies developed using traditional selective breeding methods have been used only a few times in IPM programmes (Havron *et al.*, 1995; Hoy, 1996) and continue to be explored (e.g. Devi *et al.*, 2006; Ingle *et al.*, 2007).

McDermott and Hoy (1997) reported the only experimental release of a transgenic arthropod natural enemy, a transgenic strain of the predatory mite *Metaseiulus occidentalis* with a molecular marker. While genetically modifying natural enemies may have potential for improving resistance to pesticides and improving other life-history traits, a variety of scientific, regulatory and political issues remain to be resolved before transgenic arthropod natural enemies can be used in practical pest-management programmes (Ashburner *et al.*, 1998; Hoy, 2000, 2005).

There are concerns again regarding the sustainability of this approach. While some authors have advocated the use of resistant beneficial insects in IPM programmes (e.g. Graves *et al.*, 1999), it could be argued that this approach is counterproductive to the goals of IPM because it could encourage more pesticide use (such as with herbicide-

resistant soybean cultivars; see Benbrook, 2004). However, it may also create new opportunities for other beneficials (Hale and Elliott, 2003).

4.4.4 Non-target impacts of augmentation

Impacts on non-target organisms resulting from biological control activities have long been a controversial topic and have been reviewed a number of times (Simberloff and Stiling, 1996; Follett and Duan, 2000; Bigler *et al.*, 2006; van Lenteren *et al.*, 2006). Because augmentation biological control often utilizes non-native natural enemy species that have broad host ranges, it seems reasonable to expect that some impacts on populations of non-target organisms may result from its use. Several factors act to limit these impacts. The restricted focus of industry sales and augmentative releases in the greenhouse environment (van Lenteren, 2003b) probably reduces the likelihood of impacts on non-target populations outside these enclosed environments. Because of the temporary, non-persistent activity of released natural enemies (Lynch and Thomas, 2000; van Lenteren *et al.*, 2006), augmentation does not usually face the same scrutiny as importation biocontrol over these potential non-target impacts.

There are occasional exceptions, however. An example is the recent case of a company that faced criminal conviction for ignoring national laws in importing, rearing and selling the predatory mirid *Macrolophus pygmaeus* in New Zealand (New Zealand Press Association, 2010). Commercial shipments of the ladybeetle *H. convergens* in North America routinely contain beetles that are parasitized or infected with pathogens (Bjørnson, 2008). Suppliers of these beetles collect them in large numbers from their overwintering sites. This practice could potentially increase parasitism of local populations where releases are made (Bjørnson, 2008) and has the potential for transmission of pathogens to other species of ladybeetles (Saito and Bjørnson, 2008). As most augmented natural enemies are mass reared in controlled environments, this potential problem is unlikely to be widely important, especially where careful quality-control measures are employed. Genetic pollution of local populations resulting from animal introductions, re-introductions and population reinforcements has been raised as a concern (Dubois, 2008) that could apply to open-field augmentation. This is not necessarily because of potential reductions in fitness of local populations but because of difficulties presented for future detailed phylogenetic studies of natural populations (Dubois, 2008). While these arguments have merit from an academic standpoint, they are difficult to make in an agricultural or economic context. Only one augmented natural enemy has caused significant reported harm. Commercial sales of *Harmonia axyridis* in Western Europe contributed to the distribution of this coccinellid that is now considered a pest (Brown *et al.*, 2008), warranting its own control measures (Kenis *et al.*, 2008).

Concerns over the exotic species used in augmentation may be diminishing. The proportion of indigenous versus exotic natural enemies considered for augmentation programmes in Europe over the last 50 years has shifted from favouring exotic species to overwhelmingly favouring indigenous species in the last 10 years (Cock *et al.*, 2010). This has been attributed to the avoidance of complicated legislation and registration procedures (Cock *et al.*, 2010).

4.5 Conservation Biological Control

Conservation biological control seeks to enhance the actions of resident populations of natural enemies, whether they are indigenous or introduced (Gurr and Wratten, 1999). This has significant economic potential. Natural control of native pests by native arthropod predators and parasitoids alone in the USA is estimated to avert US$4.5 billion in crop losses annually (Losey and Vaughan, 2006). There is also significant potential for adoption of conservation biocontrol by the expanding

organic agriculture sector. Warner and Getz (2008) reported that organic growers in North America are apparently relying primarily on conservation biological control techniques for pest management in field crops. However, unlike importation and augmentation biological control, economic assessments of conservation biological control programmes are not only rare but are uniquely difficult to conduct (Cullen *et al.*, 2008). These authors suggested an approach for conducting such assessments in the future.

4.5.1 Modification of existing practices

A variety of agricultural practices impact on natural enemy biology as well as natural control and biological control. A challenge for pest-management professionals is to balance the sometimes competing demands of crop production with increased incorporation of biological control methods into pest-management programmes. The use of IPM practices alone under large-scale field conditions has demonstrable value to beneficials. Furlong *et al.* (2004) examined the impact of different on-farm pest-management practices on beneficial insects in *Brassica* crops in the Lockyer Valley, Australia. Their study clearly demonstrated increased natural enemy abundance and diversity along with significantly greater predator and parasitoid efficacy on farms that practised IPM compared with farms that frequently treated with insecticides.

Presented below are the various crop production practices that impact on natural enemies and their function. Work that has been done to examine modifying these practices for enhancement of enemies and biological control is also presented.

Pesticide use

Pest management is still mostly based on broad-spectrum chemical pesticides that are harmful to natural enemies (Haskell and McEwen, 1998). Pesticide applications are probably the crop-management activity that impact on beneficial organisms most negatively in agroecosystems. Exposure to pesticides occurs through contact with spray droplets or residue on foliage, feeding on contaminated water droplets, plant-produced materials and honeydew, or feeding on the host or prey species (Jepson, 1989; Longley, 1999; Koch and Weisser, 2001).

While fungicides in general appear to have little or no direct effects on parasitoids and predators (Carmo *et al.*, 2010), they may have indirect impacts by suppressing entomopathogenic fungi (e.g. Lagnaoui and Radcliffe, 1998; Koch *et al.*, 2010). Some herbicides have been shown to have lethal and sublethal effects on arthropod natural enemies (e.g. Kühner *et al.*, 1985; Samsoe-Peterson, 1995; Paoletti and Pimentel, 2000; Manzoni *et al.*, 2006; Lacoume *et al.*, 2009; Schneider *et al.*, 2009; Benamu *et al.*, 2010; Carmo *et al.*, 2010). However, reported population effects of herbicides on natural enemies appear to be indirect. Herbicide use within crop fields simplifies plant communities and changes microclimate, which in turn can reduce or change the species composition of beneficial arthropod communities (e.g. Shelton and Edwards, 1983; Krooss and Schaefer, 1998; Bell *et al.*, 2002; Taylor *et al.*, 2006; Caballero-López *et al.*, 2010). However, advocating higher within-field weed populations is contradictory to agronomic goals and may have limited practical applications. Management decisions that increase in-field weed populations can lead to substantial reductions in yield, quality and harvestability of many crops (Bridges, 1994; Zimdahl, 2004; Oerke, 2006).

This section will focus on insecticides, as their effects are so much more significant. The side effects of insecticides on natural enemies are well known (see reviews by Haynes, 1988; Croft, 1990; articles in Vogt and Brown, 2006; Desneux *et al.*, 2007). Beneficial arthropods often display greater susceptibility to insecticides than their host or prey due to a variety of factors including their active searching behaviour, lower detoxification capacity and lower genetic variation, as well as due to food limitation

(Croft, 1990; Ruberson *et al.*, 1998; Tabashnik and Johnson, 1999).

From a pest-management perspective, pesticide effects on natural enemies can have a number of practical consequences. Indirect effects include habitat destruction and damage to nesting, oviposition, resting and mating sites (Desneux *et al.*, 2007). The lethal effects of insecticides are the most well known and can sometimes result in pest population resurgence or replacement (Hardin *et al.*, 1995). This is because, after insecticide treatment, pest and natural enemy populations may recover differently due to varying susceptibility to insecticides (Croft and Brown, 1975).

Sublethal effects of insecticides on beneficial arthropods include deleterious side effects of direct pesticide exposure on physiology and behaviour (Desneux *et al.*, 2007). The physiological effects extend to general biochemistry and neurophysiology, development, adult longevity, fecundity, sex ratio and immunology, while behavioural effects extend to mobility, navigation/orientation, feeding behaviour, oviposition behaviour and learning performance (Desneux *et al.*, 2007). Taking sublethal effects of pesticides into consideration when choosing pesticides for an IPM programme may result in improved natural enemy performance (Desneux *et al.*, 2005). An unusual sublethal effect of pesticides is enhancement of pest arthropod physiology and/or behaviour, a phenomenon known as hormoligosis (Luckey, 1968). Hormoligosis has been reported in a beneficial arthropod, the predatory mite *Amblyseius victoriensis* (Womersley); however, this phenomenon is uncommon for natural enemies and is probably of little widespread value in the integration of chemical and biological controls (James, 1997).

Insecticide use may also impact on sampling programmes. For example, estimates of natural enemy population size sometimes paradoxically increase following insecticide applications (Prasifka *et al.*, 2008).

Modifying pesticide-use practices is the most commonly implemented form of conservation biological control (Ruberson *et al.*, 1998). This approach has long been considered an important component of IPM programmes (Stern *et al.*, 1959; DeBach, 1964; Newsom and Brazzel, 1968). These modifications can be made in a variety of ways to minimize pesticide impacts on natural enemies. They include treating only when economic thresholds dictate, use of active ingredients and formulations that are selectively less toxic to natural enemies, use of the lowest effective rates of pesticides, and temporal and spatial separation of natural enemies and pesticides (Hull and Beers, 1985; Poehling, 1989; Ruberson *et al.*, 1998). While the concepts behind modifying pesticide use are relatively straightforward, implementing these modifications is not necessarily easy. One obstacle may be the variety of competing sources from which growers can get information regarding pesticide use (e.g. Rajotte *et al.*, 1987).

Pest population monitoring for pesticide-use decisions is a cornerstone of many IPM programmes and is typically based on sampling pest populations to determine whether they have reached economic threshold levels (Pedigo, 1989). A few studies have incorporated natural enemy sampling into revised economic thresholds to determine more accurately the need or timing for pesticide applications within a pest generation (Ostlie and Pedigo, 1987) or to predict the need for treatment of a future pest generation (van Driesche *et al.*, 1994). Formal revised economic thresholds incorporating natural enemy numbers are not common in IPM programmes but have been developed for several crops such as tomatoes and cotton (Hoffman *et al.*, 1991; Obrycki *et al.*, 2009).

However, pest-management professionals may informally incorporate natural enemy numbers into decision making, such as with cotton aphid management in the mid-Atlantic region of the USA (Orr and Suh, 1999). The use of economic thresholds alone in IPM does not necessarily lead to natural enemy conservation, if for example a broad-spectrum pesticide is used for treating pest populations when they exceed

threshold levels (Ruberson *et al.*, 1998). Consideration of natural enemy numbers, as well as careful selection of pesticides can lead to a more integrated approach to IPM.

Reduced-risk pesticides, including insect growth regulators, neonicotinoids, antibiotics and oxadiazines are considered by the US Environmental Protection Agency (EPA) to be safer for human health and the environment than older pesticides (EPA, 1997). The US EPA (1997) definition for these materials includes the following characteristics: 'not harmful to beneficial insects, highly selective pest impacts'. A number of studies have demonstrated that these compounds are less harmful to natural enemies than organophosphate, carbamate and pyrethroid insecticides, but they are still toxic, and deleterious effects on beneficial arthropods have been reported from exposure to reduced-risk insecticides (Sarvary *et al.*, 2007). These materials have a number of advantages over older pesticides, but their use does not necessarily lead to natural enemy conservation. Sarvary *et al.* (2007) concluded that the use of reduced-risk insecticides in individual crop fields within an agricultural landscape did not result in increased natural enemy activity in these fields, even when suitable natural habitat was interspersed with crop land. Any pest-management benefits to be gained from the use of reduced-risk insecticides may be eliminated if these products are sold in combination with broad-spectrum insecticides (Ohnesborg *et al.*, 2009).

The use of selective pesticides may be the most powerful tool to modify pesticide-use decisions to favour natural enemies (Hull and Beers, 1985) and the one that could be most readily available to growers (Ruberson *et al.*, 1998). Selecting the best insecticides for pest management that also have minimal impacts on beneficials is challenging for crop managers. A variety of databases and ranking systems have been developed that incorporate insecticide toxicities to non-target species and other information such as human toxicity and environmental contamination potential (van der Warf, 1996). These systems may be used to compare the relative impacts of various pesticides on non-target organisms and to estimate potential effects on non-target environments (Reus and Leendertse, 2000). However, they have only rarely been used to consider insecticide impacts on predators and parasitoids in agricultural environments at a landscape level (Ferraro *et al.*, 2003). To make this process more user-friendly, a beneficial disruption index (BDI) was developed by Hoque *et al.* (2002) to provide a generalized measure of insecticide impacts on beneficial arthropods in Australian cotton crops. Testing in Australian cotton by Mansfield *et al.* (2006) indicated that the BDI is an effective measure of insecticide impacts on beneficial insects.

Exposure of beneficial arthropods to pesticides may also be reduced by applying materials only where they are needed within individual crop fields. Coll (2004) reviewed the future potential for reducing the negative impacts of pesticide use on natural enemies through the use of precision agriculture technologies.

Plant breeding

Host-plant resistance and biological control are both considered valuable components of many IPM programmes (Ruberson, 1999). However, these two methods do not necessarily act on target pests independently of one another, and IPM practitioners should consider their interactions when designing management programmes (Bottrell *et al.*, 1998). Insect-resistant plants may have either positive or negative influences on natural enemies (Boethel and Eikenbary, 1986; Dicke, 1999; Simmons and Gurr, 2005; Ode, 2006). Also, beneficial arthropods could potentially contribute to the sustainability of plant resistance by slowing pest adaptation to resistant plants (Gould *et al.*, 1991; Gould, 1998).

Insect-resistant plants affect natural enemies either directly, through plant-produced chemicals or physical plant traits such as trichomes, or indirectly, through plant-mediated effects on natural enemy behaviour or host or prey characteristics

such as quality (Godfray, 1994; Kennedy, 2003; Ode, 2006). These effects can either be constitutive, acting regardless of herbivore presence, or inducible, as a result of herbivore attack (Dicke *et al.*, 2003; Kennedy, 2003; Pieterse and Dicke, 2007).

The manipulation of plant chemistry to manage populations of beneficial arthropods has tremendous potential in conservation biological control (Ode, 2006; Khan *et al.*, 2008). The approach that currently seems to have the most promise involves the manipulation of herbivore-induced plant volatiles (HIPVs) in 'push–pull' IPM strategies (Khan *et al.*, 2008). The approach involves breeding plants to express high levels of HIPVs even in the absence of pest feeding. These plants can be intercropped with the main crop and act as pest repellents (push), while other attractant plants (pull) can be planted as a trap crop around the main crop. The intercrop also attracts and conserves natural enemies, ensuring continued suppression of the pests. This system has been implemented widely in East African subsistence maize farming for the management of cereal stem borers (*Chilo partellus*) (Khan *et al.*, 2008).

Although the interactions between natural enemies and pest-resistant plants have been studied for decades (Boethel and Eikenbary, 1986), much of the recent literature on this subject has been focused on genetically modified or transgenic plants.

The transgenic plants currently deployed in IPM act on arthropods directly or indirectly through antibiosis (Gould, 1998). Most studies to date have not reported profoundly negative effects of transgenic plants on arthropod natural enemies, either in the laboratory or the field (Callaghan *et al.*, 2005).

Approximately one-third of laboratory studies reviewed by Lovei and Arpaia (2005) indicated significantly negative effects of genetically modified plants on life-history parameters of predators (30%) and parasitoids (40%). However, Lovei and Arpaia (2005) noted that there were inadequacies in the experimental methods used for these studies, including: (i) artificial test conditions unrelated to those insects would experience under field conditions; (ii) a small range of taxa tested; and (iii) variability in the types of parameters measured. Romeis *et al.* (2006) reviewed laboratory and greenhouse studies examining effects of crops expressing *Bacillus thuringiensis* (*Bt*) toxins on beneficial arthropods and concluded that there were no direct toxic effects, and that negative effects only occurred when *Bt*-susceptible, sublethally damaged herbivores were used as prey or hosts.

Bt cotton deployed in the field has a minimal impact on beneficial insect communities in cotton worldwide (Sisterson *et al.*, 2004; Naranjo, 2005). Field studies reviewed by Romeis *et al.* (2006) indicated that the abundance and activity of predators and parasitoids were similar in *Bt* and non-*Bt* crops. These authors suggested that *Bt* crops have fewer adverse effects on natural enemies than conventional insecticides and can reduce insecticide use by incorporation into IPM programmes with strong biological control components. Marvier *et al.* (2007) conducted a meta-analysis of 42 field experiments and found that non-target invertebrate populations generally were more abundant in *Bt*- versus insecticide-treated field crops, although some non-target invertebrate populations were less abundant in *Bt* versus non-*Bt* fields not treated with insecticides.

Shelton *et al.* (2002) reviewed the economic, ecological, food safety and social consequences of transgenic *Bt*-expressing plants and concluded that the risks of deploying transgenic *Bt* plants were lower than many current or alternative pest-management technologies and the benefits greater. The same pattern of results seen with *Bt*-transgenic crops has also been reported for genetically modified crops based on insecticidal proteins other than the *B. thuringiensis* delta-endotoxin (Callaghan *et al.*, 2005; Whitehouse *et al.*, 2007).

Deployment of transgenic crops has resulted in lower insecticide use. Over the 9-year period from 1996 to 2004, insecticide use on the genetically engineered maize

and cotton grown in the USA dropped by 5% (7.1 million kg) (Benbrook, 2004). *Bt* cotton has significantly reduced pesticide inputs wherever it has been commercially adopted, such as Australia where a 50% reduction was reported in comparison with conventionally sprayed cotton (Whitehouse *et al.*, 2007). In contrast, from 1996 to 2004, herbicide use on genetically engineered maize, cotton and soybeans grown in the USA increased by 5% (Benbrook, 2004). However, the use of transgenic herbicide-tolerant soybeans does not appear to have a significant effect on arthropod communities (Buckelew *et al.*, 2001). Concerns over incompatibility of transgenic crops with biological control appear largely uncorroborated by the data collected to date; however, the debate over the safety of genetically modified crops is likely to continue (Thies and Devare, 2007).

Tillage

Tillage is a primary means of disturbance in agroecosystems and is central to many agricultural practices such as preparing seedbeds and the incorporation of organic material and fertilizer, as well as the suppression of weeds and some diseases and insect pests (Gebhardt *et al.*, 1985). Tillage practices can have significant influences on arthropod populations, including natural enemies, and, in turn, pest management (Hammond and Stinner, 1999).

The influence of reducing tillage (i.e. practising 'conservation tillage') on natural enemy populations, as well as other insects, has been studied extensively. In some cases, conservation tillage has been shown to increase natural enemy populations or species diversity (e.g. Gaylor *et al.*, 1984; McCutcheon *et al.*, 1995; McCutcheon, 2000; Tillman *et al.*, 2004), while in others they were either not affected (Ruberson *et al.*, 1997; Gencsoylu and Yalcin, 2004) or reduced (Ruberson *et al.*, 1995; Cividanes and Santos-Cividanes, 2008).

Tillage can particularly affect soil-dwelling insects. Although adult ground beetles (Coleoptera: Carabidae) can disperse quickly by running or flying (Coombes and Sotherton, 1986; Wallin and Ekbom, 1988; Holland *et al.*, 2004), their less mobile life stages are far more susceptible to tillage. Ground beetle populations in crop fields can drop if tillage occurs while the beetles are in their larval stage (Fadl *et al.*, 1996; Purvis and Fadl, 1996).

The carabid beetles are significant generalist predators in annual row-crop agricultural systems (Thiele, 1977; Kromp, 1999) and much of the work on conserving soil-dwelling natural enemies has focused on them. Tillage affects carabid populations through direct mortality from tillage events or indirectly through loss of prey resources and changes in microclimate (Hance *et al.*, 1990; Thorbek and Bilde, 2004). Shearin *et al.* (2007) reported that entomophagous carabid beetles were more sensitive to tillage than herbivorous carabids. While the diversity and abundance of carabids appears to be favoured by reduced tillage (see review by Shearin *et al.*, 2007), there are examples where entomophagous beetles are significantly more abundant in conventional tillage systems (e.g. Carcamo, 1995; Menalled *et al.*, 2007, Cividanes and Santos-Cividanes, 2008).

Interpreting the results of these studies is complicated by the sampling method employed. Populations of carabids are usually sampled with pitfall traps with trap catches expressed as activity density (Thomas *et al.*, 1998). However, there are significant constraints to using this method and care should be taken when designing studies and interpreting results (Thomas *et al.*, 2006). In addition, dispersal of beetles between experimental plots may mask treatment effects (Thorbek and Bilde, 2004; Shearin *et al.*, 2007). More work appears to be needed to gain a clearer understanding of the effects of tillage on ground-dwelling arthropod natural enemies.

Along with soil-dwelling insect predators, tillage has also been found to affect foliage-dwelling arthropod predators (House and Stinner, 1983; Troxclair and Boethel, 1984; Funderburk *et al.*, 1988; Hammond and Stinner, 1999; Marti and Olson, 2007) as well as parasitoids (Nilsson,

1985; Ellis *et al.*, 1988; Hokkanen *et al.*, 1988; Runyon *et al.*, 2002; Weaver, 2004; Rodriguez *et al.*, 2006; Williams, 2006; Sharley *et al.*, 2008), either directly from soil disturbance or indirectly by altering weed communities. The direct effects of tillage are especially important where foliage predators and parasitoids pupate in the soil. For example, an outbreak of cereal leaf beetle (*Oulema melanopus*; Coleoptera: Chrysomelidae), occurred in Canada after tillage practices were changed, inadvertently killing the beetle's parasitoids that were overwintering in the soil (Ellis *et al.*, 1988).

Tillage also affects natural enemy populations by reducing ground cover by burying crop residue and killing weeds. Several studies have shown that leaving crop residues behind can conserve populations of parasitoids and predators (Joshi and Sharma, 1989; Shepard *et al.*, 1989; Mohyuddin, 1991). Many ground beetles prefer covered areas over bare ones (Thomson and Hoffmann, 2007; Shearin *et al.*, 2008).

It is clear that tillage affects insect predator and parasitoid populations in agroecosystems. Reducing tillage seems to benefit these organisms. What is less clear is the link between tillage regime, natural enemy populations and the suppression of target insect pest populations.

Cover cropping

Cover crops are employed in agricultural systems for a variety of reasons including soil fertility improvement, erosion control and, in some cases, pest management (Mangan *et al.*, 1995; Teasdale, 1996; Altieri *et al.*, 2005). In a number of agricultural systems, cover crops have been shown to disrupt behaviour of pest insects and reduce their abundance (Bugg, 1992; Bugg and Waddington, 1994; Teasdale, *et al.*, 2004).

Experiments have shown that cover crops can increase beneficial arthropod numbers and reduce pest numbers in several crops including apples, cabbage, maize, soybeans and grapes (Irvin *et al.*, 2006; Prasifka *et al.*, 2006; Ponti *et al.*, 2007; Danne *et al.*, 2010). However, study results are not always clear cut. For example, clover cover crops have been shown in some studies to enhance natural enemy populations in cotton (Tillman *et al.*, 2004), while other studies have found no effect (e.g. Ruberson *et al.*, 1997). Buckwheat (*Fagopyrum esculentum*) has been studied and shown to enhance natural enemy activity in crops as diverse as cabbage and grapes (e.g. English-Loeb *et al.*, 2003; Lee and Heimpel, 2008), but in very few cases have effects on pest densities been associated with this enhancement (e.g. Nicholls *et al.*, 2000). Even when the longevity and fecundity of field populations of the ichneumonid wasp *Diadegma insulare* were increased by the presence of flowering buckwheat, populations of the diamondback moth (*Plutella xylostella*) were not reduced (Lee and Heimpel, 2008).

When mulched, cover crops can provide microhabitats favourable to insect natural enemies and in some cases increase their numbers (Altieri *et al.*, 1985; Stinner and House, 1990; Orr *et al.*, 1997). However, the presence of standing or mulched cover crops does not necessarily produce predictable results on natural enemy populations, even within the same group of organisms (see '4.5.4 Case history: carabid beetles', below).

Operational considerations of cover-crop deployment can be challenging. Cover crops have been shown to increase the incidence of insect and disease pests in crops and compete with crops, decreasing yield (Snapp *et al.*, 2005; Bone *et al.*, 2009; Danne *et al.*, 2010). There does not appear to be any study that demonstrates an agronomically and economically acceptable method to deploy cover crops for enhancement of natural enemy populations to gain economically acceptable suppression of insect pests. Although there are several examples of farmers using cover crops (Altieri *et al.*, 2005), there does not appear to be any significant implementation by growers of cover-crop planting in any cropping system to specifically enhance biological control and manage pest insects.

Trap cropping

Recently, there has been a renewed interest in trap cropping, a traditional pest-management tool that saw widespread use prior to the advent of synthetic insecticides (Shelton and Badenes-Perez, 2006). Trap crops are deployed to intercept dispersing pests before they can enter the main crop, allowing control measures to take place in a smaller area. When natural enemies follow pests into trap crops, they may be affected as well. The effects may be positive when natural enemy populations are able to build up on concentrated pest populations and then move into the main crop (Hokkanen, 1991). However, this does not necessarily lead to increased pest reductions in the main crop (Tillman, 2006a). The trap crops may also act as a sink for insect pest populations due to increased natural enemy activity (Virk *et al.*, 2004; Khan and Pickett, 2004; Tillman, 2006b) and in these cases have been referred to as biological control-assisted trap crops (Shelton and Badenes-Perez, 2006). When control measures are directed at pests in trap crops, these positive effects could potentially be negated by the elimination of natural enemies (Hokkanen, 1991). However, this is not necessarily the case. Barari *et al.* (2005) reported that parasitism of the oilseed rape pest *Psylliodes chrysocephala* by the ichneumonid wasp *Tersilochus obscurator* was not affected by insecticide treatment of a bordering trap crop because of temporal separation of the insecticide treatment and peak parasitoid activity. Even if control measures are used in traps crops, the main impact of trap cropping on beneficial insects may be reduced pesticide usage in the main crop, resulting in conservation of beneficial insect populations (Hokkanen, 1991).

Traps and barriers

Traps and barriers are often employed in IPM programmes to reduce pest numbers directly, to deny pests access to crops or to monitor pest populations (Pedigo, 1989). In some cases, they may have side effects on beneficial organisms and thus interfere with pest management. Semiochemicals used in traps for IPM programmes can have non-target impacts on beneficial arthropods (e.g. Perez and Sierra, 2006; Franco *et al.*, 2008) because pheromones and kairomones are commonly utilized in host finding by natural enemies such as parasitoids (see reviews by Vet and Dicke, 1992; Powell, 1999). However, semiochemicals may also have the potential for manipulating populations of natural enemies to benefit pest management (e.g. Powell and Pickett, 2003; Quarles, 2007; Khan *et al.*, 2008).

In mass-trapping efforts or even monitoring with traps such as coloured sticky traps, attraction and the effect on natural enemy populations should be considered prior to implementation (e.g. Blackmer *et al.*, 2008). For example, electric traps that use ultraviolet light as an attractant killed almost exclusively non-target insects rather than the targeted biting flies, with approximately 13.5% of the catch being predatory and parasitic insects (Frick and Tallamy, 1996). Hanafi *et al.* (2007) suggested testing mesh insect barriers to determine the size that excludes specific target pests but allows natural enemies to pass through. Ultraviolet light-blocking films for greenhouse crop production that interfere with insect visual receptors and behaviour also have the potential to interfere with biological control (Kuepper and Thomas, 2002; Doukas and Payne, 2007). Studies conducted to date on vacuum systems used for organic insect management have not demonstrated any negative impact on beneficial insects in crop fields treated with these vacuums (Kuepper and Thomas, 2002).

Fertilization

Few studies have examined whether fertilization affects natural enemies and, in turn, pest management. Several studies reported that parasitoid activity was lowered under reduced nitrogen conditions (Fox *et al.*, 1990; Loader and Damman, 1991; Bentz *et al.*, 1996). However, Chen and Ruberson (2008) reported that increasing levels of nitrogen fertilization in

cotton under field conditions decreased predation but did not affect parasitism. Thomson and Hoffmann (2007) found that, even though green manures increased the populations of soil-dwelling predators as well as canopy-dwelling predators and parasitoids, they had no effect on pest populations.

Crop rotation

Crop rotation is a foundation for pest management in many cropping systems, dissociating pest populations from continued food supply from one year to the next (Pedigo, 1989). In a few instances, crop rotation can also affect the ability of some beneficials such as ground-dwelling rove beetles to find new crops (Lubke-Al-Hussein and Al-Hussein, 2006). Rotation of crops upwind (based on prevailing wind direction) in relation to previous years' crops may influence the ability of parasitoids to locate and colonize the new crop (Williams *et al.*, 2007). These effects appear to be uncommon enough that they should not affect most crop-rotation decisions.

Intercropping

Root (1973) first proposed intercropping as as a possible means to reduce pest discovery and retention in crops and to enhance natural enemy populations and activity. Andow (1986, 1988) reviewed intercropping studies in the literature and noted that pest densities were reduced in 56% of cases, increased in 16% and not affected in 28%. Russell (1989) reviewed natural enemy activity in intercropping studies and reported increased pest mortality due to natural enemies in 70% of cases, lowered mortality in 15% and no effect in another 15%. The responses of both pest and beneficial insects to intercropping are not well understood, because the underlying mechanisms at the behavioural level have not been well studied (Bukovinszky, 2007). However, manipulation of plant chemistry may allow more predictive approaches to using intercrops. An example is the highly successful management programme for cereal stem borers in eastern Africa using intercrops that express HIPVs repellent to the pests and attractive to their natural enemies (Khan and Pickett, 2004; Khan *et al.*, 2008).

4.5.2 Habitat manipulation

A major limiting factor in the life cycle of many natural enemies, particularly parasitoids, is the availability of food for adults. They depend on external food resources for not only sustaining host searching but also for the development of eggs (Vinson, 1998; Wäckers *et al.*, 2008). The availability of these adult food sources may be an important limiting factor on the effectiveness of parasitoids in pest management (Heimpel and Jervis, 2005). As a result, significant research efforts have been made over the last 50 years to try to provide provisions for beneficial arthropods to enhance their populations and improve biological control (van den Bosch and Telford, 1964; Wäckers *et al.*, 2008).

Natural enemies, like many other arthropods, visit flowers for the food resources found in nectar and pollen (Heimpel and Jervis, 2005). Many laboratory studies have demonstrated that floral resources can dramatically increase the longevity and fecundity of parasitoids; however, the provision of these resources in the field does not consistently improve biological control, even when longevity and fecundity of field populations is improved (Lee and Heimpel, 2008). Other research has focused on identifying plants that selectively provide provisions beneficial to arthropods but not to the pests that they attack. Selectivity can result from architecture of flowers and arthropod mouthparts, or nectar characteristics and availability under different environmental conditions (Winkler *et al.*, 2009).

Another important food resource for beneficials is honeydew, which may be more widespread in agricultural landscapes and more readily available than nectar (Wäckers *et al.*, 2008). However, the popu-

lation densities of parasitoids that feed on host honeydew may be strongly correlated with host densities, presenting a challenge for improving biological control through honeydew manipulation in crops (Wäckers *et al.*, 2008). The use of banker plants that host non-pest sap-feeding insects either in or around crop fields may provide opportunities for increasing the value of honeydew in pest management (Frank, 2010).

Provision of food resources for beneficial arthropods has also been attempted by application of food sprays to cropping systems for over 40 years. However, these efforts have not resulted in any practical methods for pest management (Wade *et al.*, 2008).

Another possible approach to enhance resident natural enemy populations is the provision or manipulation of non-crop vegetation as shelter habitat either within or outside crop fields (Griffiths *et al.*, 2008). Significant research efforts have shown that beneficial arthropod diversity, abundance and distribution can all be affected by shelter habitats. There may be the potential to improve pest management by manipulation of non-crop vegetation in simplified landscapes. Perović *et al.* (2010) reported that a predatory beetle (*Dicranolaius bellulus*) and a spider (*Oxyopes* spp.) both responded positively to the presence of tree lines providing connectivity between crops and non-crop resources, whereas pest response was weak. The egg parasitoid *Trichogramma* spp., however, responded most positively to the presence of cotton fields in the landscape. To date, there are few examples of shelter habitat providing demonstrated practical pest management (Griffiths *et al.*, 2008).

Based solely on personal observations and anecdotal information, many organic farmers feel they get much of their pest management from natural enemies encouraged by the ecosystem that develops with organic farming practices. It seems that at least some of these beliefs may have merit. In manipulative experiments in field enclosures, Crowder *et al.* (2010) demonstrated that organic farming methods promote species evenness in natural enemy communities and that these even communities exerted the strongest suppression of pests and produced the largest plants. Possibly overlooked by pest-management professionals, but anecdotally recognized by farmers, are complex food webs that may persist in some agricultural systems and act to stabilize both insect and disease pests of crops. Vandermeer *et al.* (2010) report on a 10-year study of a neotropical organic coffee plantation with four key pests: (i) coffee rust disease (*Hemileia vastatrix*); (ii) coffee berry borer (*Hypothenemus hampei*); (iii) green coffee scale (*Coccus viridis*); and (iv) the leaf-mining moth (*Leucoptera coffeella*). These pests all have the potential to do severe damage and yet they have not, apparently the result of complex interactions between all the species, which these authors term 'autonomous' or 'endogenous' pest management.

Fiedler *et al.* (2008) suggested that conservation biological control may see improved implementation by working towards systems that combine multiple ecological service goals. They suggest looking for synergies in various activities such as biodiversity conservation, ecological restoration, human cultural values, tourism, biological control and other ecosystem services. The concept of agrobiodiversity (see the series of 22 articles in van Rijn, 2007) has been promoted not only for the practical values provided by ecological services such as biological control and pollination but also for preserving or enhancing biodiversity in agricultural landscapes for its own sake.

4.5.3 Non-target impacts of conservation

Because conservation biological control works to enhance resident populations of beneficial organisms, it does not face the same potential for non-target impacts seen with either classical biocontrol or augmentation (van Lenteren *et al.*, 2006). However, populations of generalist predators from agriculture may spill over into natural habitats and have negative

impacts on native arthropods (Rand and Louda, 2006). Enhancing generalist predator populations through conservation biological control may exacerbate these negative impacts.

4.5.4 Case history: carabid beetles

The history of conservation biological control has provided tremendous opportunities for research but developing the research into practical strategies for pest management has been challenging (Jonsson *et al.*, 2008). The following example underscores some of the difficulties that research into conservation biological control has faced.

There are over 30,000 species of beetles in the family Carabidae (Lorenz, 2005). These ground beetles have been written about extensively, and the numerous reviews of ground beetles in agricultural settings (examples include Allen, 1979; House and All, 1981; Luff, 1987; Kromp, 1999; Holland and Luff, 2000; Holland, 2002) attest to the interest people have in these beetles as potentially important biological control agents. However, despite this history of research, it is still unclear what recommendations can be made to farmers about carabid beetles.

Many of the beetles in the family Carabidae are known predators of numerous agricultural insect pests. The ground beetles are known to eat significant crop pests such as armyworm in maize (Clark *et al.*, 1994), velvetbean caterpillar in soybeans (Fuller, 1988), black cutworm (Best and Beegle, 1977), Colorado potato beetle (Koval, 1999), Western corn rootworm (Kirk, 1975), slugs (Barker, 1991; Symondson, 1993; Bohan *et al.*, 2000) and many types of aphids (Bryan and Wratten, 1984; Mundy *et al.*, 2000; Hajek *et al.*, 2007), among other pest species (for a full review of insect pest species attacked by carabids, see Sunderland, 2002). Both larvae and adult carabids have been cited as important predators of crop pests (Brust *et al.*, 1986; Frank *et al.*, 2010).

Although carabid beetles are most commonly seen as predators, many are in fact omnivorous (i.e. polyphagous) (Allen, 1979) and some are associated with eating the seeds of agronomic weeds. Weed-seed 'predation' is a process that can significantly alter weed pest populations (Brust, 1994; White *et al.*, 2007). Some carabid adults eat the seeds of many important agronomic weeds including velvetleaf, wild mustard, yellow foxtail, common lambsquarters, redroot pigweed, hairy galinsoga (Gallandt *et al.*, 2005), large crabgrass and fall panicum (Brust, 1994), among others. Seeds are also important to the diets of some carabid larvae (Hůrka and Jarošík, 2003; Lundgren, 2006).

Because carabid beetles are known to attack and eat both insect and weed pests, much research has been devoted to increasing their numbers in agricultural settings. Ground beetle populations are sensitive to habitat disturbance (Kromp, 1999), and increasing ground cover and reducing tillage may stabilize carabid habitats. However, it is not clear whether either of these practices increases ground beetle populations. Studies show that ground beetles prefer mulched areas to bare areas (Thomas and Hoffmann, 2007) and favour cover-cropped plots over adjacent bare plots (Shearin *et al.*, 2008), and it has even been suggested that the shade from mulches and other covers may positively influence nocturnal carabid activity (Baker and Dunning, 1975). However, greater ground beetle numbers have been found in herbicide- or tillage-treated plots versus mulched ones (Miñaro and Dapena, 2003), and it has been generally stated that carabids in agricultural settings favour warm, dry areas (Kromp, 1999) not conducive to mulches and covers. Also, Menalled *et al.* (2006) proposed that, in general, even if greater ground cover did lead to greater populations, the cover may inhibit the movement of the organisms, leading to more numerous but less effective populations.

Studies of tillage on carabid beetle numbers are equally inconclusive. Ploughing and secondary tillage can disrupt or destroy ground-dwelling habitats and it is logical to conclude that ground beetle

populations would be reduced in heavily tilled environments. There are many studies that support this idea: (i) Brust *et al.* (1985) showed that deep tillage reduced carabid predator numbers; (ii) a long-term study in Norway found more carabid beetles in reduced tillage plots versus conventional ones (Anderson, 2003); and (iii) results from a large-scale soybean study showed a greater activity density of granivorous beetles in no-till plots over mouldboard-ploughed plots (Dauer *et al.*, 2001). Similarly, Menalled *et al.* (2007) found more ground beetle seed predators in no-till fields than in fields with regular ploughing, but total carabid numbers were two times greater in the conventionally ploughed fields versus the no-till ones (Menalled *et al.* 2007). Other studies have also shown carabids preferring tilled areas. Increasing tillage intensity has increased a dominant carabid's abundance (Baguette and Hance, 1997) or increased the total ground beetle population (Carcamo, 1995) in some instances. To complicate things further one study showed that fields with an intermediate level of tillage disturbance (i.e. chisel plough) harboured the greatest number of ground beetles compared with deep tillage or no-till (Shearin *et al.*, 2007).

The lack of certainty on whether more stable, less tilled fields are better for conserving ground beetles may be due to their high mobility and dispersal rates as well as the distinctive biology between species. Adult carabids can travel great distances, sometimes at great speeds, some by flying and others by walking (Coombes and Sotherton, 1986; Wallin and Ekbom, 1988; Holland *et al.*, 2004). Because of this dispersal ability adult carabids may be able to disperse away from a field during a disturbance like tillage and be able to quickly recolonize the field afterward. However, the egg, pupal and larval stages of these beetles do not share this mobility and may be more susceptible to field disturbances like tillage. Carabid species that overwinter as larvae in the soil are vulnerable to spring tillage (Fadl *et al.*, 1996; Purvis and Fadl, 1996). Other species that overwinter as adults may be less effected by spring tillage, but their larvae may need undisturbed soils during other times of the year. Therefore, conservation of carabid beetles may depend on having a suitable, habitable refuge outside the field crop for these insects to escape to and to live their less mobile life stages in.

Good refuge habitats for ground beetles can consist of hedgerows or fallow/weedy buffers or grassy strips or sown wildflower areas on the margins of crop fields. These types of vegetative refuges can be good adult carabid overwintering sites (Dennis and Fry, 1992; Thomas and Marshall, 1999; Pfiffner and Luka, 2000) or good sources of food when the crop field does not yet harbour enough prey items (Zangger, 1994). However, carabids often stay close to these beneficial borders and are often not found in the centre of these fields (Nazzi *et al.*, 1989). Intercropping with strips of flowering herbs has shown to improve carabid numbers within the field (Lys *et al.*, 1994), but intercropping is impractical for modern, mechanized intensive agriculture. To meet the needs of both improving ground beetle numbers within the crop field and the constraints of modern-day intensive farming, 'island' habitats were introduced in England in the early 1990s (Thomas *et al.*, 1991, 1992). These raised earth banks were created with regular farm implements during autumn cultivation and sown with perennial grasses. These banks were positioned in the field so that all of the farming implements could easily be driven around them during the following crop season. These initial studies showed high numbers of overwintering carabids in these 'beetle banks' that subsequently invaded the crop field during the spring. Since their inception, 'beetle banks' have become a government-sponsored conservation practice in England through the Countryside Stewardship Scheme (run by the Department for Environment, Food and Rural Affairs (Defra)).

While some strategies for conserving ground beetles in agricultural settings, such as beetle banks or establishing field border refuge habitats, are becoming more

definitive, it is still unclear whether enhancing carabid numbers actually improves pest control. In Washington state, higher beetle numbers were recorded in fields where beetle banks had been established, but pest predation did not increase in these fields (Prasad and Snyder, 2006). The authors attributed this to intraguild predation as well as feeding on non-target prey. In this study, the larger carabid beetle was eating the smaller ground beetles, which were primarily responsible for pest consumption. Also, the smaller beetles would preferentially feed on alternative prey if it was available rather than the pest species. Both of these behaviours freed the pest from predation pressure despite the increased number of predators.

The ability of carabid beetles to *interfere with* rather than support pest suppression has been shown in other studies as well. Snyder and Ives (2001) showed in a experiment on lucerne that the carabid *Pterostichus melanarius* preferentially consumed aphids that had already been parasitized by the parasitoid wasp *Aphidius ervi*. This selective predation essentially cancelled out the biological control effect of the wasp.

Cannibalism can also occur among carabids at higher densities (Brusting and Heesen, 1984; Frank *et al.*, 2010). Thus, conservation of carabids to increase their populations may not lead to greater pest suppression for a number of reasons. For these same reasons augmentation of carabids also seems ill advised.

The generalist rather than specialist nature of most carabids seems to be problematic within the complex community structures that are present in agricultural settings. This omnivorous and opportunistic behaviour may affect the seed predation abilities of granivorous carabids as well. Several species of seed-eating carabids also eat other insects (Jørgensen and Toft, 1997a,b).

However, it can be argued that making generalizations about carabids from a few specific community interactions is not conclusive (Sunderland, 2002). For one, Symondson *et al.* (2002) reviewed the literature and found that in 75% of cases, generalist insect predators were able to reduce pest numbers significantly. There are a number of examples where carabids were able to reduce the number of insect pests in crop fields (Symondson, 1993; Menalled *et al.*, 1999; Collins *et al.*, 2002). In addition, inundative releases have been deemed appropriate for at least one carabid (Symondson, 1994). For weed-seed predators, carabids have the potential to reduce weed emergence by as much as 16% (White *et al.*, 2007). There are a few studies that claim there is the potential for carabid beetles to maintain a number of pest populations below outbreak levels (Clark *et al.*, 1994) or below economic thresholds (Potts and Vickerman, 1974; Landis *et al.*, 2000).

Despite these promising results, voluntary adoption of conservation biological control techniques, such as beetle banks, has been low in Europe (Griffiths *et al.*, 2008), while in the USA, ill-advised food safety practices, like the Leafy Greens Marketing Agreement, have led to the *removal* of refuge habitat around crop fields (Beretti and Stuart, 2009). Griffiths *et al.* (2008) stated that until there is substantial evidence of the impact (on yield and profit) of these conservation practices, they will not be adopted by many farmers.

There is not enough demonstrable evidence that carabids have a significant impact on farm economics. Sunderland (2002) concluded that carabids alone cannot keep pest populations below economically viable levels, except for perhaps slugs. However, Sunderland's review also states that ground beetles can be an important component of an *assemblage* of generalist predators.

Carabids can work in tandem with other predators to create these natural enemy assemblages. For example, aphids escaping coccinellid (ladybird beetle) predation in the leaves of a lucerne crop dropped to the ground where they were consumed by carabids (Losey and Denno, 1998). There are other examples of groups

of predators that include carabids reducing pest populations. Menalled *et al.* (1999) showed that increasing ground-dwelling predator numbers, especially carabids, increases fly pupae predation and concluded that these generalist predators could keep pests below outbreak levels in annual crops. Fuller and Reagan (1988) showed that suppressing generalist predators, including carabids, with insecticide in sugarcane and sweet sorghum increased sugarcane borer (*Diatraea saccharalis* F.) numbers and reduced yield. They pointed to the importance of natural control by generalist predator complexes in preventing economic injury levels being reached in these crops. Landis and van der Warf (1997) reduced aphid predators through exclusion, which reduced carabid numbers by 90–96% in a beet field. These exclusions kept aphid populations at consistently high numbers compared with natural settings. The authors concluded that ground and foliar predators together can significantly reduce aphid populations. However, Holland and Thomas (1997) showed that groups of generalist predators, including carabid species, cannot keep aphid populations below recommended spray thresholds in wheat.

Similarly, weed-seed predation by a suite of granivorous organisms, including carabids, accounts for greater losses to seedbanks than ageing, microbial decay or even disturbances such as cultivation (Westerman *et al.*, 2003).

For this reason, conservation biological control aimed at ground beetles needs to incorporate the habitat requirements of other predators. Instead of beetle banks with one species of perennial grass that attracts adult carabids, these conservation areas should include a suite of vegetation that attracts a number of natural enemies at all life stages. Until these techniques can be tested on large, farm-scale level studies and their efficacy determined, government incentives will be needed to encourage their adoption.

4.6 Conclusions

Agriculture in today's world is facing a variety of challenges and, at times, seems to be pulled in opposite directions. This creates both challenges and opportunities for implementation of biological control. While there is a continuing need to increase global food production (Godfray *et al.*, 2010), there is also a growing awareness of the environmental degradation associated with many modern agricultural practices (IAASTD, 2009). While the US government is increasing conservation funding for farmers (Bish, 2010), market demands for crop-based biofuels are pushing farmers to turn conservation lands into maize (Streitfeld, 2008), and reactionary food safety programmes, such as the Leafy Greens Marketing Agreement, are replacing riparian buffers and other natural areas with bare ground (Beretti and Stuart, 2009). This will undoubtedly create challenges for implementing conservation biological control strategies that involve habitat manipulation.

It is clear that modern agriculture is experiencing rapid changes. There are social changes such as the increased use of genetically modified crops (Pollack, 2008) and the rising demand for organic produce (Davidson, 2005). Both of these trends present opportunity for increased implementation of augmentation and conservation biological control. At the same time, we are experiencing global transformations in our environment; global climate change is beginning to affect agricultural systems worldwide, and biological control practices may have to be altered to adapt to these changes (Stacey, 2003; Hance *et al.*, 2007).

There is no doubt that IPM will play a key role in providing the tools society will need to adapt our current agricultural practices to fluctuating market demands and shifting environmental conditions. Biological control is likely to play a significant part in this effort.

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5 Biotechnological and Molecular Approaches in the Management of Pests and Diseases of Crop Plants

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5.1 Introduction

Insect pest and disease menaces are the major factors that destabilize crop productivity in agricultural ecosystems. Tools and techniques of molecular biology and genetic engineering have provided unprecedented power to manage the biotic stresses in an effective way towards a safe and sustainable agriculture in the 21st century. One practical means of achieving greater yields is to minimize the pest-associated losses, which are estimated at 14% of the total agricultural production: 52% in wheat, 83% in rice, 59% in maize, 74% in potato, 58% in soybean and 84% in cotton (Oerke *et al.*, 1994). Insects cause loss to the agricultural produce not only directly but also indirectly due to their role as vectors of various plant pathogens. In addition to direct losses caused by insects, there are additional costs in the form of pesticides applied for pest control, valued at US$10 billion annually (Sharma *et al.*, 2000). Extensive and indiscriminate usage of chemical pesticides has resulted in environmental degradation, adverse effects on human health and other organisms, eradication of beneficial insects and the development of resurgence and resistant to pesticides in insects pests.

Over the past six decades, plant breeders have contributed significantly towards the improvement of crop productivity. However, incorporation of resistance to several pests and diseases remains elusive, which is the major limiting factor for productivity. Major technological advances in biochemistry, molecular biology, genetics and biotechnology have facilitated in designing plants with improved resistance to insect pests. The potential of genetic transformation technology has been widely recognized over the last two decades. Recent advances in molecular biology, plant tissue culture and genetic engineering have clearly demonstrated the possibility of incorporating foreign genes for desired characters while preserving the existing traits of improved genotypes. Biotechnology has the potential to reduce the severity of many pest problems, and the ways in which they are helpful are discussed in this chapter.

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5.2 Development of New Genes and Molecules for Pest Management

5.2.1 Insecticidal proteins of *Bacillus thuringiensis*

Bacillus thuringiensis (*Bt*) was first isolated by Ishiwata in 1901 from a diseased silkworm larvae (*Bombyx mori*) and was named *Bacillus sotto*. It was not characterized until a decade later, when Berliner isolated a similar *Bacillus* from a diseased Mediterranean flour moth larvae (*Anagasta kuehniella*) and named as *Bacillus thuringiensis* (Cannon, 1995). *Bt* is a Gram-positive, aerobic, sporulating bacterium that synthesizes crystalline proteins during sporulation. These crystalline (Cry) proteins are highly insecticidal at very low concentrations to lepidopterans, coleopterans, dipterans, lice, mites and nematodes, and are non-toxic to mammals and other organisms. The mode of action of insecticidal crystal proteins (ICPs) involves a cascade of events including solubilization of the crystal, activation of toxins by midgut proteases and recognition of a binding site on the midgut brush border membrane followed by pore formation and cell lysis, leading ultimately to insect death. The majority of the Cry proteins are generally of two sizes: 130–140 kDa or approximately 70 kDa. The three domains required for toxicity are present in the N-terminal half of the larger proteins, whereas the C-terminal half constitutes a pro-toxin domain and is not found in the smaller proteins.

The important subspecies of *Bt* are: (i) *Bt* subsp. *kurstaki*; (ii) *Bt* subsp. *aizawai*; (iii) *Bt* subsp. *tenebrionis*; (iv) *Bt* subsp. *israelensis*; and (v) *Bt* subsp. *thompsoni*. Dulmage *et al.* (1971) discovered the more active *Bt* subsp. *kurstaki* (HD1), which was commercialized in the USA as Dipel (Glazer and Nikaido, 1995). In the 1980s, *Bt* research was stimulated by progress in biotechnology, when Schnepf *et al.* (1981) first cloned a crystal toxin gene from *Bt* subsp. *kurstaki* into *Escherichia coli*. Since then, much research has been done to improve target spectra and to find out more infectious strains of *Bt*.

Many Cry protein genes have been cloned and sequenced, and are known as *cry* and *cyt* genes. Cry proteins toxic for lepidopteran insects belong to the Cry1 (Cry1A, Cry1B, Cry1C, Cry1D, Cry1E, Cry1F, Cry1I, Cry1J and Cry1K), Cry2 (Cry2A) and Cry9 (Cry9A, Cry9B and Cry9C) groups. Cry toxins against coleopteran insects are known as Cry3 (Cry3A, Cry3B and Cry3C), Cry7 (Cry7A), Cry8 (Cry8A, Cry8B and Cry8C), Cry1Ia1, Cry14A and Cry 23A proteins. The Cry2Aa, Cry4A, Cry10A, Cry11 (Cry11A and Cry11B), Cry16A, Cry17, Cry19A, Cry20A, Cry21A and Cyt proteins are toxic to dipteran insects, while Cry5, Cry12, Cry13 and Cry14 proteins are nematicidal. Each *Bt* strain can carry one or more crystal toxin genes, and therefore strains of the organism may synthesize one or more crystal proteins. As of January 2012, 224 Cry and 11 Cyt holo toxins belonging to 74 families had been reported.

By using biotechnological approaches, it has become possible to use *Bt* more effectively and rationally by introducing the ICPs of *Bt* into crop plants. The crystal toxins are classified on the basis of amino acid sequence homology. The first transgenic tobacco plants using *cry* genes were developed against tobacco hornworm in 1987. The commercialization of *Bt*-transgenic crops started in 1996 with the introduction of bollworm-resistant cotton (Bollgard) carrying the *Bt* toxin gene in the USA, while *Bt* cotton was commercialized in India in 2002.

Vegetative insecticidal proteins of Bt

Certain isolates of *Bt* produce novel insecticidal proteins during the vegetative stage, known as vegetative insecticidal proteins (VIPs). These proteins do not show any structural similarity to Cry proteins and this structural dissimilarity is indicative of a possible divergent insecticidal mechanism from the other known *Bt* toxins. VIPs with their significantly different mode of action from ICPs (Lee *et al*., 2003) and their broad range of host specificity seem to be potential

candidates for gene pyramiding, and are currently regarded as second-generation insecticidal toxins. The known VIP toxins from *Bt* can be classified into three groups – VIP1A and VIP2A (coleopteran specific) and VIP3A (lepidopteran specific). The effectiveness of VIP3A has been proved against several lepidopteran insect larvae including black cutworm, fall armyworm and tobacco budworm (Estruch *et al.*, 1996; Selvapandiyan *et al.*, 2001).

5.2.2 Pesticidal proteins from plants

Plants have evolved a certain degree of resistance through the production of defence compounds, which may be aproteic (antibiotics, alkaloids, terpenes and cyanogenic glucosides) or proteic (chitinases, β-1,3-glucanases, lectins, arcelins, vicilins, systemins and enzyme inhibitors). The enzyme inhibitors impede digestion through their action on insect gut digestive α-amylases and proteinases, which play a key role in the digestion of plant starch and proteins (Franco *et al.*, 2002). Several kinds of α-amylase and proteinase inhibitors (PIs), present in seeds and vegetative organs, act to regulate numbers of phytophagous insects

Proteinase inhibitors

Plants possess a wide array of defence proteins including the PIs and lectins induced in response to insect attack. PIs are the most well-studied plant defence proteins and are abundantly present in the storage organs (seeds and tubers). They inhibit the activity of gut proteinases, which in turn affects protein digestion, leads to deficiency of essential amino acids and exerts physiological stress in the insect, leading to growth retardation. PIs of the plant families *Fabaceae*, *Graminaceae* and *Solanaceae* are well characterized. Several classes of PI corresponding to different types of insect gut proteases have been observed (Winterer, 2002). Serine PIs inhibit trypsin and chymotrypsin, which are the primary digestive PIs of many lepidopteran insects. Insects also produce their own serine PI for regulation of their digestive proteases. Cysteine proteinases acting on papain and cathepsin are also present in some plants. Some unusual PIs, such as metallo- and aspartate PIs inhibiting carboxypeptidase and cathepsin, have also been found (Koiwa *et al.*, 1997). The effectiveness of PIs is influenced by several factors such as insect gut pH (Michaud *et al.*, 1993), stage of larval development (Orr *et al.*, 1994) and the amount of PI in the insect diet (Bolter and Latoszek-Green, 1997). It was demonstrated that the resistance of a cowpea variety to the bruchid beetle was due to elevated trypsin inhibitor levels in the seeds (Gatehouse and Boulter, 1983). Direct proof of the protective role of PIs against insect herbivory was provided by Hilder *et al.* (1987) who showed that transgenic tobacco plants expressing cowpea trypsin inhibitor were resistant to tobacco budworm (*Heliothis virescens*). PIs have not had as much success as *Bt* toxins for insect control (Winterer, 2002). The most widely believed reason for the failure of PIs to protect plants from insect infestation is the insects' ability to counter the action of PIs by inducing new proteases that are insensitive to these inhibitors (Brito *et al.*, 2001). New hybrid PIs, incorporating active domains of several different naturally occurring PIs, and thus designed to minimize the chances of adaptation in insects to all active domains, can be created through genetic engineering (Jermutus *et al.*, 2001).

Plant lectins

Lectins are carbohydrate-binding proteins (or glycoproteins) of non-immune nature, and bind reversibly to specific mono- or oligosaccharides. Lectins have been reported to affect the survival and development of insect pests, as listed in Table 5.1.

Although the effects of lectins on insects have been recorded for a number of years, the molecular basis of these effects remains largely unknown. As our knowledge on insect glycobiology and lectin structure and specificity are steadily

Table 5.1. Lectins and their effective host range.

Lectin	Effective against pest species	Reference(s)
Snowdrop (*Galanthus nivalis*) lectin (GNA)	*Diatraea saccharalis*, *Lacanobia oleracea*, *Myzus persicae*, *Nephotettix virescens*, *Nilaparvata lugens*, *Rhopalosiphum maidis*, *Sogatella furcifera*	Powell *et al.* (1995); Sauvion *et al.* (1996); Foissac *et al.* (2000); Sétamou *et al.* (2002); Nagadhara *et al.* (2004); Wang *et al.* (2005)
Garlic leaf lectin ASAL	*Aphis craccivora*, *Lipaphis erysimi*, *Nephotettix virescens*, *Nilaparvata lugens*, *Spodoptera littoralis*	Hossain *et al.* (2006); Sadeghi *et al.* (2008); Yarasi *et al.* (2008); Chakraborti *et al.* (2009)
Griffonia simplicifolia (GSII lectin)	*Callosobruchus maculatus*	Zhu *et al.* (2006)
Pisum sativum agglutinin (PSA, pea lectin)	*Meligethes aeneus*	Melander *et al.* (2003)
Wheat germ agglutinin (WGA)	*Helicoverpa armigera*, *Lipaphis erysimi*, *Nilaparvata lugens*	Kanrar *et al.* (2002); Gupta *et al.* (2005)
SNA-I or SNA-I′ (ricin B lectin)	*Myzus persicae*, *Spodoptera exigua*	Shahidi Noghabi *et al.* (2009)
Cinnamomin (ricin B lectin)	*Helicoverpa armigera*	Wei *et al.* (2004)

increasing, new insights in both fields may merge into a better understanding of the mechanisms behind the lectin toxicity towards insects (Michiels *et al.*, 2010). However, it currently remains controversial to use plant lectins to produce transgenic crops as they can agglutinate mammalian red blood cells and are toxic/allergenic to mammals, which restricts the use of these proteins in developing transgenic organisms safe for human consumption.

α-Amylase inhibitors

The α-amylase inhibitors produced by plants have different types of structure and modes of action and also differ in their target insect specificity. The potentials of six different α-amylase inhibitor classes, lectin-like, knottin-like, cereal-type, Kunitz-like, c-purothionin-like and thaumatin-like, were tested for pest control. These classes of inhibitor show remarkable structural variety leading to different modes of inhibition and different specificity profiles against diverse α-amylases. The common bean (*Phaseolus vulgaris*) contains a family of related seed proteins called phyto-haemagglutinin, arcelin and α-amylase inhibitor. α-Amylase inhibitor forms a complex with certain insect amylases and takes part in plant defence against insects. The introduction and expression of the bean α-amylase inhibitor gene in pea has been shown to confer resistance to bruchid beetles (Schroeder *et al.*, 1995; Ishimoto and Chrispeels, 1996).

Plant metabolic enzymes

Transgenic tobacco expressing tryptophan decarboxylase from periwinkle was found to induce the synthesis of tryptamine, while tryptamine-based alkaloids reduced the pupal emergence of whitefly (Schuler *et al.*, 1998). Other enzymes such as polyphenol oxidase and lipoxygenase have been also found to be toxic to insects.

Novel genes of plant origin

Through recent developments in molecular biology and biotechnology, it is possible to clone the genes from higher plants that are resistant to insect pests. Cloning the *Mi-1* gene from wild tomato (*Lycopersicon peruvianum*) has allowed the simultaneous control of root-knot nematode and peach-potato aphid (Vos *et al.*, 1998). Future bioprospecting needs to be done to identify the genes from plant sources to provide resistance to insect pests and diseases.

5.2.3 Insect chitinases

Insect cuticle contains chitin, an insoluble structural polysaccharide that protects the insect against water loss and abrasive agents. Dissolution of chitin by chitinase is known to perforate the peritrophic matrix and exoskeleton and to make insects vulnerable to attack by different pathogens. Expression of proteins that interfere with chitin metabolism is likely to have a serious effect on the growth and moulting of insects. In this aspect, chitinase produced by insects themselves has been used as an insecticidal protein. Larvae feeding on transgenic tobacco plants expressing the cDNA for chitinase obtained from the tobacco hornworm (*Manduca sexta*) exhibited partial protection against *H. virescens* (Ding *et al.*, 1998). Exposure of insect larvae to high levels of chitinases in conjunction with *Bt* toxins may enhance their vulnerability to *Bt* toxins and lead to more effective control of insect pests.

5.2.4 Insecticidal viruses

There are many viruses pathogenic to insect pests and these are being used in insect pest-management programmes. Viruses with a small genome size can be introduced into crop plants, which will synthesize the viral particles and acquire their entomocidal property. *Helicoverpa armigera* stunt virus (HaSV) is a tetravirus specific to lepidopteran insects and is very remotely related to viruses of plants and animals. HaSV is harmless to beneficial insects and the environment (Gordon *et al.*, 1995). A bioprospecting approach is required to develop further sources of such small genome size entomopathogenic viruses.

5.2.5 Genes from bacteria other than *Bt*

Bacteria belonging to the genera *Photorhabdus* and *Xenorhabdus* are symbiotically associated with the entomopathogenic nematodes of the Heterorhabditidae and Steinernematidae families, respectively. These bacteria are present in the gut of the nematodes and are released into the insect upon invasion by the nematodes. The Pht toxins from *Photorhabdus luminescens* have been exploited for insect control in the development of transgenic tobacco tolerant to *M. sexta* (Bowen *et al.*, 1998). Development of resistance to toxins produced by *P. luminescens* and *Xenorhabdus nematophilus* has not been reported to date and is unlikely to occur due to the physical macroscopic nature of the infection (Chattopadhyaya *et al.*, 2004). Secondary metabolites from bacteria are also being screened for their insecticidal potential. The isopentenyltransferase (*ipt*) gene affects cytokinin biosynthesis in insects, leading to increased levels of toxins in insects. Such genes from bacteria have been transferred to some crops (Ebinuma *et al.*, 1997). Cholesterol oxidase isolated from bacteria reduces the growth rate of insects by destroying cell membranes in the digestive tract.

5.2.6 Biotin-binding proteins

Biotin-binding proteins are insecticidal to a very wide range of insects. Biotin-binding proteins are effective across a broad range of insect orders (Lepidoptera, Coleoptera, Orthoptera, Diptera and leaf-eating Hymenoptera) and other invertebrates, and the expression levels required are generally low (approximately 100 p.p.m.). Avidin and streptavidin, in particular, have been reported as causing death or severe growth reduction in at least 40 species of insects and mites, and no adverse impacts on non-target microorganisms or invertebrates have been recorded. Two properties of the biotin–avidin complex that make it highly suitable for use in transgenic plant crop protection strategies against a large range of insects are its extreme stability and its resistance to proteolysis. However, the nutritional value of the plant could potentially be com-

promised in the absence of biotin supplementation. Hence, its use in non-food crops such as fibre and in forestry and biofuel crops is seen as the most suitable focus for this technology (Christeller *et al.*, 2010).

5.2.7 Pathogenesis-related proteins

A group of plant-encoded proteins induced by different stress stimuli, named pathogenesis-related (PR) proteins, has been assigned an important role in plant defence against pathogenic constraints and in general adaptation to stressful environments. Originally, five main groups of PR proteins (PR-1 to PR-5) were characterized by molecular and genetic techniques in tobacco, numbered in order of decreasing electrophoretic mobility. Each group consists of several members with similar properties (Bol *et al.*, 1990). Group PR-1 is the most abundant, reaching up to 1–2% of total leaf proteins. The PR-5 group members share significant amino acid sequence homology with the sweet-tasting protein in the fruits of the tropical plant *Thaumatococcus daniellii* and have been named thaumatin-like (TL) proteins (Cornelissen *et al.*, 1986). Osmotins of the same group display similarity to TL proteins (Singh *et al.*, 1987). Therefore, in 1994, a unifying nomenclature for PR proteins was proposed based on their grouping into families sharing amino acid sequences, serological relationships and enzymatic or biological activity. By then, 11 families (PR-1 to PR-11) were recognized and classified for tobacco and tomato, with the families PR-8 and PR-10 being also present in cucumber and parsley, respectively (van Loon *et al.*, 1994). Later, three novel families (PR-12, PR-13 and PR-14) were recognized in radish, *Arabidopsis* and barley, respectively (van Loon and van Strien, 1999). Germins and germin-like proteins have been classified as PR-15 and PR-16; PR-16 has been isolated from hot pepper during the resistance response to bacterial and viral infection (Park *et al.*, 2004).

5.2.8 Silencing of genes using an RNAi approach for development of pest-resistant plants

RNA interference (RNAi) is the specific downregulation of gene expression by double-stranded RNA (dsRNA). The specificity is sequence based and depends on the sequence of one strand of the dsRNA corresponding to part or all of a specific gene transcript. RNAi is an emerging genetic tool in developing pest-resistant crops and has been used successfully in generating pathogen resistance in different crop plants (Escobar *et al.*, 2001; Mikhail *et al.*, 2003). In 2007, plant-delivered RNAi technology was used to engineer resistance against plant-parasitic nematodes (Dubreuil *et al.*, 2009; Patel *et al.*, 2010), herbivorous insects (Price and Gatehouse, 2008; Huvenne and Smagghe, 2009), parasitic weeds (Tomilov *et al.*, 2008) and fungi (van de Craen *et al.*, 2006; Roberts *et al.*, 2008). RNAi-mediated plant resistance offers several advantages over conventional bioengineered crop resistance. It is possible to develop durable and multiple-pathogen resistance by silencing conserved and essential genes. The possibility of the pests overcoming this resistance is likely to be difficult if the right target genes are identified.

5.3 Pest-resistant Transgenic Plant Development in Different Crops

Recombinant DNA technology offers the possibility of developing transgenic plants. In addition to widening the pool of useful genes, genetic engineering also allows the use of several desirable genes in a single event and reduces the time to introgress novel genes into an elite background. Biotechnology has provided several unique opportunities that include access to novel molecules, the ability to change the level of gene expression, the ability to change the expression pattern of genes and the development of transgenics with different insecticidal genes. The potential of this technology has now been widely recognized

(Burke and Thomas, 1997). The engineering of plants to express *Bt cry* genes has been especially helpful against pests that attack parts of the plant that are usually not well protected by conventional insecticide application. *Bt* genes conferring resistance to insects have been inserted into crop plants such as maize, cotton, potato, tobacco, potatoes, rice, broccoli, lettuce, walnuts, apples, lucerne and soybean.

5.3.1 Transgenic crops with *Bt* genes

The first transgenic tobacco plants containing *Bt* genes were produced in 1987 (Barton *et al.*, 1987) and different steps in successful transgenic plant development system have been developed by many workers (Perlak *et al.*, 1991; Carozzi *et al.*, 1992; Koziel *et al.*, 1993; Hilder and Boulter, 1999). However, the expression level was quite low in tobacco plants, resulting in only 20% mortality of tobacco hornworm (*M. sexta*) larvae. In 1996, three insect-resistant crops developed by the Monsanto Company received regulatory approvals and were grown commercially for the first time in the USA. The transgenes incorporated into these crops were modified *cry1Ab* in maize (Carozzi and Koziel, 1997), modified *cry1Ac* in cotton (Perlak *et al.*, 1990) and modified *cry3Ab* in potato (Perlak *et al.*, 1993). All three insect-resistant crops had genes that produced insecticidal proteins for protection against the notorious European corn borer (*Ostrinia nubilalis*), the Colorado potato beetle (*Leptinotarsa decemlineata*) and the cotton bollworm complex, which includes the tobacco budworm (*H. virescens*), bollworm (*Helicoverpa zea*) and pink bollworm (*Pectinophora gossypiella*).

The large-scale commercial cultivation of genetically modified (GM) crops started with 1.7 million ha in 1996 and has since increased at a rapid pace in terms of cultivated acreage, amounting to a global 134 million ha in 2009. Twenty-five countries across the world are cultivating GM crops, and among these 15 are considered as mega-biotech countries. The main countries for GM crop cultivation are the USA, Brazil, Argentina, India, Canada, China, Paraguay and South Africa. India ranks fourth in GM crop cultivation. The GM crops that are cultivated worldwide are soybean, maize, cotton, canola, squash, papaya, lucerne, sugarbeet, tomato, poplar and sweet pepper. The most important traits that have been conferred by genetic modification are herbicide tolerance and insect resistance.

In India, transgenic *Bt* cotton was commercialized in 2002 and was adopted in 0.045 million ha in 2002. By 2009, this area had increased to 8.4 million ha. Globally, 14 million small- and large-scale farmers are involved in cultivation of GM crops. The largest increase in the number of beneficiary farmers in 2009 was in India – 0.6 million more small-scale farmers planted *Bt* cotton, which was 87% of total cotton, up from 80% in 2008. In the short span of 7 years, *Bt* cotton has generated economic benefits for farmers valued at US$5.1 billion, has halved insecticide requirements and has contributed the doubling of yield, transforming India from cotton importer to a major exporter (James, 2009). The approved events (i.e. the unique DNA recombination events that take place in one plant cell and are then used to generate entire transgenic plants) in the case of *Bt* cotton were: (i) MON531 (Bollgard I) by Mahyco-Monsanto Biotech, containing the *cry1Ac* gene (approved in 2002); (ii) MON15985 (Bollgard I II) by Mahyco-Monsanto Biotech containing the *cry1Ac* and *cry2Ab* genes (approved in 2006); (iii) 'GFM Cry1A' containing *cry1Ac–cry1Ab* fusion gene by Nath Seeds (approved in 2006); (iv) Event-1 containing the *cry1Ac* gene by IIT Kharagpur/JK AgriGenetics (approved in 2006); (v) BNLA 601 containing the *cry1Ac* gene by CICR, Nagpur (not commercialized, although approved in 2008); and (vi) MLS-9124 carrying the *cry1C* gene by M/s Metahelix Life Sciences (approved in 2009).

According to 2009 statistics, trait-wise herbicide-tolerant GM crops occupied an area of 83.6 million ha (62%), whereas stacked-traits and insect-resistant GM crops

occupied 28.7 (21%) and 21.7 million ha (16%), respectively. The virus-resistant and other traits were adopted in 0.1 million ha (<1%). The dominant biotech crops worldwide are: herbicide-tolerant soybean (69.2 million ha), stacked-traits maize (26.1 million ha), *Bt* cotton (12.4 million ha), *Bt* maize (9.2 million ha), herbicide-tolerant maize (6.4 million ha), herbicide-tolerant canola (6.4 million ha), stacked-traits cotton (2.6 million ha) and herbicide-tolerant cotton (1.1 million ha). The predominant GM crops are: soybean (69.2 million ha, 77%), cotton (16.1 million ha, 49%), maize (41.7 million ha, 26%) and canola (6.4 million ha, 21%) (James, 2010).

5.3.2 Transgenic crops for disease resistance

Experimental evidence has substantiated the utility of PR genes to develop disease resistance in transgenic plants. This practical aspect of PR gene research has resulted in the release of agronomically important crops resistant to various diseases of economical interest. One promising strategy is based on the exploitation of genes encoding antifungal hydrolases, such as β-1,3-glucanase and chitinase, which are associated with systemic acquired resistance (SAR) response in plants. Increased resistance of tomato plants against fungal pathogens was achieved by simultaneous expression of a class I chitinase and β-1,3-glucanase (PR-3 and PR-2 families, respectively) from tobacco. Transgenic tomato plants expressing either of these genes alone were less protected. Field evaluation of transgenic carrot plants containing the same genes has shown a high level of resistance against major fungal pathogens of carrots. An important feature is that the majority of the transgenic lines that had resistance to one pathogen exhibited significant resistance to the other pathogens (Melchers *et al.*, 1998). The constitutive overexpression of tobacco class I, PR-2 and PR-3 transgenes in potato plants enhanced their resistance to *Phytophthora infestans*, the causal agent of late blight (Bachmann *et al.*, 1998). Similar results for the effectiveness of the co-expression of chitinase and β-1,3-glucanase in plant disease resistance were reported by Kombrink *et al.* (2001). Transgenic *Brassica napus* plants constitutively expressing a chimeric chitinase gene displayed field tolerance to fungal pathogens (Grison *et al.*, 1996). Overexpression of the cloned rice thaumatin-like (PR-5) gene in transgenic rice plants enhanced the environmental friendly resistance to *Rhizoctonia solani* causing sheath blight disease (Datta *et al.*, 2001).

Several attempts have been made to engineer durable disease resistance in economically important crop plants, but many have failed due to the complexity of disease-resistance signalling and the sheer diversity of infection mechanisms that different pathogens use. Although disease-resistant transgenic plants or seeds are not yet available commercially, future product development seems likely as our current level of understanding of pathogenesis and plant defence improves (Stuiver and Custers, 2001). Genetic manipulation of the regulatory mechanisms and signalling processes controlling the coordinated activation of multiple defence responses such as SAR may be the ultimate approach to modifying plant resistance. However, this requires precise knowledge of both the signalling pathways involved and the subsequent metabolic pathways that are triggered. When exploiting the genes in signalling pathways to produce fungus-resistant transgenic plants, one needs to be cautious about the role of the signalling gene in various other pathways, which may lead to undesirable side effects in transgenic plants. The earlier the gene function in the pathway, the greater the intricacies of regulation that will have to be addressed. Correct temporal and spatial expression of the transgene will be of critical importance and will require the availability of well-defined, pathogen-inducible promoters with the desired properties (Grover and Gowthaman, 2003). Resistance genes (*R* genes) and PR genes seem to be a good class of gene to use for transgenic approaches.

Plants overexpressing these genes display very few side effects but show strong (but probably not durable) protection effects for *R* genes as well as for PR genes (Delteil *et al.*, 2010).

Virus resistance is usually achieved through the antiviral pathways of RNA silencing, a natural defence mechanism of plants against viruses. The experimental approach consists of isolating a segment of the viral genome itself and transferring it into the genome of a susceptible plant. Integrating a viral gene fragment into a host genome does not cause disease (the entire viral genome is needed to cause disease). Instead, the plant's natural antiviral mechanism that acts against a virus by degrading its genetic material in a nucleotide sequence-specific manner via a cascade of events involving numerous proteins, including ribonucleases (enzymes that cleave RNA), is activated. This targeted degradation of the genome of an invader virus protects plants from virus infection (Waterhouse *et al.*, 2001; Voinnet, 2005).

Transgenic tobacco plants expressing *hrmA*, a gene that encodes an avirulent factor from *Pseudomonas syringae* pv. *syringae* conferred resistance to tobacco vein mottling virus (Shen *et al.*, 2000). Expression of viral avirulence factors, such as coat proteins (CPs), has been shown to confer resistance to the same virus that the CP was derived from and sometimes to closely related viruses. Transgenic tobacco expressing the CP of tobacco mosaic virus or tomato mosaic virus showed resistance to tobacco mosaic virus (Nelson *et al.*, 1988; Sanders *et al.*, 1992). CP-mediated resistance has also been reported in transgenic plants expressing cucumber mosaic virus (Namba *et al.*, 1991; Quemada *et al.*, 1991), potato virus X (Hemenway *et al.*, 1988), potato virus Y (Perlak *et al.*, 1994), alfalfa mosaic virus (Loesch-Fries *et al.*, 1987), potato leaf roll virus (Kaniewski *et al.*, 1993) and papaya ring spot virus (Chiang *et al.*, 2001). Virus-resistant transgenic squash and papaya cultivars have been developed and were released commercially in 1996 and 1998, respectively (Gonsalves, 1998). These were the first transgenic disease-resistant transgenic crop and first transgenic fruit crop, respectively, to be deregulated and introduced in the market. The squash cultivars are resistant to cucumber mosaic virus, zucchini yellow mosaic virus and watermelon mosaic virus because they express the CP gene of these three aphid-borne viruses. Similarly, the papaya cultivars expressing the CP gene of papaya ringspot virus are resistant to this aphid-borne virus. Thus, the exploitation of the sequence-specific antiviral pathways of RNA silencing, a potent natural plant defence mechanism against viruses, has facilitated the development of virus-resistant crop plants. The transgene-mediated resistance by insertion of avirulence factors of pathogens has been found to protect plants from several diseases.

Some virus strains are able to overcome engineered resistance because RNA silencing is a defence mechanism based on nucleotide sequence identities. Resistance is more likely to be achieved against virus strains with high sequence homology to the strain(s) from which the transgenes are derived than with those that are more distantly related. In addition, viruses encode some proteins that can act as suppressors of RNA silencing. Activation and regulation of the antiviral pathways of RNA silencing are dynamic processes. Therefore, monitoring the introduction of virus-resistant transgenic crops and the eventual emergence of new viral strains is key to maximize the effectiveness and durability of engineered virus resistance (Tepfer, 2002; Fuchs and Gonsalves, 2007).

As the genome sequencing projects and functional analysis of several genes are completed for both plants and pathogens, a better understanding of the function of *R* genes along with their interactions with pathogens will be obtained. In addition, the function of avirulent factors will be better understood, which will facilitate the development of more durable disease-resistant crops.

5.3.3 Transgenic crops for nematode resistance

Resistance to nematodes has been characterized as a reduction in the level of nematode reproduction. Transgenic plants for nematode resistance could be achieved by *R* gene-mediated resistance, transformation of genes that result in disruption of the development of specialized feeding structures of nematodes or transformation of genes that interfere with the digestive system of nematodes. Several genes that confer resistance to nematodes have been cloned, but only a few have been transformed successfully into different crop plants. These include *R* genes and genes encoding PR proteins (Grundler, 1996; Williamson and Hussey, 1999). Examples of nematode resistance genes that have been cloned and are well characterized are $Hs1^{pro\text{-}1}$, *Mi* and *Gpa2* (Cai *et al.*, 1997; Rouppe van der Voort *et al.*, 1999; Urwin *et al.*, 2000).

5.4 Issues in Adopting Pest-resistant GM Crops

Detailed analysis of any potential effects of transgenic crops on the environment and human health is crucial before commercial release (Dale *et al.*, 2002; Conner *et al.*, 2003; Nap *et al.*, 2003). The Cry proteins are known to have selective toxicity to certain categories of insects and require specific conditions for their effective action. The protein has to be ingested by the target insects, solubilized and activated and toxins have to be bound to the receptor sites for effective action. Predators with chewing mouthparts, such as lady beetles (Coccinellidae), are expected to ingest the toxin when preying on *Bt*-fed arthropods because they ingest the gut where most of the toxin is located. For predators with sucking mouthparts such as predatory bugs (Hemiptera), this is likely but less clear because they might selectively feed on body tissues that do not contain the toxin. Until now, toxin uptake by predators has only been measured directly by means of an immunological test (ELISA) (Meissle *et al.*, 2005).

Cry1A toxins do not show specific binding to brush border membrane vesicles from the midgut of common green lacewing (*Chrysoperla carnea*) larvae, which is a prerequisite for toxicity. When *C. carnea* larvae are fed with lepidopteran larvae reared on Cry1Ab-expressing maize, larval development was prolonged and mortality increased significantly (Hilbeck *et al.*, 1998; Dutton *et al.*, 2002). Recent studies by Wei *et al.* (2007) reported that Cry proteins were non-toxic to *C. carnea*.

Predators attacking sap-feeding herbivores, such as aphids and planthoppers, are unlikely to be exposed because the Cry proteins do not appear to be transported in the phloem (Raps *et al.*, 2001). To date, only trace amounts of Cry proteins have been detected in sap feeders on different *Bt*-transgenic events of maize (Head *et al.*, 2001; Dutton *et al.*, 2002), oilseed rape (Schuler *et al.*, 2005) and rice (Bernal *et al.*, 2002). Thus, predators feeding preferentially on aphids, such as most lacewings and lady beetles, are unlikely to be at risk.

Reports of potentially toxic effects of *Bt* maize pollen and flower parts eaten by monarch butterfly larvae have captured widespread attention (Losey *et al.*, 1999). A flurry of subsequent research demonstrated that the effects of *Bt* pollen on monarch larvae are highly variable, depending on factors such as pollen density, the crop's *Bt* genotype and environmental factors (Sears *et al.*, 2001). This illustrates that risk assessment research can clarify whether a putative risk is, in fact, a problem. Many scientists have carried out a meta-analysis of all published non-target studies with *Bt* crops (Marvier *et al.*, 2007; Naranjo, 2009; Duan *et al.*, 2010).

Transgenic plants expressing a single *cry* gene throughout the season and in all parts of the plant can accelerate the onset of resistance in pests (Gould, 1997). Pest populations exposed to *Bt* crops continuously for several years have the potential to develop resistance to Cry proteins (Kaur *et al.*, 2004). There is a serious threat of the development of

resistance in *H. armigera* to Cry1Ac protein following large-scale cultivation of *Bt* cotton. In view of this, proactive insect resistance management strategies have been developed and are in place to prevent or delay resistance development. A key element of these plans is that growers should plant sufficient non-*Bt* crops to serve as a refuge for producing *Bt*-susceptible insects. The recommendation includes growing 20% non-*Bt* cotton around the periphery of the *Bt* cotton crop as refuge and taking the necessary control measures against bollworms in the refuge crop as and when required. Fortunately, except for the diamondback moth (*Plutella xylostella*), none of the other insect species have developed resistance to *Bt* biopesticides under field conditions so far. A resistant strain of diamondback moth was able to complete its life cycle without any adverse effects on *Bt*-transgenic canola, which produced high levels of the Cry1Ac protein (Ramachandran *et al.*, 2000). The recommendations for deployment of *Bt*-transgenic crops in an integrated pest management (IPM) strategy advocate the use of as-high-as-possible toxin doses to minimize the chances of survival of the resistant heterozygotes, planting non-transgenic refuges to sustain the homozygous susceptible insect population and the deployment of multiple toxin genes acting on different midgut receptors (Shelton *et al.*, 2000). Transgenic plants carrying more than one *cry* gene (gene pyramiding/stacking) with different binding sites has been suggested as a resistance management strategy (van der Slam *et al.*, 1994). The long-term transgenic deployment strategy to minimize the development of pests with resistance to *cry* genes should involve the incorporation of novel insecticidal genes, either native or engineered, into transgenic crops.

The best-known mechanism of resistance is through reduced binding of Cry proteins to receptors in the target insect's midgut epithelial membrane. As many Cry proteins share these binding sites, the development of resistance to one leads to cross-resistance for other Cry proteins (Ballester *et al.*, 1999; Griffitts *et al.*, 2001; Li *et al.*, 2004). The linkage of Cry1A resistance to mannose phosphate isomerase isoenzymes in *P. xylostella* and *H. virescens* suggests the occurrence of homologous resistance loci (Herrero *et al.*, 2001). The cadherin locus has also been proposed to be associated with the development of resistance in *P. gossypiella* (Tabashnik *et al.*, 2005) but not in field-evolved resistant strains of *P. xylostella* (Baxter *et al.*, 2005). As differential expression of the *cry* gene in various parts of the plant may affect larval survival and pest population dynamics, it is also a critical factor in resistance management (Adamczyk *et al.*, 2001). Furthermore the efficacy of *Bt*-transgenic crops can be prolonged in multiple cropping situations, having crops with different transgenes serving as refuges for each other (Caprio and Suckling, 2000). In India, *H. armigera*, by far the most predominant bollworm, besides cotton, has a large number of alternative host crops such as chickpea, pigeon pea, tomato, sunflower, maize and sorghum, which are grown substantially around the same area at the same time as cotton. These crops, especially chickpea and pigeon pea, support large populations of *H. armigera*, thereby serving as a natural refuge and helping insect resistance management.

5.5 Genetic Engineering of Insects

Genetic transformation of insects, which involves the introduction of DNA from external sources, was first tried on a scaleless mutant of the stored-grain pest *Ephestia kuehniella* in 1965. Injection of wild-type DNA resulted in the production of adults with wing scales. Some agriculturally important pests such as cotton pink bollworm (*P. gossypiella*) can be managed effectively by employing a technique called autocidal biological control (see below), which greatly reduces insecticide usage with a subsequent reduction in environmental pollution. There are many methods available for delivering the gene of interest into the target species. The most common is

microinjection, followed by lipofection (a process by which DNA or RNA encapsulated in an artificial phospholipid vesicle is delivered into eukaryotic cells). In addition to these direct methods, indirect methods such as paratransgenesis (genetic alteration of microbes living in association with insects for various purposes) is also used on insects that are less amenable for laboratory rearing or that have a long generation time. The genetically engineered insects are selected using the *nptII* gene (which confers resistance to neomycin analogues), the organophosphorus dehydrogenase (*opd*) gene (which confers resistance to paraoxon) and the gene for dieldrin resistance (*rdl*). Other useful visual markers are the green fluorescent protein and its spectral variants. The practical utilities of genetic engineering of insects are: (i) as bioreactors; (ii) as genetically improved biocontrol agents; (iii) for impairing disease transmission by insect vectors; and (v) for insect pest management through the sterile insect technique (SIT) and release of insects carrying a dominant lethal gene (RIDL®).

5.5.1 Sterile insect technique and release of insects carrying a dominant lethal gene

SIT uses the mass release of sterile insects as a highly effective area-wide, environmentally safe method of pest control. The advent of modern biotechnology has made available a wide variety of tools to manipulate and express genes within insect pests on shorter timescales and with a wider range of accessible phenotypes than is possible through classical genetics. A population reduction method using a strain of insects homozygous for a dominant lethal genetic system (Alphey and Andreasen, 2002; Gong *et al.*, 2005), known as RIDL®, is now available for field use. RIDL technology offers a solution to many of the drawbacks of traditional SIT that have limited its application, while maintaining its environmentally friendly and species-specific utility (Alphey *et al.*, 2008). This autocidal biological control was applied to the development of conditional-lethal pink bollworm strains. When these strains are mass reared, the lethal gene expression is suppressed by a tetracycline repressor element, which is activated by the presence of chlortetracycline, a normal component of the mass-rearing diet. Once removed from the tetracycline diet, the lethal genes are passed on to offspring when ordinary lab-reared pink bollworms mate with special lethal strains. Lethality is dominant (i.e. one copy is sufficient for lethality), and is expressed in the egg stage and affects all eggs (100% lethal expression). The current state of the biotechnology behind genetic modification of insects is in a state of high flux. Deeper analysis is needed on the potential risks and benefits involved in the release of GM insects into the environment along with international and domestic legislation that regulates their release.

5.6 Marker-assisted Breeding for Pest Resistance

Marker-assisted breeding will be helpful for improving the efficiency of conventional breeding methods and can be used as a diagnostic tool to identify elite pest-resistant seedlings. Markers are used as tags for genes located near them, as they are simpler to detect than the gene itself that determines a pest-resistance characteristic. This technique is used as a way of introducing resistance towards pests and diseases that will enhance durability and enables halving of the time to introduce new genetic characters into new varieties. In the early 1980s, indirect selection in plant breeding using DNA markers became technically feasible through the development of restriction fragment length polymorphism (RFLP) markers. However, the laborious nature of the RFLP technique prevented the broad application of RFLP markers for marker-assisted breeding. In the late 1980s and the early 1990s, molecular diagnostic methods based on PCR technology were developed, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and the use of microsatellites. These

methods allowed efficient detection of DNA fragments, even with small amounts of DNA.

Extensive research, including the generation of many genetic maps in different crops, has demonstrated that the majority of amplified restriction fragments correspond to unique loci in the genome. Reference molecular-linkage maps have been constructed in, for example, *Arabidopsis*, maize, wheat, barley, rice, oilseed rape, soybean, sunflower, potato, tomato, lettuce, pepper, cucumber and watermelon.

Genetic distance analysis can be a powerful tool for breeders to identify different heterotic groups and to increase the efficiency of finding crosses with good combinability. To determine the genetic distance between lines and groups of lines, the lines are fingerprinted and the marker presence or absence is scored for each line. Based on the obtained score table, similarity indices can be calculated for all combinations of lines. Subsequently, the relatedness among the lines can be visualized using a dendrogram display or principal component analysis plot. Indirect selection using markers can be an advantageous method of selection in plant breeding, especially for traits whose phenotypic tests are unreliable or expensive.

5.6.1 Monogenic resistance traits

Different approaches can be followed for the identification of markers linked to monogenic traits. The preferred approaches are all based on screening a limited number of samples with a relatively large number of primer pairs. In this way, many loci can be screened with limited effort. The number of lanes per fingerprint can be limited by screening sets of near-isogenic lines, if these are available. Candidate markers that are identified in this way are then screened on a panel of phenotypically well-characterized lines to confirm their linkage and to determine the predictive value of the markers. Another efficient approach is bulk segregant analysis (BSA) method (Michelmore *et al.*, 1991). In this method, individuals from a segregating population are pooled on the basis of their phenotype, and the pools are then screened until a sufficient number of markers emerge. This method can be used for both dominant and recessive monogenic traits. For dominant genes, *cis* markers (linked to the trait of interest) will emerge from the screening, whereas *trans* markers (linked to the opposite allele) will be identified for recessive traits.

5.6.2 Oligogenic resistance traits

The BSA approach may also be useful for the identification of linked markers for oligogenic traits. This has been demonstrated in an AFLP marker screening for one type of disease resistance in tomato. The BSA screening yielded *trans* markers for a recessive gene involved in the resistance. When screening the *trans* markers in individuals of the segregating population, the resistance could only be predicted in 75% of individuals, while the remaining 25% of individuals could not be explained as recombinants. The results suggested that an additional (dominant) gene is needed for full resistance. By bulking the sensitive individuals in which the resistance cannot be predicted by the markers for the recessive gene, and creating a 'positive' bulk of resistant individuals on the other hand, a screening can then be performed for markers linked to the putative dominant gene.

5.6.3 Polygenic (quantitative) resistance traits

The classical approach for the identification of loci involved in complex polygenic traits consists of the screening of a large number of individuals from a segregating population with a set of markers that are evenly distributed throughout the genome. Statistical analysis is then performed to identify regions in the genome that are involved in the trait. The laborious nature of this approach makes it unrealistic to screen sufficiently large populations to

locate the quantitative trait loci (QTL) precisely. As a consequence, the QTL cannot be localized precisely on the map and closely linked markers cannot be obtained, thereby preventing the broad-scale application of indirect selection for quantitative traits. The BSA strategy may also be useful for identification of markers for quantitative traits. In this method, the F_2 population is segregated for quantitative traits. Based on the phenotypic scores, bulks are composed and loci are screened on these bulks using the AFLP fingerprinting technique. Candidate markers that are identified on the bulk screening are then analysed in randomly chosen individuals of the F_2 population. The results obtained by this method have been confirmed in independent studies in which the same map positions were identified to be involved in the respective traits (Toroser *et al.*, 1995; Jourdren *et al.*, 1996).

5.6.4 Marker-assisted backcross breeding

Marker-assisted backcross breeding is now becoming a standard application in modern plant breeding. In selection for highly recurrent parental genomic content, the DNA fingerprints are used to calculate the percentage recurrent parental genome in each backcrossed individual, thereby taking the genome representation of the markers into account. When negative characteristics are linked to the trait that needs to be introgressed, molecular markers can be used to select for recombinants in the region. After phenotypic testing of these recombinants, individuals may be selected in which the region responsible for the linkage drag has been removed from the locus of interest.

Since the advent of the first DNA markers, marker-assisted selection (MAS) has been viewed as a promising approach to streamline resistance breeding. Molecular markers are now used routinely in plant cultivar development to assist backcrossing of major genes into elite cultivars and to select alleles with major effects on high-value traits with relatively simple inheritance. Molecular markers have become important tools for enhancing the selection efficiency for various pest-resistance traits in precision plant breeding (Table 5.2). Traditional breeding efforts are being greatly enhanced through the integration of comparative genomics. In addition, by tagging several genes with closely linked molecular markers, MAS strategies facilitate the development of lines with stacked

Table 5.2. Marker-assisted breeding for pest and disease resistance.

Crop	Pest	Reference(s)
Rice	Brown planthopper, gall midge	Biradar *et al.* (2004); Sharma *et al.* (2004)
Wheat	Hessian fly	Behura *et al.* (2004)
Maize	Corn earworm, southwestern corn borer and sugarcane borer	Guo *et al.* (2001)
Sorghum	Sorghum midge	Tao *et al.* (2003)
Groundnut	Root-knot nematode	Choi *et al.* (1999)
Cotton	*Helicoverpa armigera*	Liu *et al.* (2000)
Cowpea	Aphid, bruchid	Githiri *et al.* (1996)
Pea	Pea common mosaic virus, *Erysiphe polygoni*, Russian wheat aphid	Dirlewanger *et al.* (1994)
Bean	Common bean mosaic virus, bean pod weevil (*Apion godmani* Wagner) in common bean	Blair *et al.* (2007)
Tomato	*Helicoverpa armigera*, *Meloidogyne incognita*, *Phytophthora infestans*, tomato mosaic virus, cucumber mosaic virus, yellow mosaic virus, tospovirus	Yaghoobi *et al.* (1995); Brommonschenkel and Tanksley (1997); Moreau *et al.* (1998); Diwan *et al.* (1999); Hanson *et al.* (2000); Sobir *et al.* (2000); Stamova and Chetelat (2000)

resistance genes, giving the cultivar more durable protection than that afforded by a single resistance gene (William *et al.*, 2007). Yencho *et al.* (2000) reviewed how molecular markers can be used to increase our understanding of the mechanisms of plant resistance to insects and to develop insect-resistant crops. Genes controlling resistance to different races or biotypes of a pest or pathogen, or genes contributing to agronomic or seed quality traits, can also be pyramided together to maximize the benefit of MAS through simultaneous introgression (Dwivedi *et al.*, 2007). The use of molecular markers is a very reliable method to diagnose the integration of the two genes in the plants.

5.6.5 Characterization of induced pest resistance in plants using molecular tools

In plant–pathogen and plant–herbivore interactions, early events, such as changes in plasma membrane potential (V_m) changes, protein phosphorylation or activation of plasma membrane proteins, directly or indirectly trigger diverse signalling networks, interconnecting branch pathways that amplify and specify the physiological response. The early events measurable in plant–insect interactions starting from damage includes V_m changes at the plasma membrane, immediately followed by changes in the intracellular Ca^{2+} concentration and the generation of H_2O^2. Within minutes, kinases and the phytohormones jasmonic acid (JA) and salicylic acid (SA) are detectable. Gene activations and subsequent metabolic changes are first seen after about 1 h. The plant signalling compounds SA and JA have been found to be of major importance in regulating two different branches of induced resistance in plants. SA was recognized first as the key regulator of pathogen-induced SAR. Limited infection increases SA levels in the plant systemically, thereby protecting previously uninfected, and even newly emerging, tissues from further attack by the same or other pathogens. JA was found to be responsible for wound-induced resistance against insect attack and to be elicited by, and effective against, mainly chewing insects. In nature, plants may be attacked simultaneously by both microbial pathogens and insects. Unfortunately, the two signalling pathways have been found to interfere: in particular JA-dependent defences are suppressed by the activated SA-dependent defences. Such antagonism may be one reason why SA- and JA-dependent induced resistances have not evolved to become part of the constitutive resistance repertoire of plants. It also follows that caution is needed when exploiting induced resistance in practice, because activating SAR will most probably increase vulnerability to insect attack. Recent studies have advanced our understanding of the mechanisms by which plants recognize pests and subsequently activate direct and indirect defence responses. Introduction of plant growth-promoting rhizobacteria (PGPR) for increasing plant growth promotion during the 1950s from research findings in the Soviet Union and in Western countries (Backman *et al.*, 1997) opened new vistas to use PGPR as an alternative to chemical pesticides for the management of soil-borne pathogens (Kloepper, 1993). The application of PGPR, either as single strain or strain mixture-based formulations, was found to check pest and disease spread as well as increasing growth and yield.

5.7 Molecular Characterization of Pests, Pathogens and Natural Enemies

With the development of PCR and DNA sequencing technologies associated with high-throughput screening systems, marker polymorphisms are now the choice for molecular-based surveys of genetic variation in pests, pathogens and natural enemies. DNA barcoding employs sequence diversity in short, standardized gene regions to aid species identification and discovery in large assemblages of life. A 648 bp region of the cytochrome *c* oxidase I (*COI*) gene forms the primary barcode

sequence for members of the animal kingdom. The early goals of DNA barcoding focused on the assembly of reference libraries of barcode sequences for known species. Current results show that these libraries will be very effective in generating identifications; more than 95% of species in test assemblages of varied animal groups have been shown to possess distinctive COI sequences. The Barcode of Life Data Systems (BOLD, www.boldsystems.org) is an online workbench that aids collection, management, analysis and use of DNA barcodes. As of December 2010, 93,599 insect and 6500 fungi species barcodes are available in the database.

5.7.1 Characterization of biotypes of crop pests

Insect biotypes are populations able to kill or injure crops with resistance genes and complicate pest-management programmes based on host-plant resistance. Unlike the use of plant host differentials for identifying biotypes, the molecular assay is fast, reliable and unaffected by environmental factors. This assay is used in *Orseolia oryzae* (Wood Mason) to discriminate the five different biotypes of *O. oryzae* Indian isolates and the Asian rice gall midge, African rice gall midge (*Orseolia oryzivora*) and the paspalum midge (Behura *et al.*, 1999). Many molecular studies have been conducted to differentiate biotypes B and Q in *Bemisia tabaci* and to study the genetic relationships within and between populations (Brown, 2000; Hsieh *et al.*, 2007). Research in population genetics and population ecology with the help of molecular markers helps to characterize pest populations across hosts and geographical areas.

Biotypes occur in Russian wheat aphid (*Diuraphis noxia* Kurdjumov), a worldwide pest of wheat, (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). Five *D. noxia* biotypes were described in the USA and given the number designations 1 to 5. A single RAPD polymorphism was found in only two individuals. However, no DNA sequence variation in the *COI* mitochondrial gene was found among 26 *D. noxia* clones and no variation was found at seven examined simple sequence repeat loci. This indicated that a limited genetic variation existed within and between Russian wheat aphid biotypes in the USA (Shufran and Payton, 2009).

5.7.2 Characterization of invasive pests

In recent years, phylogenetic analysis of invasive species and their relatives has at times been effective in identifying their geographical origin. *Apis mellifera*, which is native to Europe, Asia and Northern Africa but has been introduced to North America, South America and Australia, was differentiated using nuclear and mitochondrial markers (Lobo, 1995), allozymes and mitochondrial DNA (Oldroyd *et al.*, 1992). The characterization of red imported fire ant (*Solenopsis invicta*) and the Argentine ant (*Linepithema humile*) in North America was differentiated using mitochondrial and nuclear DNA markers (Tsutsui *et al.*, 2000), as well as the Formosan termite (*Coptotermes formosanus*) in the USA using allozymes (Korman & Pashley, 1991) and the wasp *Vespula germanica* in Australia using DNA microsatellite markers (Goodisman *et al.*, 2001).

Grapputo *et al.* (2005) studied the voyage of an invasive species across continents in the genetic diversity of North American and European Colorado potato beetle populations. From an analysis of mitochondrial DNA and AFLP markers, they found the highest genetic diversity in beetle populations from the central USA.

5.7.3 Molecular markers for characterization of insecticide resistance in insect pest populations

Insecticide resistance is an important focus of entomological research and has medical and agricultural relevance. Molecular markers are used for identification and mapping of resistance genes in insects against insecticides. In malaria control

programmes, difficulties arise because of emerging resistance in the mosquito vectors to dichlorodiphenyltrichloroethane (DDT). DDT resistance in the major malaria vector in Africa, *Anopheles gambiae*, is associated with increased metabolism of the insecticide. Use of microsatellite markers in mapping experiments has identified QTL in *A. gambiae* that determine the DDT resistance phenotypes (Ranson *et al.*, 2000). Using RFLP markers, it was discovered that DDT resistance in houseflies (*Musca domestica*) was associated with the 'knockdown' *kdr* trait (Knipple *et al.*, 1994). The knockdown *kdr* trait is associated with reduced neuronal sensitivity to pyrethroid insecticides, in turn related to a mutation of the sodium-channel gating system. Gene-specific PCR markers have also been used to examine expression patterns of cytochrome P450 genes near a DDT resistance gene in *Drosophila* (Brandt *et al.*, 2002). Similarly, using RAPD markers, genetic loci have been mapped in lesser grain borer (*Rhyzopertha dominica* Fabricius) that determine high-level resistance to phosphine (Schlipalius *et al.*, 2002).

Use of AFLP markers has facilitated the identification of resistance loci in Colorado potato beetle to pyrethroid (Hawthorne, 2001) and in diamondback moth to *Bt* toxins (Heckel *et al.*, 1999). Biochemical markers have also been used successfully for molecular diagnostic purposes for screening resistance to methyl-parathion in Western corn rootworms (Zhou *et al.*, 2002) and neonicotinoid cross-resistance in *B. tabaci* (Rauch and Nauen, 2003). RAPD fingerprints were used to analyse the genetic variability in three separate strains of the stored-grain pest *R. dominica* (Schlipalius *et al.*, 2002) in Australia.

Biochemical and molecular comparison of one susceptible and three resistant *H. virescens* strains identified alterations that correlated with *Bt* toxin resistance. Following this approach, Jurat-Fuentes and Adang (2006) identified an alkaline phosphatase (HvALP) as a potential receptor and tested the utility of this protein as a marker for resistance to Cry1Ac. Comparison of brush border proteomes from susceptible and resistant larvae identified additional molecules directly involved in the toxicity process.

5.7.4 Molecular markers in biological control

Molecular identification of prey in the diets of predators can provide important information on trophic interactions that may be difficult to obtain in any other way. Monoclonal antibodies are excellent for studies that seek to identify and quantify the predators feeding on a target prey species and have thus mainly been used to study the natural enemies of single invertebrate pests. Jones *et al.* (2005) designed PCR primers and tested for identification of immature parasitoids in small grain cereal aphids and estimated the parasitism rates. They concluded that PCR is a useful tool for providing accurate estimates of parasitism rates and especially for identification of immature parasitoids to species level. Agustí *et al.* (2005) used primers to amplify fragments of the *COI* gene for species specificity to differentiate *Lydella thompsoni* and *Pseudoperichaeta nigrolineata* from field populations.

Studies on strain-specific detection of the insect-pathogenic fungus *Beauveria bassiana* in agricultural fields by use of sequence-characterized amplified region markers (OPA14 F/R(445), OPA15 F/R(441) and OPB9 F/R(677)) resulted in the detection of as little as 100 pg of *B. bassiana* strain GHA genomic DNA in the field (Castrillo *et al.*, 2004). Monitoring and evaluation of the application of *B. bassiana* was done with molecular markers in south-east China by Wang and Porter (2004). *Beauveria brongniartii* strains applied as biocontrol agents against *Melolontha melolontha* in Italy was studied with RAPD markers for monitoring of five *B. brongniartii* strains in field trials (Dolci *et al.*, 2006).

5.7.5 Markers for detection and diagnostics of pest problems

Plant diseases caused by fungal, bacterial, viral and other pathogens in agricultural and horticultural crops around the world lead to major losses. An essential precursor of the implementation of control measures is an accurate diagnosis of diseases and mapping of their geographical and temporal distribution in an area or crop. Many methods have been developed for the detection and identification of plant diseases. In the case of viruses, there are no direct methods available yet for their control and, consequently, current measures rely on indirect tactics to manage viral diseases. Hence, methods for detection and identification of viruses, in both plants and vectors, play a critical role in virus disease management.

Diagnostic techniques fall into two broad categories: (i) biological properties related to the interaction of the pathogen with its host and/or vector (e.g. symptomatology and transmission tests); and (ii) intrinsic properties of the pathogen itself (CP and nucleic acid, in the case of viruses). Detection methods based on the CP (in the case of viruses) include precipitation/agglutination tests, ELISAs and immunoblotting. Viral nucleic acid-based techniques such as dot-blot hybridization assays and PCR are more sensitive than other methods. The availability of these diagnostic methods provides greater flexibility and increased sensitivity and specificity for rapid diagnosis of virus diseases in disease surveys, epidemiological studies, plant quarantine and seed certification, and breeding programmes. A single diagnostic test or assay may provide adequate information on the identity of a pathogen, but a combination of methods is generally needed for unequivocal diagnosis. The detection of viral pathogens is another application for which microarrays have potential as a diagnostic tool. Low-density, spotted oligonucleotide arrays that define either short sequences of interest or individual mutations could be used to identify virulence markers that distinguish viral vaccine strains from wild-type isolates. Microarrays that include both generic and strain-specific probes could also be used for identifying both previously recognized strains and new or variant viruses associated with outbreaks. DNA chips have the capability to genotype viral pathogens and may be useful in determining virus transmission pathways and the source of outbreaks. Another application for arrays could include the identification of distinct subspecies of vectors and reservoirs that harbour zoonotic pathogens such as hantaviruses and various kinds of arboviruses.

5.8 Conclusion

With the advent of genetic transformation techniques based on recombinant DNA technology, it is now possible to insert genes into the plant genome that confer resistance to pests. Insect-resistant transgenic plants evolved based on ICPs from the soil bacterium *Bt* have been cultivated by farmers since 1996 in the USA and from 2002 in India. Several other pesticidal genes such as VIPs from *Bt*, trypsin inhibitors, lectins, ribosome-inactivating proteins, secondary plant metabolites, *R* genes, PR proteins and small RNA viruses are being used along or in combination with *Bt* genes in transgenic plants. Transgenic crops have ushered in an era of low insecticidal use management of lepidopteran insect species. However, suitable technology to manage sucking pests is vital. Today, the need for additional *Bt* crops that are well adapted to diverse ecological conditions has been realized and the development of many crops is in progress so that the technology gains are not curbed by a germplasm disadvantage. It is clear that molecular biology will be able to make increasing contributions to the advancement in this field. It has been shown that GM pest-resistant crops are easy to manage for pests at the farm level and could reduce the gap between attainable and actual yield, especially among smallholder farms. Various studies on MAS will also

greatly accelerate the acquisition of knowledge about the genetic control of pest resistance, with further development of tools for analysing and modifying the genome of many crop species. The use of biotechnology tools in breeding programmes will continue. Advances in biotechnology will accelerate the development of pest-resistant plants. However, transgenic plants need to be integrated into pest-management strategies. In another step, field trials are being performed in different locations and for several seasons to promote ecologically safer cultivars with durable resistance genes and, in future, biotech approaches should provide a sustainable eco-friendly component of IPM. Better consumer information is necessary to allow well-informed decisions based on comparison of the potential benefits of GM transgenic crops against complete reliance on chemical insecticides. With proper research, the integration of molecular technology, proper plant breeding, IPM and insect-resistance management strategies, better pest management can be achieved.

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6 Botanicals in Pest Management

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6.1 Introduction

Plants could not have survived in the course of their evolution without acquiring characteristics that enabled them to reproduce and defend themselves. Some of the numerous compounds they harbour are involved as chemical mediators as well as in the cellular biochemical mechanisms of resistance of the plants against pathological agents. Having observed that some plants protect themselves better than others, humans soon developed the use of plants or plant extracts as pesticides. Although these compounds offer many environmental advantages, their uses during the 20th century have been rather marginal compared with other biocontrol methods of pests and pathogens. In this chapter, we examine their biocontrol successes through examples of the most representative botanicals currently commercialized and also look at the factors that hamper this development. A better understanding of their mechanisms of action and of the risk assessments linked to their use today offers new prospects for using these substances in integrated pest management (IPM). We discussed some of these clues in regard to sustainable agriculture.

6.2 Ecological Advantages of Botanicals: a Result of Co-evolution

In a pioneering observation, Whittaker (1970) pointed out that there were chemical mediators biosynthesized by organisms that affected the behaviour or the physiology of other organisms 'for reasons other than food as such'. Studying the biochemical ecology of higher plants, he suggested that the term allelochemics underlined the point that 'ecologists may think of community metabolism in term of three major group substances – inorganic nutrient, foods and allelochemics – by which the species are in a community linked with one another and their environment.' These allelochemicals (coming from 'allelochemics') are involved in interspecific relationships (Whittaker and Feeny, 1971). They were further divided into allomones, which were advantageous to the emitter (e.g. defensive secretions), and kairomones, which were advantageous to the receiver (e.g. secretions that can be detected by a predator or parasite) (Blum, 1977). Allelochemicals were considered in the past to be secondary compounds of plants or waste compounds, because the role they played was not well understand.

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The study of allelochemicals and the interactions they mediate contributes to an understanding of the behaviour and the evolution of organisms. Through the modifications of the behaviour and physiology of other organisms that they cause, allelochemicals present interesting features not only for interspecific communication but also potentially for controlling pest populations.

In fact, plant defence allomones act in several ways. Because they are repulsive or irritating substances, they repel predators or prevent them from taking food. They give an unpleasant taste to the food (astringency, for example) to avoid food-taking. They disturb the digestive system (antifeedent, antinutritional) or neurological system (neurotoxicity), and more generally they affect the biotic potential of parasites and pests. Plant allelochemicals act on a broad diversity of species: insects, which have been the most studied because of easily identification, as well as nematodes and phytopathogenic microorganisms (fungi and bacteria) and other species of plants (allelopathy). For decades, the use of plant allelochemicals and botanicals was focused on the control of insects more than other pest organisms (Regnault-Roger *et al.*, 2008).

Beside these activities in chemical communication, they also are now known to be involved in the mechanisms of resistance of plants to pest aggression through phytoalexins. Their synthesis is induced in response to a biotic or inorganic stress (Grayer and Kokubun, 2001). Their chemical structures are highly diversified (Hammerschmidt, 1999), but most are products of plant secondary metabolism (e.g. flavonoids and terpenes) and must consequently be classified as allelochemicals (Regnault-Roger, 2005a). Among the 500,000 estimated secondary plant compounds, less than 5% of allelochemicals have been characterized (Regnault-Roger and Philogène, 2008). According to Harborne (1989) – and this classification is still relevant – they belong to three main groups: (i) phenylpropanoids and phenolics; (ii) terpenoids and steroids; and (iii) alkaloids and nitrogen compounds. Consequently, plants contain a true arsenal of allomones resulting from co-evolution of the species to defend themselves.

Very early on, humans developed the use of plants as pesticides. Historically, botanicals, readily provided by nature and agriculture, were used before other kind of pesticide. They are mentioned in Chinese, Greek and Roman antiquity. In India, the Veda, a body of holy Hinduist manuscripts dating from at least 4000 years ago, reported the use of the neem tree (*Azadirachta indica* A. Juss.; *Meliaceae*) (Philogène *et al.*, 2008). It is thus difficult to assess exactly where and when plants or plant extracts were first systematically used in plant protection or more generally in agriculture. The first botanicals and allelochemicals to be used as pesticides came from readily available products. Pest insects were targeted more than pathogens because they could easily be identified. Several recent books and chapters have reviewed biopesticides of plant origin (Weinzeirl, 1998; Koul and Dhaliwal, 2001; Thacker, 2002; Regnault-Roger, 2005b; Isman, 2006; Regnault-Roger *et al.*, 2008; Copping, 2009; Koul and Walia, 2009).

Because they are the result of co-evolution, botanicals have developed interesting ecological properties that have been noted. In fact, they have many environmental advantages that improve their use in IPM (Regnault-Roger, 2005c). Because they possess selectivity and specificity in their effects on the target species, their effects on beneficial insects and other non-target species are limited. Because they are biosynthesized, they are enzymatically biodegradable, generally having a short half-life, and thus persistence is avoided. As the part of the complex secondary metabolism of plants, their properties result from the interactions of numerous compounds. The association of several compounds can be synergistic, decreasing the effective amount of active ingredient required. Because they belong to several different chemical families, they

contribute to the diversification of the biochemical and molecular targets towards pests. By increasing the choice of the molecules available for IPM, they limit or delay the resistance phenomenon.

Regarding all these ecological advantages, botanicals and plant allomones have to be taken into consideration for plant protection as biocontrol agents for sustainable agriculture. They have great potential against bioagressors and in the last few decades they have been considered for their insecticidal properties.

6.3 The Range of Active Compounds and Commercialized Botanicals

A wide range of active compounds exists but only a few botanicals have been commercialized. The search for botanicals for use as insecticides resulted historically from two kinds of method: (i) the observation of the traditional uses of plants and extracts for cattle and crop protection, followed by checking the efficiency of these practices and identification of the active molecules; and (ii) a systematic screening of families of plants collected during prospecting campaigns, followed by biological tests to discover the active molecules. Before the Second World War, four main groups of compound were in fact commonly used: (i) nicotine and alkaloids; (ii) rotenone and rotenoids; (iii) pyrethrum and pyrethrins; and (iv) vegetable oils. These are considered to be the first generation of biopesticides of plant origin. However, the use of these substances, because of their toxicity to non-targeted species (e.g. the use of nicotine) or the instability of the molecules (e.g. pyrethrum), decreased with the increasingly commercialization of chemically synthesized insecticides that were easier to handle and less expensive during and after the Second World War. However, the success of organochlorides, organophosphates and carbamates induced such a massive and inappropriate use that the negative effects on non-target species and environmental risks renewed interest in botanicals. Research on biopesticides of plant origin was pursued in order to improve the stability of biochemicals such as pyrethrum or to discover new molecules and new sources of molecules. The development of pyrethrinoids (synthesized molecules derived from pyrethrum) and the rediscovery of neem, extracted from *A. indica* L. (Schmutterer, 1990) illustrate these approaches. The search for other sources of plant biopesticide from plants of North America and from tropical or wood species from Central America (Philogène and Arnason, 1992; Arnason *et al.*, 2008), as well as from aromatic Mediterranean plants (e.g. thyme, rosemary and citronella) (Regnault-Roger, 2008), together with common plants such as garlic and onion classified in the genus *Allium* (Auger *et al.*, 2008), increased in the 1980s and 1990s. The insecticidal properties of compounds such as hydroxamic acids and monoterpenes such as carvacrol, linalool and eugenol, as well as glucosinolates, polyphenols and flavonoids, were highlighted. These belong to chemical families described previously by Harborne (1989). Most of these compounds were well known for other properties but, because of the focus on their pesticide activities, they are considered the second generation of biopesticides of plant origin. Nowadays, numerous works are devoted to discovering the pesticide properties of botanicals or plant allelochemicals extracted from traditional flora in several developing countries from all continents, including Asia, Africa and South America. Discovering new molecules may not only provide an economical development for developing countries but also contributes to diversifying the wide range of useful molecules for IPM.

The identification of useful properties does not necessarily mean that the new plant extract or compound will succeed in commercialization of new biopesticides of plant origin. Because plant phytochemical profiles are not constant, experiments must take into consideration numerous factors according to the standardization of

protocols following scientific criteria (relevance, reliability and reproducibility of results, i.e. controls, replicates, statistical analyses, etc.), the variability resulting from the extraction method (e.g. solvents, pH and temperature) and environmental or physiological factors (e.g. climate and soil conditions, stage of plant growth, the intensity of plant metabolism, and chemotypes). The spectrum and the specificity of activities of the compounds as well as potential synergies between molecules, together with studies of the mechanism of action (if there is enough time and money) have to be determined between biology and chemistry laboratories. After these various steps, the industrial research and development stage follows. This includes tests under operating conditions, toxicological and ecotoxicological considerations, the choice of a commercial strategy, market profitability, etc. However promising the biopesticide molecules may be, the commercialization of a new plant insecticide is not yet achieved. At this stage, scientists do not manage the project alone – there is also input from industrials and commercials.

In fact, during the whole of the 20th century, only a limited number of botanicals or plant allelochemicals were used for crop protection. Isman (2006) found that four substances are mainly used: pyrethrum, rotenone, neem and essential oils, followed by nicotine, ryania and sabadilla for minor uses. Thus, the question is: which factors impede the commercial development of botanicals?

Some of the impeding factors that limit the development of the botanicals on the market are linked to economical and commercial considerations. The availability of the raw material and its accessibility, standardization and refinement of plant commercial products and the regulation rules in several countries are some of the parameters of note (Isman, 1997b, 2008; Regnault-Roger, 2005d; Regnault-Roger and Philogène, 2008). Others take into consideration the risk assessment for the use of botanicals in IPM. Today, the use of plant-protection products requires strict regulations for food and feed and the need to be environmentally friendly. Although they are natural, botanicals are not all necessarily safe for people and for the environment. However, the current claims that natural plant-protecting products should not pose unreasonable risks to people or to the environment means that the evaluation of these compounds meets today's most stringent standards of scientific knowledge. The case of some commercialized botanicals is given below to illustrate this situation.

6.4 Features of Some Current Commercialized Botanicals

6.4.1 Nicotine

Nicotine was one of the first molecules used as an insecticide, with the first mention of the use of aqueous extracts of tobacco against the sucking/piercing insects of cereals in 1690. However, the active molecule of this plant, nicotine, was isolated only in 1828 and synthesized in 1904 (Matsumura, 1985; Ware, 2000). This very stable alkaloid in its levogyre form is neurotoxic for insects, mammals and birds. It is an acetylcholine mimic, binding to post-synaptic receptors and interfering with the transmission of signals in nerves. This causes stimulation followed by depression of muscles and the central nervous system. Nicotine is acutely toxic (Toxicity category I) by all routes of exposure (oral, dermal and inhalation). The 50% lethal dose (LD_{50}) of nicotine is 50 mg/kg for rats and 3 mg/kg for mice. A dose of 40–60 mg can be a lethal dosage for adult humans through paralysis of respiratory muscles, and doses as low as 1–4 mg can be associated with toxic effects in some individuals. Nicotine is neither an initiator nor a promoter of tumours in rodents but it is toxic for birds. Some countries such as China and Bolivia use nicotine to protect rice cultivation (by immersing the stems of tobacco in the plantations) and potato fields (spraying) (Thacker, 2002). In the USA, since 21 May 2008, because of risks (i) for applicators

both during and after application, (ii) for consumers of plants from treated greenhouses and (iii) for people who might be exposed to nicotine residues in treated greenhouses, the sole remaining nicotine registration is a restricted pesticide use only in greenhouses for ornamentals against adult whiteflies, aphids and thrips (EPA, 2008). In the European Union (EU), the Commission decided to not include nicotine in Annex I of Directive 91.414/EEC, because it was not demonstrated to be a safe use with respect to operators, workers, bystanders and consumers. Consequently, plant-protection products containing nicotine are withdrawn but the uses of those plant-protection products, according to the existence of stocks, remain available for farmers for 18 months after the adoption of the decision in December 2008 (OJEU, 2009).

6.4.2 Sabadilla

Sabadilla is a mixture of alkaloids extracted from an endemic plant of the Balkans, *Veratrum album* L., and also from a Venezuelan *Liliaceae*, *Schoenocaulon officinale* (Schlect. & Champ.), also known as Indian caustic barley or sabadilla. This mixture of alkaloids includes several compounds (veratridine, cevadine, sabadine, sabadinine and sabadilline) and was called veratrine. Sabadilla possesses, like pyrethrins, a neurotoxic activity by slowing the shutting of Na^+ channels and disturbing membrane depolarization. They cause paralysis before death. They are contact and non-systemic insecticides. As they break down quickly in air or sun (inactive in less than 24 h), they are not very toxic for mammals. According to the US Environmental Protection Agency or EPA (EPA, 2004), the LD_{50} is greater than 5000 mg/kg for rats and no death occurs via dermal and inhalation routes (Toxicity categories III and IV). Available and estimated data on toxicity and exposure indicate that sabadilla alkaloids present minimal risks to small mammals on an acute basis. Because sabadilla is used on an annual basis with only a small volume of end-use product and on a limited number of minor crops in a limited geographical area, it is not considered to exhibit an ecological risk for the endangered species or non-target species (e.g. birds, fish and green algae, aquatic invertebrates) at the typical application rates. It controls grasshoppers, lice of cattle and house flies. Currently, these compounds are only used marginally by farmers in developing countries who have been using them for a long time, or before 1997 in the USA (especially in California), in organic farming against thrips on citrus, avocados and mangos. Because sabadilla was considered to be a lower risk pesticide, the exemption from the requirement of tolerance was reassessed in the USA in 1994 under the Reregistration Eligibility Decision (EPA, 2004), but since February 1996, the registration status has been cancelled on the request of the company Woodstream Corporation (Kegley *et al*., 2010). Nowadays, the actual procedure of regulation for plant-protection products requires more data than previously and there is lack of additional data for sabadilla.

6.4.3 Ryania

Ryania is an extract from another member of the *Liliaceae*, *Ryania speciosa* Vahl, from the Amazonian basin with two alkaloids extracted from the ground stems, ryanodine and its derivative, dehydroryanodine. Ryania is a complex mixture of many compounds and no single structure represents it. It was originally used as a rodenticide, and the insecticidal properties of the compounds were only highlighted after the Rutgers University–Merck prospecting campaign in the 1940s. In the early 1950s, at its maximum use, approximately 2,000,000 kg of active ingredient of 40% ryania was used each year. By contrast, only about 1000 kg of ryania was used per year in the 1990s. Ryania interferes with nerve impulses at the Ca^{2+}-channel level and generates a sustained contraction of the muscle and paralysis. The toxicity of ryania extracts towards mammals varies according to the sensitivity of the species.

Toxicity of powdered stems of *R. speciosa* was evaluated with an LD_{50} of 1200 mg/kg in rats, 150 mg/kg in dogs, greater than 400 mg/kg in monkeys, 650 mg/kg in rabbits, 650 mg/kg in mice and 2500 mg/kg in guinea pigs. Despite the fact that its mode of action is different from phosphate/carbamate, because of its neurotoxicity, ryania causes similar clinical signs to those of organophosphorous insecticides with asthenia, slowing of the respiratory rate, nausea, vomiting and diarrhoea, potentially worsened by tremors, convulsions and coma, leading to death in cases of ingestion of a lethal dose. Eye and dermal tests show no to moderate irritation and no skin sensitization. The lack of persistence of ryania or ryanodine in the environment means that residues do not appear in the food chain or in the human diet. Ryania is highly toxic to the fruit moth, coddling moth (*Cydia pomonella*), corn earworm, European corn borer (*Ostrinia nubilalis*) and citrus thrips but is ineffective against the cabbage maggot, cauliflower worm and boll weevil. Its insecticide use has thus been limited to susceptible species, such as *C. pomonella*, *O. nubilalis* and citrus thrips (Weinzeirl, 1998), and against pests of maize, apple and pear crops. All uses in the USA were cancelled voluntarily in 1997 (EPA, 1999). In the EU, ryania is not included in Annex I of Directive 91/414/CE.

6.4.4 Quassin

The exotic trees *Cassia amara* L. from Surinam and *Picrasma excelsa* Swz. from Jamaica provide quassin, an alkaloid that includes two dextrogyre isomers: quassin ($C_{22}H_{28}O_6$) and neoquassin. It is effective against plant lice and several species of Lepidoptera. It was cultivated intensively in the 1940s in Venezuela for export to Europe and North America (up to 500 t). Insecticidal formulations were traditionally prepared by maceration of trunks and branches, followed by concentration by boiling the aqueous extracts for an unspecified period of time. The efficiency of the commercialized product varied widely and this affected the commercialization of quassin. It was easily supplanted by synthesized chemical insecticides. Recently, quassin has been assessed in trials in Australia to control pests of *Brassicae*, and some Indian farmers still use extracts from *P. excelsa* to protect native cultures (Thacker, 2002). There has been a renewed interest in quassin for the control of apple sawfly (*Hoplocampa testudinea* Klug) (Kienzle *et al.*, 2006). However, like other botanical insecticides that were used in North America in the 1950s, it lost its regulatory status as an approved product (Isman, 1997a) but is registered for use in Australia (Kegley *et al.*, 2010). In the EU, the Commission decided to not include quassin in Annex I of Directive 91/414/EEC because the notifiers voluntarily withdrew their support for an inclusion. However, as this non-inclusion was not 'based on the presence of clear indication of harmful effect', the decision did not prejudice the submission of a new application (OJEU, 2008a).

6.4.5 Pyrethrum

Pyrethrum is a powder obtained by crushing the dried flowers of daisies belonging to the family of *Asteraceae*: *Chrysanthemum* spp., *Pyrethrum* spp. and *Tanacetum* spp. *Chrysanthemum cinerariaefolium* Benth & Hook was first used in Europe in the 1800s against lice and flies (Regnault-Roger and Philogène, 2008). Other species of *Chrysanthemum* such as *Chrysanthemum roseum*, *Chrysanthemum tamrutense* and *Chrysanthemum carneum* also contain significant amounts of pyrethrum. Pyrethrum or pyrethrins are a mixture of six esters: (i) pyrethrins I and II (the most abundant); (ii) cinerin I and II; and (iii) jasmoline I and II. Pyrethrin I is the most toxic ester. It alters nerve transmission by slowing the shutting of Na^+ channels during the recovery phase of neuronal action potentials. The insect consequently presents hyperactivity followed by convulsions. Pyrethrins are very toxic and act very quickly on insects. However, they have low to moderate

toxicity towards mammals (oral LD_{50} is 1400 mg/kg for rats, dermal LD_{50} is greater than 2000 mg/kg for rabbit, inhalation LC_{50} is 3.4 mg/l for rats), are a moderate eye irritant and mild dermal irritant and do not cause skin sensitization. Massive intoxication by pyrethrum cause a tremor followed by convulsions, and nervous system lesions are observed in rats and mice following an acute exposure. A thyroid effect is observed following chronic exposure in rats and dogs, as well as liver effects in rats, dogs and mice following a short or long exposure. Pyrethrum is quickly hydrolysed in the digestive tract, although it is more toxic by inhalation or if administered intravenously. Pyrethrins are classified as having 'suggestive evidence of carcinogenicity' because of the occurrence of benign tumours in female rats. However, there is insufficient evidence to assess their human carcinogenic potential. Toxicity is noted for non-targeted species, especially fish, invertebrate and bees. However, its instability in light, air and moisture considerably reduces risks related to its use. Despite its high production cost, it is a natural insecticide that is currently widely used (1000 t of pyrethrum are sold every year, with about 90% being used on non-agricultural sites in the USA) (EPA, 2006). It is recommended for the control of flying and crawling insects and arthropods and mites on fruits, field crops, ornamentals, greenhouse crops and house plants, as well as on stored products and domestic and farm animals. It is normally applied in combination with piperonyl butoxide, a synergist that inhibits detoxification (Copping, 2009). Because pyrethrum is registered for use in agricultural, residential, commercial, industrial and public health sites in the USA, several scenarios of use to evaluate risks for human health (e.g. dietary risk, residential) and non-target organisms (e.g. the ecological risk for aquatic and terrestrial organisms) have been tested. The EPA (2006) concluded that the 'currently registered uses of pyrethrins are eligible for reregistration provided mitigation measures … are implemented through label amendments'. These mitigations focus on: (i) the restriction for using the end product in specific places (e.g. nursing homes, hospitals and schools); (ii) the method of application of the end product and the protection equipment required for applicators; (iii) the number of applications for agricultural use in relation to the season; and (iv) the pest pressure. This example demonstrates that the most popular botanicals must be used cautiously. In the EU, pyrethrins were included in Annex I of Council Directive 91/414/EEC in December 2008. It came into force in September 2009 up until August 2019 for use as an insecticide only (OJEU, 2008b).

6.4.6 Rotenone

Rotenone is widespread in the *Fabaceae* (ex. *Papilionaceae*) growing in Asia (*Derris* spp.) and in America (*Lonchocarpus* spp.). Rotenone is one of the oldest insecticides used all over the world. The use of crushed roots of *Fabaceae* to catch freshwater fish by native populations of South America was mentioned as early as 1665, while it was reported that these extracts were added to insecticidal soaps in 1848 (McEwen and Stephenson, 1976). The active ingredient, belonging to the flavonoids, was isolated by Emmanuel Geoffroy in 1895 from *Lonchocarpus nicou*, an American member of the *Fabaceae*, and was called 'nicouline'. Similar work was carried out in 1902, with the roots of *Derris elliptica* (roten in Japanese) by Kazuo Nagai who called the compound rotenone, and this name prevailed (Dajoz, 1969).

Rotenone inhibits cellular respiration and energy metabolism at the level of the mitochondrial respiratory chain. Although harmless for warm-blooded animals, it is very active against cold-blooded animals such as amphibians, fish and reptiles. Although some accidents were reported with enzymatic inhibition, rotenone was regarded for a long time as being moderately toxic for mammals. Although its acute dermal LD_{50} in rabbits was over 5000 mg/kg and no skin sensitization was noted, its acute oral LD_{50} in rats was 39.5 mg/kg for

females and 102 mg/kg for males. The acute inhalation LC_{50} in rats was 21.2 μg/kg. These latter figures resulted in rotenone being classified in the highest category of toxicity (EPA, 2007). Cases of chronic toxicity leading to kidney and liver damage were noted, and it was also found to be carcinogenic for rodents (Weinzeirl, 1998). More recently, a link between rotenone and Parkinson's disease was hypothesized (Betarbet *et al.*, 2000). Rotenone persists for 3–5 days on foliage after application and is easily biodegradable. Its half-life in warm and cold water is 1.5 and 20 days, respectively. Rotenone used alone is non-toxic for bees but is lethal in combination with pyrethrum (Copping, 2009).

This compound was used until the 1940s, but, as with many other insecticides extracted from plants, its use declined at the end of the Second World War (Philogène *et al.*, 2008). Until recently, rotenone was used in organic agriculture alone or associated with other ingredients such as pyrethrinoids, synergists (piperonyl butoxide), sulfur or copper to control a wide range of arthropod pests including aphids, thrips, moths, beetles and spider mites (ACTA, 2008). However, following the regulatory update 46/2007 within the frame of Directive 91/414/EEC and EC Decision 2008/317/EC published on 10 April 2008, because of a lack of required information, it was decided that rotenone should not be included in Annex I to Directive 91/414/EEC and consequently it was withdrawn from the EU plant-protection products market on 10 October 2009 (OJEU, 2008c). Nevertheless, rotenone has been granted essential use in the UK, Italy and France until 2011 on fruit trees, ornamentals and potatoes only. This derogation is limited to professional users with appropriate protective equipment. The uses of rotenone were also restricted in the USA for livestock, residential and homeowner use, domestic pet uses and all other uses except for piscicide uses. Consequently, rotenone is now registered to be applied directly to water to manage fish populations in lakes, ponds, reservoirs, rivers, streams and in aquaculture, to eliminate completely or partially undesired fish species in the treatment area (EPA, 2007). The risk assessment was evaluated according to human health, the occupational risks (workers), non-target aquatic (fresh water fish other than the target species, invertebrates) and terrestrial (piscivorous birds, wild mammals, plants, bees) species. It is classified by the EPA (2007) as a Restricted Use Pesticide (due to acute inhalation, acute oral and aquatic toxicity).

6.4.7 Neem

Neem is extracted from *A. indica* A. Juss, which is native to arid regions of India. The ability of the oil to repel pests has been known for thousands of years. The oil has also been used on skin and medicinally. Neem is a part of the traditional practices in India. It is a mixture of more than 100 limonoid compounds including azadirachtin, salannin and nimbin and their analogues. All these compounds act differently, and numerous effects of neem on insects have been reported. Salannin causes repellence and feeding deterrence, while azadirachtins are the only compounds that have a significant activity as inhibitors of insect growth (Schmutterer, 1990). This results from an inhibition of the synthesis of ecdysteroids with, as a consequence, a disruption of moults and of the reproductive cycle of the insect. Neem oil was classified by the EPA (1995) in Category IV (practically non-toxic) because of its acute oral LD_{50} on rat of greater than 5000 mgs/kg (no mammal toxicity) and all routes of exposure were classified as being the same. It has a mild (minimal) effect on skin sensitization and eye irritation but is not cytotoxic or mutagenic according the Ames test. However, Kleter *et al.* (2006) reported that, according to Boeke *et al.* (2004), some unknown hazards with new extraction methods could produce toxic effects of neem extract in mice and guinea pigs, with gastrointestinal spasm, hypothermia and death with 200–400 mg/kg of leaf extract. Neem and azadirachtin were recently suspected to be endocrine disruptors

(Falconer *et al.*, 2006; PAN, 2010) although there is a contradictory claim (Pfau *et al.*, 2009).

In relation to its environmental impact, neem is sensitive to light and degrades in water (Isman, 1997b). Consequently, it has limited persistence in the environment. The half-life of azadirachtin A after spraying on leaves of tomato or potato was 1 day (Kleeberg, 2006). A study on six aquatic organisms (including crayfish, shrimps, mosquitoe larvae and water fleas) concluded that the risk values of azadirachtin and neem-based insecticides (e.g. Neemix and Bioneem) did not exceed the criteria, and consequently no ecological hazard was likely to result from their use (Goktepe *et al.*, 2004) or from their application to aquatic macroinvertebrates (Kreutzweiser, 1997). Azadirachtin acts on a wide range of insects, such as balsam fir sawfly (*Neodiprion abietis* Harris), thrips, leaf miners, aphids, caterpillars and pine false webworms. It deters certain insects, such as locusts, from feeding, and interferes with the normal life cycle of insects, including feeding, moulting, mating and egg laying. It was tested on over 300 species and 90% were found to be susceptible, with a large variability in LD_{50} (Philogène *et al.*, 2008). Recommended by the National Research Council as a 'tree for solving global problems' (NRC, 1992), neem is considered by many experts to be a superior biocontrol agent (Brahmachari, 2004; Kleeberg, 2008). Despite such qualities, the development of this insecticide has been hampered by: (i) cultivating the plant on a commercial scale; (ii) extraction of the active ingredients; and (iii) development of persistent formulations and shelflife (Philogène *et al.*, 2005). According to Kleeberg and Ruch (2006), the standardization of neem seeds extracts, which show a large variation in azadirachtin content, is one of the key factors for enhancing the commercialization of neem products. Neem and azadirachtin are currently registered in several countries. In the USA, azadirachtin and extract of neem oil were registered by the EPA between 1985 and 1995 (EPA, 2001). However, in the EU in 2008 like quassin, azadirachtin was not included in Annex I of Directive 91/414/EEC for a similar reason (because the notifiers voluntarily withdrew their support for an inclusion). But in April 2011, in amending Commission Decision 2008/941/EC it was decided to include azadirachtin as active substance in the Annex 1 (OJEU, 2011).

6.5 Suggestions for Uses of Botanicals in IPM and Sustainable Agriculture

It is apparent from these examples of the main botanicals that have been available on the market over the last several decades that the situation is complex and that only a few compounds used in insecticide formulations really appear to have a future as biocontrol agents.

Biotechnological developments could provide future use of allomones and botanicals in plant protection. The use of molecular biology has improved our knowledge of the mechanisms of resistance of plants to the attacks of bioaggressors and of the role of allelochemicals, opening up the prospects of using allelochemicals in two main approaches: (i) by improving plant resistance by increasing the level of constitutive allelochemicals of interest in plant varieties or cultivars; and (ii) by stimulation of plant-induced resistance. These approaches, which aim to reduce the use of synthetic pesticides in agriculture, include not only plant allelochemicals and botanicals but also other microbial, fungal, mineral or organic molecules.

Considering the first approach, one of the basics of modern agronomy is the selection of characteristics of interest to improve the quality of crop plants. Selection of varieties, which was carried out by traditional methods, now uses biotechnology tools such as marker-assisted selection (MAS). MAS is a powerful tool to help breeders identify resistance genes to specific diseases by identification of the genomic regions contributing to pathogen resistance by characterization of quantitative trait loci. It was observed that

there existed, in certain cases, a direct relationship between the degree of tolerance of a plant to a disease and particular allelochemicals. Positive correlations between the content of allelochemicals and resistance to pathogens were highlighted. As an example, Bily *et al.* (2003) noted that a variety of maize that was resistant to the ear rot disease caused by *Fusarium graminearum* had higher concentrations of diferulate than a susceptible variety. Subsequent research has aimed to establish whether it is possible to build a hierarchy from diferulate levels between the various varieties of maize according to ear rot disease resistance. The aim is contribute to the development of maize varieties resistant to *F. graminearum*.

The second approach involves the enhancement of induced resistance. This technology has been developed for over 15 years. This innovating approach enhances plant resistance to pathogen infection by treatment with a variety of biotic and abiotic inducers, also called elicitors. These agents could be virulent or avirulent pathogens, non-pathogens, cell-wall fragments, plant extracts or synthetic chemicals. They can lead to the induction of resistance to subsequent pathogen attack, both locally and systemically. The activation of defence responses includes an oxidative burst, which can lead to cell death, trapping the pathogen in dead cells, or changes in cell-wall composition that can inhibit the pathogen (Walters *et al.*, 2007). Plant allelochemicals, especially polyphenols, are strongly implicated in these mechanisms (El Modafar *et al.*, 2008). The elicitors currently identified are mainly of microbial origin, but some plant extracts and allelochemicals also induce the natural defence of plants. Extracts from *Hedera helix* L., *Salix alba* L., *Viscum album* L., *Alchemilla vulgaris* L. and *Reynoutria sacchalinensis* (F. Schmidt) were identified as inducers of resistance against fire blight of apple and of *Cotoneaster watereri* (Zeller, 2006). *R. sacchalinensis* induced phenolic phytoalexines. Marketed under the name of Milsana (KHH Bioscience), it is used particularly in North America for the protection of ornamental plants such as roses and begonias, and also against various *Oidium* spp. of vegetables and fruit (Konstantinidou-Doltsinis *et al.*, 2006). *Macleaya cordata* extract, registered under the name of the fungicide Qwel (Camas Technologies Inc.), induces increased amounts of polyphenolic phytoalexines, as well as systemic acquired resistance (Copping, 2009). The extract laminarin, a polymer of β-glucan 1.3 and 1.6 purified from the brown algae *Laminaria* sp., was included in Annex I of Directive 91/414/EEC. Recently, a renewal of the authorization for the use of sea algae extracts was given from September 2009 until August 2019 with the inclusion in Annex I of Directive 91/414/CE but for use as a plant growth regulator only (OJEU, 2008b). Another plant extract, *Trigonella foenum-graecum* L., marketed under the name of Stifénia, was recently approved in France against the vine powdery mildew (Pajot and Regnault-Roger, 2008). Plant inducers act on a very broad spectrum of plant species, as well as on fungal and viral pathogens, although their efficacy can be cultivar dependent. In the same context, studies have highlighted that the physiological stage of the treated plants plays a significant role in the expression of the stimulation of plant defence, for example Stifénia, whose use is recommended before flowering. To be efficient, elicitors must be used at a receptive physiological stage of the plant. The limit of this technology is probably the incomplete control of disease (20–85%) or non-significant results under field conditions because the expression of induced resistance is influenced by environmental conditions, genotype and crop nutrition. An important challenge will be to convince farmers and growers that stimulation of natural plant defences will provide a useful and practical approach to be used in association with fungicides to decrease the frequency and amount of chemical treatment (Walters *et al.*, 2005). This will enhance sustainable development.

6.6 Conclusion

During the 20th century, botanicals succeeded in diversifying approaches to plant protection. However, they faced two main factors that hampered progress: (i) more stringent standards for risk assessment according to the claims that plant-protection products should not pose unreasonable risks to people or the environment; and (ii) an economic challenge because alternative approaches do not control pests as perfectly as chemical pesticides and are also more expensive. In this context, the use of botanicals in insecticide formulations is questionable. The key for their future in IPM probably lies in their lack of persistence because of their biodegradability, and also in more specific modes of action of some of them that improve selectivity to target species (e.g. insect growth regulators) and decrease the risks of non-intentional effects. Because they diversify the active ingredients for plant protection, they also decrease the risk of insect resistance as long as they are used in rational agricultural practices. The technologies involving plant extracts or allelochemicals to enhance plant resistance to bioaggressors by elicitation, or the selection of improved resistant varieties, are probably the most promising approaches, because of the lack of associated significant risks for human health and the environment. The renewal of agriculture for the sake of sustainable development requires that pollutant practices are reduced. Among alternative approaches for plant protection, botanicals and plant allelochemicals still present qualities as potentially significant tools for IPM.

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7 Biopesticides in Ecologically-based Integrated Pest Management

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7.1 Introduction

India ranks second worldwide in farming output. Agriculture and allied sectors such as forestry play a significant role in the overall socio-economic development and livelihoods of 120 million families in India. This is set against the background of a world that will be demanding 50% more food, 30% more water and 50% more energy by 2030 and an even more challenging scenario for 2050 by which time food production will need to have doubled in a sustainable way in order to feed the world's growing population, which is set to rise to 9 billion. In contrast, the indiscriminate use of pesticides and fertilizers has had an adverse impact on ecological balance. Integrated pest management (IPM) was developed in 1970 as a response to the negative side effects of using pesticides. Pests were becoming resistant to chemical treatments, and the health of farmers and consumers was in danger. These hazards have been far greater in developing countries, and current evidence suggests that the situation has become even more volatile.

Sustainable agriculture should combine the wisdom of traditional and natural farming practices with modern technologies. Traditional agricultural systems are the products of centuries of accumulated experience. Farmers all over the world have developed their own systems of farming within the framework of local opportunities and ecological limitations, and within the socio-economic and political structure of their own countries, exemplified by efficiency and the careful management of soil, water, nutrients and biological resources. Inter-cropping, agro-forestry, shifting cultivation and other traditional farming practices are all based on a thorough understanding of the elements and interactions between vegetation and soil, animals and climate. Strengthening such systems through village-based initiatives and actively involving local peasants is the key to successful sustainable agriculture and rural development programmes. Increasing quantities of chemicals are used for agricultural intensification to feed an ever-growing population. However, pest-induced loss is on the rise, despite the increasing usage of pesticides. Fortunately, a realization of the negative effects of these chemicals on nature and on natural resources, such as pollution, pesticide residues and pesticide

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resistance, have forced us to shift our focus on to more reliable, sustainable and environmentally friendly agents of pest control, the biopesticides. In spite of the claimed efficacy, however, their use has remained low due to a number of socio-economic, technological and institutional constraints. None the less, a rise in income levels due to a growing economy coupled with an increasing awareness of the health-related effects of chemical pesticides has increased the demand for organic food. In view of this demand and government efforts to mitigate climate change, biopesticides are likely to play an important role in future pest-management programmes.

IPM is essential for sustainability, as insect pests, pathogenic microorganisms and weeds pose a considerable quantitative as well as qualitative threat to yields of agricultural produce. Development of effective means for managing pests is essential for social and economic development of agriculture. Production systems that do not include effective pest regulation cannot sustain long-term profitability. Reliance on a single control strategy, particularly chemical control, has led to increasing difficulties in controlling these pests over the last 50 years and has resulted in a number of direct and indirect consequences to the agriculture production system. Rachel Carson was the first to voice these consequences in her classic book entitled *Silent Spring* (Carson, 1962). Since 1962, it has become increasingly apparent that applying chemical control unilaterally will not provide safe and effective regulation over the long term (Cuperus *et al.*, 1990; Zettler and Cuperus, 1990; Read *et al.*, 1993). Problems ranging from resistance in target organisms to environmental degradation and contamination of food products by pesticide residues have proven that using a single tactic seriously detracts from sustainability. Therefore, the goal is the deployment of ecologically-based IPM practices (Kennedy and Shutton, 2000), the call for which was made in 1996 (Overton, 1996) along with biointensive IPM (Benbrook *et al.*, 1996).

Like virtually all other organisms, arthropods are susceptible to microbial infections. These disease-causing microbes may cause significant mortality, which can, under appropriate conditions, substantially control and even decimate arthropod populations and may do so before host populations reach economic threshold levels (Boucias *et al.*, 2000). Biopesticides are a vital component in sustainable agriculture. Biopesticides have been defined as 'certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals' (http://www.epa.gov/opp00001/about/types.htm). The US Environmental Protection Agency (EPA) separates biopesticides into three major classes based on the type of active ingredient used, namely microbial, biochemical or plant-incorporated protectant. For example, garlic, mint, neem, papaya and baking soda, as well as entomopathogenic agents such as fungi, bacteria, viruses, pheromones and plant derivatives, all have pesticidal applications and are considered biopesticides. According to the EPA, at the end of 1998 there were approximately 175 registered biopesticide active ingredients and 700 products. The most commonly used biopesticides are living organisms (bacteria, viruses and fungi) that are pathogenic for the pest of interest. These include biofungicides, bioherbicides and bioinsecticides. In this chapter, the emphasis has been laid on an understanding of entomopathogenic agents, or bioinsecticides.

7.2 The Advantages of Using Biopesticides

There are a number of advantages of biopesticides compared with chemical pest-control measures:

1. Biopesticides are often effective in very small quantities, thereby offering lower exposure.
2. Biopesticides decompose quickly, leaving virtually no harmful residue and allowing field re-entry almost immediately after application, thus avoiding the kind of environmental problems associated with chemical pesticides.

3. Biopesticides generally target a specific pest or a small number of related pests, in contrast to broad-spectrum chemical pesticides, which may affect other beneficial insects, birds and mammals in addition to the pest.
4. When used in IPM programmes, biopesticides can greatly reduce the use of conventional pesticides, while the crop yield remains high.

At present, the demand for biocontrol as a viable commercial option at different junctions of sustainable agriculture (Butt *et al.*, 2001) occurs only: (i) where conventional chemical control does not give sufficient control or where there is a case of insecticide resistance; (ii) where conventional chemicals are too expensive; or (iii) where the government restricts the application of chemicals. This may be because of a lack of awareness as far as the hazards of chemical pesticides are concerned or a general lack of understanding of the efficacy of biocontrol methods. To overcome this, biological pest-control methods must be given a high priority, and people in general – and agriculturists in particular – must be educated about the dangers posed by handling and the use of chemical pesticides. The general public should also demand farm products where chemical pesticides are not used. All this will lead to a general enlightenment about the benefits of biopesticides and will force governments to make policy decisions reducing the use of chemical pesticides and increasing the use of a greener alternative.

7.3 Biopesticide Production

7.3.1 The global scenario

There has been an increasing trend in the global pesticide market, which increased from approximately US$40 billion in 2008 to US$43 billion in 2009. It is further expected to increase at a compound annual growth rate (CAGR) of 3.6%, reaching a level of US$51 billion in 2014. Interestingly biopesticides have shown a very encouraging trend and are expected to increase at 15.6% CAGR from US$1.6 billion in 2009 to US$3.3 billion in 2014. The greatest share of the market is occupied by synthetic pesticides, which are valued at US$41 billion in 2009 and are expected to reach an estimated value of $48 billion in 2014 at 3% CAGR.

7.3.2 India

In 2005, biopesticides represented only 2.89% of the overall pesticide market in India, with the expectation that this would increase at an annual growth rate of about 2.3% in the coming years (Thakore, 2006). In India, to date only 12 types of biopesticide have been registered under the Insecticides Act, 1968 (http://www.ncipm.org.in/biopesticides-registered.htm). Neem-based pesticides and those based on *Bacillus thuringensis* (*Bt*)-transgenic organisms, nuclear polyhedrosis virus (NPV) and *Trichoderma* spp. are the major biopesticides produced and used in India (http://coe.mse.ac.in/taxproj.asp). In contrast, more than 190 synthetic agents are registered for use as chemical pesticides. Most biopesticides are used in public health, except for a few that are used in agriculture. In addition, transgenic plants and beneficial organisms (bioagents) are used for pest management in India.

The consumption of biopesticides has increased from 219 t in 1996–1997 to 683 t in 2000–2001, and about 85% of biopesticides used are neem-based products (Table 7.1). In contrast, the use of chemical pesticides has fallen significantly from 56,114 million t to 43,584 million t during the same period.

7.4 Types of Biopesticide

Biopesticides can be divided into three classes.

Table 7.1. Annual use of biopesticides in India.

Biopesticide/bioagent	Quantity per annum (approx.)
Neem 300 ppm	1 million l
Neem 1500 ppm	250,000 l
Bt	50,000 kg
NPV (liquid)	500,000 l
Beauveria	Minimal
Pheromone traps	500,000 (number)
Lures	2 million
Trichogramma	1 million
Chrysoperla and other biocontrol insects	Minimal
Trichoderma	500 t

Source: Kalra and Khanuja (2007).

7.4.1 Plant-incorporated protectants

Plant-incorporated protectants refer to substances that are produced from genetic material that has been added to a plant. For example, scientists have taken the gene for the pesticidal toxin protein of *Bt* and introduced it into the plant's own genetic material, resulting in *Bt*-transgenic plants which then themselves manufacture the substance that destroys the pest.

7.4.2 Biochemical pesticides

Biochemical pesticides are substances that occur naturally but can control pests by non-toxic mechanisms. They include insect sex pheromomes, which interfere with mating as well as attracting the insects.

7.4.3 Microbial pesticides

Microbial pesticides contain a micro-organism (e.g. a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides are probably the most widely used biopesticide and are cheaper than other methods of pest bioregulation (Szewczyk *et al.*, 2006). The microbes currently marked as insecticides are very effective against their target pests and are virtually non-toxic to other living organisms except the target pest. In other words, they are highly selective in their nature. Besides their selectivity, they also have a much reduced potential for environmental contamination compared with conventional pesticides as they are much more biologically fragile and are therefore more biodegradable. Moreover, the use of microbials is far less likely to produce pest resistance or resurgence. The rest of this chapter will look at microbial pesticides in more detail.

7.5 Entomopathogenic Fungi

Worldwide interest is growing in the area of biological control using entomopathogenic fungi, and a significant advance in the development and mass production of these agents has been witnessed over the last few decades. A plethora of reviews is available on arthropod-associated fungi (Ferron, 1978; Roberts and Humber, 1981; Hall and Papierok, 1982; Zimmermann, 1986; McCoy *et al.*, 1988; Roberts, 1989; Ferron *et al.*, 1991; Roberts *et al.*, 1991; Goettel, 1992; Roberts and Hajek, 1992; Leathers *et al.*, 1993; Sosa-Gómez *et al.*, 2010). A brief discussion on production and formulation has also been given by Goettel and Roberts (1992).

Much useful literature is also available on entomopathogenic fungi (Table 7.2). Over 700 species of entomopathogenic fungi have been reported so far (Roberts, 1989; Roberts *et al.*, 1991) and it would be difficult to discuss all entomopathogenic fungi and their potential as biological control agents in this chapter, so we will

Table 7.2. Some of the literature available on entomopathogenic fungi.

Review area	References
Epizootiology and ecology of entomopathogenic fungi	Carruthers and Soper (1987); Fuxa (1987); Carruthers and Hural (1990); Onstad and Carruthers (1990); Glare and Milner (1991); Meyling and Eilenberg (2007); Quesada-Moraga *et al.* (2007)
Physiology and genetics of entomopathogenic fungi	Khachatourians (1991); Wang *et al.* (2005)
Insecticidal efficacy of entomopathogenic fungi against the insects	Roberts (1981); Gillespie and Claydon (1989); Wright and Chandler (1992)
Stability of entomopathogenic fungi in response to abiotic factors	Roberts and Campbell (1977)
Safety of entomopathogenic fungi to non-target invertebrates	Goettel *et al.* (1990); Hokkanen and Lynch (1995)
Safety of entomopathogenic fungi to vertebrates	Siegel and Shadduck (1990); Semalulu *et al.* (1992)
Commercial approaches for the use of entomopathogenic fungi	Bartlett and Jaronski (1988); Powell and Faull (1989); Bradley *et al.* (1992)
Review on specific fungal agents, e.g. *Beauveria*	Feng *et al.* (1990b)
Review on specific fungal agents, e.g. *Metarhizium*	Ferron (1981)
Review on specific fungal agents, e.g. *Verticillium lecanii*	Hall (1981)
Review on specific fungal agents, e.g. *Hirsutella thompsonii*	McCoy (1981)
Review on specific fungal agents, e.g. *Nomuraea rileyi*	Ignoffo (1981)
Entomopathogenic fungi	Wilding (1981, 1990); Wilding *et al.* (1986); Wolf (1988)
Mass production and formulation of entomopathogenic fungi	Soper and Ward (1981)
Mass production and formulation of other microbial agents	Couch and Ignoffo (1981)

limit our discussion to a number of examples.

In general, fungal pathogens persist at low levels within insect populations and are responsible for a small amount of sickness and/or death in individuals of a field population. According to Benjamin *et al.* (2004), the entomopathogenic fungi can be classified into two main groups, biotrophic fungi and necrotrophic fungi.

7.5.1 Biotrophic fungi

Biotrophic fungi require living cells of their hosts (Lacey and Kaya, 2000). Some are commensals that obtain nutrients from the digestive tract of the insect. This class of fungus is widespread in many regions, but is not broadly used for pest control as the fungi are either asymptomatic in insects or the changes caused by pathogens are difficult to observe.

7.5.2 Necrotrophic fungi

Necrotrophic fungi live at the expense of dead cells and have to kill their hosts before consuming it. Fungi belonging to this group are potential agents of biological control of insects and are very effective in their attack. Their host range includes the orders Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, Orthoptera, Homoptera and Diptera. Attack can occur at several stages of the life cycle. A large number of compounds with insecticidal properties

have been isolated from species of fungi belonging to the genera *Beauveria*, *Metarhizium*, *Nomuraea*, *Aspergillus*, *Verticillium*, *Paecilomyces*, *Isaria*, *Fusarium*, *Cordyceps* and *Entomophthora*.

Morphologically, fungi may occur as single cells (such as yeast) or branched filaments (hyphae) that form mats or mycelia, and they may reproduce sexually, asexually or in both ways. Sexual reproduction involves some sort of fusion between two structures such as gametes or hyphae. The conidial spore is the most commonly used infective stage of fungal microbial pesticides. Other stages (i.e. mycelial fragments and blastospores) have been investigated but without significant applications. Commercial mycopesticides are based primarily on the conidia of Deuteromycota. Most fungi do not invade hosts through the gut, even if the conidia are ingested. The spores of entomopathogenic fungi usually enter the host through the integument. The host ranges of fungi vary from narrow to broad, but some species with broad ranges may consist of a series of more specific pathotypes. There is a great variation in fungal infections, but the following description is typical for Zygomycota and Deuteromycota. Fungal infections begin after conidia or other infective stages randomly make contact with a susceptible host after transport by wind, rain or animals or, in the case of biopesticides, by direct application to the target. Following contact, adhesion and germination of the conidia on the host cuticle must occur. Fungi may grow as hyphae, yeast-like bodies and wall-less protoplasts. Protoplasts help overwhelm the host defences because they are not recognized by the immune system. The physical and chemical properties of the insect cuticle affect this process, influencing the host range of the fungus. Adhesion of conidia is often promoted by mucilaginous materials. After it has been deposited on the insect's cuticle and under appropriate humidity conditions, the conidium produces a germ tube that grows and branches through the host's integument.

7.5.3 Mass production of entomopathogenic fungi

Mass production of mycopesticides is carried out using species that are capable of growing on non-living medium. For this purpose, naturally occurring substances such as rice bran is suitable rearing media. Conidia are harvested by washing fungal mats with distilled water. It has been found that effective control of target pests with fungi requires 10^5–10^6 conidia/cm^2 of leaf surface or /cm^3 of soil. Federici (2007) found that production of this conidial quantity consumed 10–15 kg of rearing substrate /ha, making treatment of large areas of field crops expensive.

The production of fungi on solid medium lacks a satisfactory economy of scale or potential for automation. Only a few species such as *Beauveria bassiana* (Balsamo) Vuillemin and *Hirsutella thompsonii* Fisher will sporulate in submerged culture (van Whinkelhoff and McCoy, 1984). An alternative method for the commercial production of entomopathogenic fungi is to develop application methods to use mycelial fragments, rather than conidial spores as the infective propagule to be applied. This approach has been explored with *H. thompsonii*, and a patented process has been developed in which mycelia can be produced in submerged culture, dried and stored by refrigeration until applied (McCoy *et al.*, 1975; McCabe and Soper, 1985).

Beauveria bassiana

Two species of the genus *Beauveria* have been examined for biological control potential. *B. bassiana* and *Beauveria brongniartii* have been shown to produce a cyclodepsipeptide called beauvericin responsible for toxicity against insects. Of several species in this genus, *B. bassiana* has been studied the most extensively since it was first reported as a pathogen of the silkworm, *Bombyx mori* L., by Agostino Bassi in 1834 (Glare and Milner, 1991), and experiences gained with *B. bassiana* have

served as a basis for the current status of microbial control of pest insects by fungi.

MODE OF INFECTION The insects are infected by conidia (asexual propagules) of *B. bassiana*, which attach to the host cuticle and require a high humidity to germinate. The germ tubes developing from the conidia penetrate the host cuticle and invade the haemocoel. Infection by *B. bassiana* is dependent primarily on various enzymatic activities for degradation of proteins, chitin and lipids in the insect integument (Ferron *et al.*, 1991; Khachatourians, 1991). Instead of the general mode of infection through the integument, *B. bassiana* can also infect insects in other ways, specifically insects with chewing mouthparts, e.g. the red imported fire ant (*Solenopsis invicta* Buren), German cockroach (*Blattella germanica* L.) (Siebeneicher *et al.*, 1992), Colorado potato beetle (*Leptinotarsa decemlineata* Say), pine caterpillar (*Dendrolimus punctata* Walker) (Long and Du, 1988), cabbage looper (*Trichoplusia ni* Hübner) (Ignoffo *et al.*, 1982), lesser cornstalk borer (*Elasmopalpus hgnosellus* Zeller) (McDowell *et al.*, 1990), mulberry silkworm (*B. mori* L.) (Huang, 1988) and migratory grasshopper (*Melanoplus sanguinipes* F.). The fungus proliferates quickly after invading the haemocoel. Mycelia from the elongated germ tubes are septate and release blastospores. Host insects are killed due to depletion of their haemolymph nutrients and/or due to toxaemia caused by fungal toxic metabolites (Roberts, 1981; Khachatourians, 1991). Under moist conditions, the fungus emerges and produces a layer of aerial conidia on the surface of host cadavers. In addition to its lethal effect, infection by *B. bassiana* may have sublethal or secondary effects. For example, doses of *B. bassiana* near the 50% lethal does (LD_{50}) reduced the reproductive potential of adult spotted pea weevils (*Sitona lineatus* L.) and the fertility and fecundity (Faizi, 1978) of adult asiatic rice borers (*Chilo suppressalis* Walker) surviving infection. The fungus also declined the fecundity and fertility e.g. colorado potato beetles (Fargues *et al.*, 1991), and rice plant and leafhopper (Zhang and Huang, 1988).

OCCURRENCE IN NATURE More than 200 insect species belonging to nine orders (Prasad and Syed, 2010) have been recorded as hosts of *B. bassiana* (Li, 1988). Despite this wide range of hosts, epizootics caused by *B. bassiana* in natural insect populations occur infrequently. Feng *et al.* (1990a) reported that in 2930 aphid cadavers examined, ten species of fungal pathogens, including eight Entomophthorales and two Hyphomycetes, were recovered. Of the fungal infections, less than 1% each were attributed to infection by *B. bassiana* or *Verticillium lecanii*, and the remainder were from the Entomophthorales. However, a large swarm of the red locust (*Nomadacris semptemfasciata* Serville) was killed by a *B. bassiana* epizootic (Schaefer, 1936), and several *B. bassiana* epizootics were observed to decrease the populations of pine caterpillars (*Dendrolimus* spp.) in southern China (Chen *et al.*, 1990).

A considerable suppression by *B. bassiana* was recorded on Russian wheat aphid (>60%) in Moscow (Knudsen and Schotzko, 1999) and for rice leafhoppers and the elm leaf beetle (*Pyrrhalta luteola* Müller) (>50% mortality) in Hubei and Hunan during the 1970s (Lü and Zhao, 1988).

POTENTIAL Shi *et al.* (2008) studied the efficacy of *B. bassiana* against the red spider mite (*Tetranychus urticae*; Acari: Tetranychidae) and found ovicidal potential. The egg mortality caused by the fungal formulation fell in the range of 62.5–87.9%. The virulence of the fungus (Fargues, 1981) and pathogenicity of isolates towards any insect varies. There is no clear definition of virulence for entomopathogenic fungi. The term virulence has been defined as the 'degree of pathogenicity within a group or species' of entomopathogenic fungi, in the context of a particular host bioassay (Khachatourians, 1991). Often virulence is measured in terms of 50% lethal concentration (LC_{50}),

but bioassays can be performed under different relative humidity and temperature conditions because of the experimenter's choice or the particular requirements of an insect. In this chapter, we have adopted the terminology used in the publications and hope to avoid further confusion. Different publications on bioassays of over 50 *B. bassiana* isolates from around the world on the Colorado potato beetle produced 50% lethal time (LT_{50}) values ranging from 2 to 10 days. Generally, *B. bassiana* isolates tend to have higher virulence towards their original hosts, or to species closely related to the original hosts (Xu, 1988). However, exceptions exist: for instance, an isolate from the Colorado potato beetle was more virulent to the Russian wheat aphid (*Diuraphis noxia* Kurdjumov) than isolates derived from other homopteran hosts. Interestingly, high concentrations of a suspension of conidia of an isolate highly virulent to the European maize borer (*Ostrinia nubilalis* Hübner) caused only 4–10% mortality in the Chinese oak silkmoth (*Antheraea pernyi* Guérin-Méneville), even under laboratory conditions (Xu *et al.*, 1988), while repeated use of a formulation of this isolate yearly for control of maize borer in the fields never resulted in white muscardine in the silkmoth populations located in nearby fields. Kononova (1978) classified 190 *B. bassiana* isolates into two groups: on solid media, one group had characteristic suppressed vegetative growth and abundant sporulation, whereas the other had fluffy, cottony colonies (excessive vegetative growth) and suppressed sporulation.

In the laboratory, repeated subculture of an isolate sometimes leads to attenuation of its original features such as growth, sporulation and virulence (Khachatourians, 1991; Hayden *et al.*, 1992). It can be speculated that this problem may be a consequence of genetical change through a parasexual cycle in *B. bassiana*, as reported by Paccola-Meirelles and Azevedo (1991), and perhaps could be minimized by routine host passage and single spore isolation (Hayden *et al.*, 1992). Although evidence for the genetic basis for variation is still to be determined, once discovered, through *in vitro* recombinant DNA-derived vectors and electroporation-mediated transformation (Pfeifer and Khachatourians, 1992) or the parasexual cycle, one could methodically approach the genetical stabilization or enhancement of *B. bassiana* isolates.

Verticillium lecanii

This fungus has been developed against aphids, whiteflies, thrips and red spider mites. The fungal spores are dried to form a wettable powder formulation, which can be mixed with water to produce a suspension suitable for immersion of plant cuttings. Strains of this fungus have been shown to be highly pathogenic to several crucifer pests, yet are harmless to honeybees.

Metarhizium anisopliae

Driver *et al.* (2000) have recently carried out a taxonomic revision of the genus *Metarhizium* based on phylogenetic analysis of rRNA gene sequence data and reported that only four species – *M. anisopliae*, *Metarhizium album*, *Metarhizium flavoviride* and *Metarhizium taii* – exist. *M. anisopliae* and *M. flavoviride* have a wide geographic distribution. The cosmopolitan distribution of these species has enabled them to be exposed to a broad diversity of environmental factors and insect host species. Tulloch (1976) categorized two varieties in *M. anisopliae*. *M. anisopliae* var. *major* (Johnston) Tulloch has long conida of about 10.0–14.0 μm in length (range 9.0–18.0) and is common. The short conidial type, with conidia of 5.0–8.0 μm (range 3.5–9.0), has a diverse host range. The species *M. flavoviride* var. *flavoviride* Gams and Rozyspal with conida of 8.0–9.0 μm (range 7.0–11.0) and *M. flavoviride* var. *minus* Rombach, Humber and Roberts with conida of 4.5–5.5 μm (range 4.0–6.5) long are confined to grasshoppers and locusts. *M. anisopliae*-infected cadavers show typical green aerial mycelia and sporulation on the surface. The colony of this fungus appears white in the

initial stages upon conidiation, with the colour eventually turning to dark green. The mycelium is composed of hyaline, septate, branched hyphae and short, erect, hyaline conidiophores, which are septate, simple or branched, terminating in single or a cluster of phialides. The conidia are one-celled, hyaline, smooth and long ovoid to cylindrical (4.8 × 1.6 mm), abstracted from the tips of the phialides and forming long basipetal chains or columns.

MODE OF ACTION This fungus acts in two ways. The first is a pathogenic phase, which begins when the conidia come into contact with the epicuticle of the host integument or are ingested. The conidia adhere and germinate forming a germ tube that penetrates directly or grows over the surface of the epicuticle. Successful penetration depends on the inherent capabilities of the germ tube and the physiological state of the host, and involves mechanical and enzymatic processes. The germ tube penetrates by lysing both the epicuticle, a layer characterized by a cross-linked network of lipids and protein, and the procuticle, a layer of chitin and protein. An active digestion and absorption of cuticular components occurs, colonizing the host's tissues and producing elongated hyphae and/or hyphal bodies (blastospores). Before extensive invasion of the host tissues, the insect dies as a result of the production of an array of secondary metabolites called destruxins which affect ion transport channels involved in muscle response and cell-membrane integrity. Insects exhibit various symptoms including restlessness, cessation of feeding and loss of coordination. As the host nutrients are depleted, the blastospores differentiate into elongated hyphae that extend outwards from the body forming a mycelial mat of conidiophores over the surface of the integument, ultimately resulting in mummification. Under proper environmental conditions, the conidiophores mature giving rise to conidia. The conidiophores of *Metarhizium* are characterized by palisade-like conidial masses with compact conidial chains, which are olive green in colour.

The second mode of action is that achieved by the insecticidal activity of destruxins, which have a knockdown effect on insects. Destruxins are secondary metabolites of *M. anisopliae* produced both *in vitro* in culture and *in vivo* in mycosed insects, such as cyclodepsipeptides produced during blastospore replication. These insecticidal toxins of fungus can speed up the fungus action to cause early mortality of the targets. Target insects are killed by ingestion of these metabolites, which are activated in the alkaline environment of the insect stomach. These metabolites can also penetrate through the spiracles of the insect cuticle. Symptoms of the action of these metabolites are discoloration, loss of mobility and coordination, cessation of feeding and death with in 72 h. These destruxins can be extracted from the fungus grown by fermentation and can be formulated as a biological insecticides.

APPLICATIONS Although *Metarhizium* was described and its importance in insect pest control indicated as early as in 1988, few attempts were made to use it experimentally for biological control. Exploitation on a large scale in different crop ecosystems, with the intention of establishing and exploring *Metarhizium* for use as a full biopesticide remains untried. However, attempts have been made to manage insect pests using *Metarhizium* spp. Douro-Kpindou *et al.* (1997) recorded a considerable reduction in grasshopper populations when they were sprayed with *M. anisopliae*. In the case of termites, infection in both soldiers and workers was witnessed and 100% mortality was observed when the fungus was applied to termatorium (Zobari, 1994; Alves *et al.*, 1995). Sundarababu (1985) applied the fungus at four doses (1.72×10^4, 8.6×10^4, 17.2×10^4 and 34.4×10^4 spores/g of manure) in both heaps and pits. At the highest dosage, the pest was not seen in the treated manure pits for up to 12 months. When applied at a rate 1.5×10^8 conidia/ml in Brazil against the coffee berry borer (*Hypothenemus hamphei*), 60% control was observed in 4 days after treatment.

When dry mycelial particles were incorporated into the soil at a depth of 15 cm at a concentration of 0.93–3.0/g of particles/m row at the time of maize planting, there was an increase in mortality of maize root worm larvae (Krueger and Roberts, 1997).

MASS PRODUCTION Grain-based production and liquid fermentation are the two major techniques for mass cultivation of *M. anisopliae*.

The convenient and durable developmental stage of the dusty spore hyphomycetes for application and storage is the conidium, which is the natural distributive stage (Burges, 1998). In nature, this forms on the surface of the insect or internally in dry situations. Bartlett and Jaronski (1988) reported that cereal grains were the simplest and lowest cost type of nutrient granules. Medonca (1992) developed a low-cost technology that could generate an *M. anisopliaea* yield of 10^{13} conidia/kg of rice. They found that maize, potato, bean, sorghum and soya gave a lower yield than rice. However, recent reports by other researchers have indicated consistent yields of only 1.5×10^{12} conidia/kg of rice (Dorta and Arcas, 1998; Jenkins *et al.*, 1998; Rachappa *et al.*, 2005).

Liquid fermentation has been used to produce *M. anisopliae* blastospores (Humphreys *et al.*, 1989; Kleespies and Zimmermann, 1992) and mycelial formulations (Pereira and Roberts, 1990). Mycelial growth of fungus was better on rice bran, barley or maize extracts than on SMAY (sabouraud, maltose, agar and yeast extract) medium (Burges, 1998). Furthermore, they found that silkworm pupal and wax moth larval extract medium was similar to that of SMAY medium in producing mycelia. Coconut water has been reported to be better than potato dextrose broth (Feng *et al.*, 1990b). Medonca (1992) reported that molasses, soy flour and mineral salts gave a lower conidial yield, while bean broth gave maximum yield. Molasses yeast broth produced 8×10^8 conidia/ml (Sharma *et al.* 1999). Coconut water and rice gruel fortified with yeast (1%) supported growth and conidial production better (10.9×10^8 and 8.32×10^8 conidia/ml, respectively) than molasses fortified with 1% yeast (Rachappa *et al.*, 2005).

M. anisopliae and *B. bassiana* can successfully be mass produced on whey-based media. A good yield of spores can be obtained depending on the strain and production process used. Studies have revealed that spores produced by all strains in whey-based solid and liquid media showed between 73 and 99% viability; germination rates were comparable with those obtained using the standard Sabouraud dextrose agar medium. In the two-stage production process, the viabilities of conidia produced by *B. bassiana* isolates GHA-726 and CA-603, and *M. anisopliae* isolate CA-1 were 35–86, 32–98 and 6–29%, respectively; viability was correlated with whey concentration and isolates (Kassa *et al.*, 2008).

7.6 Entomopathogenic Viruses

Viral diseases have been found in 13 insect orders and probably occur in all orders. Viruses are the simplest 'life forms' recovered so far from insects, and consist of a nucleic acid core and a protein shell or capsid. This nucleocapsid may also be surrounded by a lipid bilayer envelope. Some viruses are additionally occluded into a protein matrix called an occlusion body. Occlusion bodies are found in three families of viruses and appear to have evolved independently in each family. Viruses are causative agents of naturally occurring infections that have been observed for centuries in insect populations including the jaundice disease in the silkworm, the wilting disease of the nun moth described by Tubeuf in 1892 and more recently virus epizootics in the gypsy moth (Stiles *et al.*, 1983). Their ability to devastate insect populations has stimulated interest in deploying pathogenic viruses as insecticidal agents. Insect virology is an immensely broad and diverse field encompassing many kinds of insects in many different ways.

Of the several kinds of viruses that have been used to control insect pests, the rod-shaped DNA viruses, particularly members of the family *Baculoviridae*, have received the most attention. Viruses of this type have been reported only to infect arthropods, principally insect pests, and their absence as pathogens of plants or higher animals implies that they are safe for large-scale environmental use to control insect pests, posing no threat to non-target organisms. The baculoviruses have also been of special interest because of the broad range of insect species they are known to infect. In addition, baculoviruses are embedded within paracrystalline proteinaceous occlusion bodies, which confers environmental stability and allows baculoviruses to be formulated and applied in much the same way as traditional insect pest-control agents.

The earliest example of pest suppression by an introduced insect virus was recorded in the 1930s. The European spruce saw fly (*Gilpinia hercyniae*) was brought accidentally from Scandinavia to Canada and classical biological control using parasitoids was undertaken to manage this insect. Several parasitoid species were introduced and a few became established; however, some of them carried a virus disease of the host, described as a polyhedrosis, which spread effectively throughout a severely infested area of about 3 million ha by 1940.

G. hercyniae has ceased to be a pest in Canada (Balch and Bird, 1944). Although this was an impressively successful example of permanent introduction using a viral disease of an insect pest, most viruses used subsequently as control agents have been applied by inundative release or inoculative release approaches. To date more than 40 viral pesticides have been registered worldwide for insect control (Stock *et al.*, 2009). *Helicoverpa zea* nucleopolyhedrosis virus was the first baculovirus registered for commercial use in the USA and was sold from 1975 to 1982 (Ignoffo and Couch, 1981).

Insect viruses may contain double- or single-stranded DNA or RNA, may be enveloped or non-enveloped, and may be occluded in a protective protein matrix or non-occluded. Viruses that are primarily or exclusively found in insects are currently placed in 12 families and one unclassified group; viruses in families that have been isolated but are not common in insects are not included in this chapter.

7.6.1 Baculoviruses

Morphologically, baculoviruses are rod-shaped nucleocapsids containing double-stranded DNA. The virus particles, or virions, responsible for infection are occluded in protein bodies called polyhedra (in nucleopolyhedrosis viruses or NPVs) or granules (in granuloviruses or GVs). Infection with baculoviruses occurs when a target host feeds on the polyhedra or granules in infected food, which are dissolved in the basic digestive gut juices. The polyhedra of NPVs are larger than the virions (usually 1–15 μm) and may contain many virions. The virions are released when the protein matrices dissolve. After entering the nuclei of midgut cells, the virions replicate and are released to infect many of the tissues and organs in the insect, mainly the fat body, epidermis and blood cells. Non-occluded baculoviruses, or nudiviruses, are not occluded in polyhedra and have recently been removed from the family *Baculoviridae*. Their taxonomic status is currently uncertain.

Baculoviruses are environmentally benign and do not replicate in vertebrates. However, use of these viruses requires a thorough understanding of the host range and the mechanism that controls host specificity because they may also cause disease to beneficial insects present in the environment (Miller, 1998). After infection of the insect epidermis, the host appears to disintegrate and releases virus particles into the environment. The tissues of the host liquefy, hence infection of insects with baculoviruses was initially termed ‘wilt disease’.

NPVs

NPVs are largely restricted to insects and most species are relatively host specific. They are known to infect more than 500 species of insects, and are best known for infection of Lepidoptera. They have also been found to infect Hymenoptera, Diptera, Thysanura, Trichoptera and Crustacea (e.g. shrimp) (Federici, 1997). The NPV of *Helicoverpa armigera* (HaNPV) is the one of the most intensively studied species. The polyhedra may contain from a few to many virions. After ingestion by the host and reproduction in the midgut cells, other tissues and organs in the insect become infected, primarily the fat body, epidermis and blood cells.

The LT_{50} of NPVs usually ranges from 5 to 12 days after infection depending on the viral dose, temperature and the larval instar at the time of infection. Before dying, the infected larvae often crawl to the tops of plants or any other available structure where they hang upside down, die and decompose. Millions of polyhedra are contained in the fluid mass of the disintegrating larvae and fall into feeding zones (leaves and leaf litter) where they can be ingested by other conspecific larvae. NPV epizootics are very impressive and, although they are important as naturally occurring mortality factors for many insect species, they often occur after the pest insect has exceeded the economic injury level. This is especially true when the crop that is being damaged by this insect has a relatively low economic threshold.

APPLICATION As NPVs are obligate parasites, they cannot be produced on synthetic culture media, i.e. they cannot be produced commercially without living cells. This means that the production of NPV isolates as microbial insecticides requires a colony of insects or an acceptable insect tissue culture. This is more expensive than producing organisms such as the bacterium *B. thuringiensis* in an artificial medium. Used as a microbial insecticide, NPVs can provide a relatively fast kill and many are very host specific. All are specific to arthropods, most to a narrow range of Lepidoptera. Research on the production of NPVs as microbial insecticides has been aimed primarily at developing more efficient methods of production in insect tissue culture, as well as developing more virulent NPV strains. Most of the research on virulence involves inserting genes that produce toxic substances into the polyhedron envelope protein gene. For example, genes for insect-specific toxins, inserted into the polyhedron gene locus are expressed at the time that the polyhedron gene would be expressed. The toxins then kill the insect at an earlier stage than occurs in a normal infection (Black *et al.*, 1997). To date, a number of NPVs have been developed and are commercially available for the control of insect pests (Lynn *et al.*, 1989).

GVs

GVs are closely related to NPVs and are similar in structure and pathogenesis. They have a very similar mode of action and reproduction. There are three major genetic types. Type 1 GVs, such as those isolated from the cabbage looper (*T. ni*) only infect midgut cells and subsequently the fat body cells. Because they do not infect the tracheal matrix or epidermis, infected larvae may live longer than NPV-infected insects. Type 2 GVs, first isolated from the codling moth (*Cydia pomonella*) are similar to NPV infections (Payne, 1982). Type 3 GVs, known only from the western grapeleaf skeletonizer (*Harrisina brillians*) infect only the midgut tissues (Federici, 1997).

APPLICATION Several GVs have been formulated as microbial insecticides, for example, GVs from the codling moth. Like NPVs, these viruses are generally produced *in vivo* because of difficulties producing them in cell culture. *In vivo* production costs and their narrow host spectrum limits their attractiveness to industry.

7.6.2 Ascoviruses

These are double-stranded DNA viruses that possess a large genome with a unique

hexagonal envelope. Although not occluded in a single-protein matrix the ascovirus virions are intermixed with proteins and microvesicles to form a vesiculate occlusion body (Tanada and Kaya, 1993). Ascoviruses cause hypertrophy of host nuclei and cells in their lepidopteran hosts (Federici, 1983), and eventually the nucleus ruptures. Cytoplasmic membranes form in sheets in the cell, resulting in the formation of vesicles that are released into the host tissues and haemolymph when the cell basement membranes break down. Ascoviruses infect a range of tissues, but the fat body is always infected. The LT_{50} for ascoviruses is 12–21 days, and infections are typically chronic, producing colour changes in the host and difficulties in moulting. These viruses appear to be transmitted more efficiently via parasitoids rather than orally (Tanada and Kaya, 1993).

Application

Ascoviruses are readily transmitted by injection (Govindarajan and Federici, 1990), suggesting that these viruses are vectored mechanically via the ovipositor of parasitoids during oviposition. The use of ascoviruses in commercial cultivation is rare, which may be attributed to there being very little information on the ecology or genetics of this group. In noctuid populations, they have been reported to cause between 1 and 25% mortality, indicating that they may be important components of the natural enemy complex of some noctuid species.

7.6.3 Iridoviruses

Viruses from the family *Iridoviridae* infect both vertebrate and invertebrate organisms and are large, non-enveloped, non-occluded double-stranded DNA viruses that are icosahedral in shape (Williams *et al.*, 2000). The term was derived from 'iridescent virus', which refers to the characteristic microcrystalline lattice arrangement of virions in the host cells that results in Bragg reflection of visible light (Williams and Smith, 1957; Williams, 1998). This family of viruses has been isolated from Coleoptera, Diptera, Hemiptera, Lepidoptera (Hall, 1985) and Orthoptera (Boucias *et al.*, 1987), as well as some non-insect arthropods. Most iridoviruses are transmitted transovarially and are not easily transmitted orally. Transmission may depend on the massive dosages that can be encountered as a result of cannibalism or by mechanical transmission by parasitoids (Williams, 1998). Infections are chronic and tend to be systemic, but the fat body and epidermis of hosts are particularly affected. The nuclei of cells are destroyed, resulting in the destruction of haemocytes.

Application

Researchers have generally concluded that iridoviruses have little utility as microbial insecticides because of their low infectivity and chronic infection nature. In field populations of some pest species such as mosquitoes, however, the viruses may be important components of the natural enemy complexes (Hembree, 1979; Fedorova, 1986).

7.6.4 Parvoviruses (densoviruses)

Densonucleosis viruses or densoviruses (DNVs) are invertebrate viruses belonging to the subfamily *Densovirinae* within the family *Parvoviridae* (Bergoin and Tijssen, 2000). Only a single genus (*Densovirus*) in the family *Parvoviridae* has been recovered from insects. This non-enveloped, single-stranded DNA virus is characterized by its effects on the nucleus of host cells, and it attacks most host tissues. Infection results in extreme hypertrophy of the cell nucleus and filling of the nuclei with small isometric particles. The nuclei become strongly eosinophilic (dense nuclei, thus the name densovirus). Discoloration and paralysis of the host often occur. These viruses can be very virulent and infectious, and the type species, *Galleria mellonella* DNV (GmDNV), has caused serious problems for the waxworm bait industry in the Midwest in

recent years. Despite their high virulence, DNVs do not replicate in vertebrates (Bergoin and Tijssen, 1998).

Application

Although highly virulent, DNVs appear to be safe for non-target organisms such as mammals, including humans (Giran, 1966; Fédière *et al.*, 1991; Jousset *et al.*, 1993; Fédière, 1996; Masri, 1997; El-Far *et al.*, 2003, 2004). The use of DNVs as a viral pesticide has not yet been investigated in detail; however, it is important to mention that some DNVs, for instance, GmDNV, *Sibine fusca* DNV, *Casphalia extranea* DNV and *Aedes aegypti* DNV have been used very successfully to control insect pests (Lavie *et al.*, 1965; Fédière *et al.*, 1986; Buchatsky *et al.*, 1987; Lebedinets and Kononko, 1989). These spectacular field results could be encouraging for utilization of other DNVs in biological control (Bergoin and Tijssen, 1998; Fédière, 2000). The use of infected waxworms in beehives to eliminate waxworms from the hives is one such successful example (Lavie *et al.*, 1965). Other pest species have also been treated successfully, for example, the palm oil defoliator (*Sibine fusca*). However, sequence homologies between DNVs and vertebrate parvoviruses have raised concerns about their use, despite a lack of evidence that this genus can infect vertebrates.

7.6.5 Polydnaviruses

The name polydnaviruses comes from the fact they are DNA viruses that are 'poly-dispersed'. They are enveloped, double-stranded DNA viruses, and are unique because of their obligatory mutualistic relationship with their hymenopteran hosts. Polydnaviruses have a narrow host range and recognize only a few genera of ichneumonid and braconid hosts. The relationships of the viruses within host genera parallel those of their hosts, which is expected as the virus integrates into the wasp host DNA as proviral DNA and is inherited by the host progeny (Whitfield, 1997). Polydnaviruses have a unique infection mechanism, in which they excise themselves from the host genome and replicate only in the calyx cells of the host ovaries. When the female wasp oviposits into a host, the virus particles are secreted with the eggs. There are no known pathogenic effects on the wasp host. The virus does not replicate in these tissues but acts to suppress the host immune system, possibly in conjunction with teratocytes and wasp venoms. Immune suppression and the host developmental delays that result favour the survival of the parasite eggs and larvae (Tanada and Kaya, 1993) and, in fact, may be required for survival of the parasitoids (Webb, 1998).

Application

For commercial utilization, there is a need to investigate the comparative ecological and behavioural aspects along with the immune suppression role of polydnaviruses, as well as the ability of the viruses to delay development and alter the physiology of the parasitoids' lepidopteran hosts. There is potential for use of these viruses or their viral genes to suppress the immune system of a targeted host (Beckage, 1996; Washburn *et al.*, 1996).

7.6.6 Poxviruses

Poxviruses belong to the family *Poxviridae*, which is divided into two subfamilies: (i) the *Entomopoxvirinae*, which comprises invertebrate poxviruses and can be further subdivided into three 'types' that are restricted to several insect families; and (ii) the *Chordopoxvirinae*, which is subdivided into eight genera that infect vertebrates (Barrett *et al.*, 2006). The insect-specific poxviruses, the entomopoxviruses (EPVs), are occluded, much like the baculoviruses, into protein matrices called spheroids. The occlusion bodies arise independently in poxviruses, as well as in baculoviruses and reoviruses, probably as protection from environmental degradation. EPVs have been found infecting Coleoptera, Lepidoptera and Orthoptera, Diptera and Hymenoptera (Arif, 1984; King *et al.*, 1998). Lepidoptera

infected with EPVs show swelling and whitening due to infection of fat body cells, which causes cell proliferation and hypertrophy. Mortality is not rapid – the larvae may live for 12–72 days after infection occurs – and development of infection may be even slower in Coleoptera (Tanada and Kaya, 1993).

Application

EPVs may have great usefulness in pest management as they have been found in a number of insect orders containing pest species. It is possible that these viruses can be manipulated via molecular techniques to increase the speed of kill and improve other desirable characteristics (Palmer *et al.*, 1995).

7.6.7 Reoviruses

Cytoplasmic polyhedrosis viruses or cypoviruses (CPVs) have a ten segment, double-stranded RNA genome and are classified as members of the genus *Cypovirus* within the family *Reoviridae*. Superficially resembling NPVs, CPVs are occluded (occlusion bodies of 0.1–10 μm) and non-enveloped. Easily mistaken for NPVs when seen outside the cell, CPV can be diagnosed from intact host cells because the polyhedra are formed only in the cell cytoplasm, unlike the NPVs, which replicate and form polyhedra in the host cell nucleus. Vertebrate and plant reoviruses do not produce polyhedra. CVP particles are icosahedral in shape and have 12 spikes or projections at each of the 12 vertices of the particles. CPVs have been isolated from Lepidoptera (80%), Diptera (16%) and Hymenoptera (3%) (Graham *et al.*, 2006), and Coleoptera and Neuroptera (<1%) (Hukuhara and Bohami, 1991). In Lepidoptera, CPVs are primarily midgut pathogens, but some CPV species also infect the fat body and other tissues.

After feeding of the target host on infected food, the polyhedra dissolve in the midgut juices and the virions are released. The infective virus particles attach to the midgut cells and the infective core is injected through the spikes (Kobayashi, 1971). New virus particles appear in the cell cytoplasm within 3 h of infection. CPV infections tend to be more chronic and less lethal to host larvae than other viruses, but destruction of the midgut tissues leads to nutritional deficiencies and physiological consequences (Billoncik and Mori, 1998). CPVs seem to have little or no effect on dipteran hosts (Tanada and Kaya, 1993). CPVs can cause problems in colonies of insects raised in laboratories, as large numbers of polyhedra are shed in the host faeces and the disease can easily be transmitted within a colony.

Application

Besides being highly virulent, CPVs are protected by polyhedra and can also synergize other pathogens; however, they are also relatively slow acting and chronic in nature (Billoncik and Mori, 1998). They probably have more potential in systems where the economic threshold is high (not unlike most other insect pathogens), or as inoculatively or augmentatively released natural enemies.

7.6.8 Nodaviruses

Members of the family *Nodaviridae* are small RNA viruses with a single-stranded, positive-sense genome. One of the best-studied nodaviruses is nodamura virus (NOV), which infects both insects and mammals, with mosquito transmission to pigs in Japan being documented. Other nodaviruses include black beetle virus, flock house virus, pariacoto virus, New Zealand virus, Boolarra virus and Manawatu virus. Many isolates are from the South Pacific (Ball and Johnson, 1998).

Only NOV has been studied *in vivo*. This virus produces localized lesions in the cytoplasm of muscle, nerve, salivary gland and moulting gland cells. Wax moth larvae become paralysed at 4–6 days post-inoculation and die between 7 and 14 days post-inoculation (Garzon *et al.*, 1978).

7.6.9 Picorna-like viruses

Until recently, several small RNA-containing viruses (called SRVs) were placed in the *Picornaviridae*, a family of non-enveloped, non-occluded, single-stranded RNA viruses that includes human viruses such as the common cold virus and polio virus (Christian and Scotti, 1998). The best known of the picornaviruses from insects are cricket paralysis virus (CrPV) and *Drosophila* C virus (DCV). CrPV and DCV are the best studied because they can be grown in tissue culture. CrPV has one of the broadest natural host ranges known for insect viruses – it has been detected in 22 species in five insect orders (Christian and Scotti, 1998). DCV, on the other hand, seems to be limited to a few *Drosophila* spp. CrPV can be transmitted *per os* and may also be transmitted on the surface of eggs (transovum transmission). Viruses of the honeybee (*Apis mellifera*) such as those that cause sacbrood disease, acute bee paralysis and chronic bee paralysis are three of seven different picorna-like bee viruses. Picorna-like viruses have also been isolated from mosquitoes and hemipterans.

Application

On account of their apparent homologies to mammalian picornaviruses, little effort has been done to incorporate insect-specific picorna-like viruses into biological control programmes. Nevertheless, Christian and Scotti (1998) suggested that some of these viruses have potential for use in biological control.

7.6.10 Tetraviruses

Members of the family *Tetraviridae* are specific to insects isolated from Lepidoptera. They are the causative agent of viral epizootics in the pine emperor moth (*Nudaurelia cytheria capensis*), emperor gum moth (*Opodiphthera eucalypti*) and cotton bollworm (*Helicoverpa armigera*). The family *Tetraviridae* contains 18 members, ten known members and another eight that are as yet unassigned (Gordon and Hanzlik, 1998).

Tetravirus infections cause symptoms ranging from inapparent infections to acutely lethal infections. In lethal infections, the larvae become moribund, discoloured and flaccid at about 7–9 days post-infection, and hang from their prolegs much like NPV-infected larvae. Replication appears to occur only in the midgut cells. The tetraviruses studied have shown a huge range of effects on hosts depending on larval age and dosage, and it is possible that they are successful in controlling populations due to survival of the host and hence also of the virus, which promotes further spread and epizootics. They appear to be transmitted horizontally by oral ingestion, although there is some evidence for vertical transmission. The capsids of the virus appear to be sensitive to direct sunlight but are environmentally resistant to desiccation and proteases (Hanzlik *et al.*, 1993).

Application

As with many other RNA viruses, little attention has been paid to tetraviruses as biological control agents, despite their known effects on some pest hosts. Some laboratory studies suggest that this group may have good potential as microbial insecticides. The biggest obstacle is the difficulty in producing the virus, but their small genome may make it relatively simple to overcome these difficulties in tissue culture production, or by production in plant tissues. They also have potential as vectors of targeted RNA delivery to cells (Gordon and Hanzlik, 1998).

7.6.11 Mass production of insect viruses

As obligate intracellular parasites, viruses can develop only in live hosts, either intact animals, such as caterpillars or live cell cultures. For mass production, host larvae are reared in cups containing an artificial diet and infected by spraying virus on the food 1 week after the host eggs have been

added to the diet cups. At the end of the second week, most larvae are dead and the cadavers can be collected, homogenized and strained through cheese cloth, and the virus particles harvested via centrifugation. Optimal viral inoculation rates can be determined by comparing yields from a series of different doses per cup. Low doses may not infect all larvae. High doses kill larvae while they are still small, reducing viral yield per larva. In Brazil, laboratory production was replaced with outdoor virus farming, in which natural host outbreaks were located and infected and the virus-infected insects later harvested.

Insect cell cultures can be used to produce insect viruses (Granados *et al.*, 1987; King *et al.*, 1988; Lynn *et al.*, 1990; Lenz *et al.*, 1991) and these cell lines are being used by the pharmaceutical industry to produce genetically modified NPVs for the production of materials for medical use.

7.7 Bacterial Insecticides: *B. thuringiensis*

The Enterobacteriaceae, Micrococcaceae, Pseudomonadaceae and Bacillaceae are the major families containing insecticidal bacteria. The active ingredient in most bacterial insecticides is rod-shaped bacteria from the genus *Bacillus*. About 100 species are reported to attack insects, and four of them – *B. thuringiensis*, *Bacillus popilliae*, *Bacillus lentimorbus* and *Bacillus sphaericus* – have been studied extensively as insect-control agents. The chief advantages of bacterial species are their narrow host range and their lack of toxic effects on non-target species, including natural enemies of the pest and humans.

Bacillus infection was first reported on Japanese beetles as 'doom' or 'milky spore disease' in 1948. It was composed of two bacterial species, *B. lentimorbus* and *B. popilliae*, milky spore disease is still marketed, while the second species to be marketed was *B. thuringiensis* var. *kurstaki* (BTK), which is the most effective against caterpillars. Subsequently, a number of strains were discovered and formulated commercially for the management of a variety of insect pests. The mosquito pathogen *B. sphaericus* has also been studied as a commercial possibility (Olkowski *et al.*, 1995).

The four *Bacillus* species described above are commonly found in soil and all are spore forming. The spore is the stage that tides the bacteria over from one favourable period to next, and it has the ability to withstand degradation by ultraviolet light, drought and other unfavourable environmental conditions, at least for a short time. *B. thuringiensis* and *B. popilliae* also produce protein crystals with their sporulating cells. These crystals are their important toxic agents. Bacteria that do not form spores do not persist long enough to control pests once applied to a crop; consequently, they are considered poor candidates for development as insecticides (Olkowski *et al.*, 1995).

B. thuringiensis (*Bt*) was named after Thuringia, the town in Germany where it was discovered in 1911 in diseased Mediterranean flour moths. *Bt* is a highly effective insecticide against lepidopteran larvae (Rogoff and Yousten, 1969; Bulla *et al.*, 1975). It is a Gram-positive, soil-dwelling bacterium, commonly used as a biological alternative to a pesticide; alternatively, the Cry toxin produced by the bacterium may be extracted and used directly as a pesticide. *Bt* also occurs naturally in the gut of caterpillars of moths and butterflies, as well as on the dark surface of plants (Madigan and Martinko, 2005).

At least 35 *Bt* varieties have been identified, so far each of which attacks different groups of insect hosts through the toxic protein crystals present in its spores. The number of strains under investigation for possible culture for various uses has increased the commercial interest of growers. Some companies maintain hundreds of cultures or more. The first commercial *Bt* product entered the market in 1958. It was formulated from the variety *kurstaki* (BTK) and was effective only against the caterpillar (larval) stage of moths and butterflies of order Lepidoptera. Over

the years, improved BTK strains have been formulated as a variety of products. In the 1980s, additional *Bt* stains that kill insects in other orders are *Bt* var. *israelensis* (BTI), which attacks and kills the larvae certain mosquitoe larvae, black flies and fungus gnats in the insect order Diptera, and *Bt* var. *san diego* and *tenebrionis*, which are toxic to certain beetles of order Coleptera. *Bt* appears as fine tan to brown powder or as reddish-orange granules consisting of spores or toxic crystals and inert ingredients (Olkowski *et al.*, 1995).

7.7.1 Mode of Action

Feeding by the target organism is necessary as the *Bt* toxin is a stomach poison. The crystals and subunits inside the spores ingested by an insect are dissolved by the high alkaline pH of the midgut (Karim *et al.* 2000). The components of the crystal attach to the gut wall, blocking the enzyme systems that protect the insect gut from its own digestive juices. In a short time, holes appear in the gut wall, allowing the gut contents to enter the insect's body cavity and bloodstream. This initial poisoning cause the insect to stop feeding and may also lead to paralysis. Upon ingestion of the proteinaceous crystal by susceptible larvae, proteolytic enzymes in the larval gut juice hydrolyse the crystal protein into as yet undefined toxic moieties. This is followed by paralysis and eventual death of the larvae (Angus, 1954, 1956; Angus and Heimpel, 1959).

A caterpillar that ingests *Bt* may live for several days, but after infection it does not continue to feed and therefore causes no further damage to the plant. Dead and dying caterpillars turn a dark colour, remaining attached to leaves for a few days, usually hanging at a 90° angle towards the ground (Olkowski *et al.*, 1995).

Although *Bt* multiplies within the body of the infected insect, spores and toxic crystals are almost never produced. Consequently, insects killed by *Bt* do not serve as a source of new infections, and several applications of *Bt* may be required to control an insect infestation, as with a conventional insecticide. Also, when sprayed outdoors, *Bt* is broken down with in 1 to several days by ultraviolet radiation – another reason why repeated application may be necessary (Olkowski *et al.*, 1995).

Bt is non-toxic to natural enemies of *Bt*-susceptible insects; thus, target pests that escape the effect of *Bt* application are often attacked afterwards by their predators and parasitoids, further lowering the pest population and reducing the amount of pesticide needed (Olkowski *et al.*, 1995).

7.7.2 Application

All *Bt* pesticides are regarded as environmentally friendly, by virtue of being highly target-specific in general with little effect on humans, wildlife, pollinators and most other beneficial insects. Spores and crystalline insecticidal proteins produced by *Bt* have been used as an active ingredient to control insect pests since 1920 (Dean, 1984). They are now used as specific insecticides under trade names such as Dipel and Thuricide. The Belgian company Plant Genetic Systems was the first company (in 1985) to develop genetically engineered (tobacco) plants with insect tolerance by expressing *cry* genes from *B. thuringiensis* (Höfte *et al.*, 1986; Peggy, 2008).

Bt-based insecticides are often applied as liquid sprays on crop plants, where the insecticide must be ingested to be effective. It is thought that the solubilized toxins form pores in the midgut epithelium of susceptible larvae. Recent research has suggested that the midgut bacteria of susceptible larvae are required for *Bt* insecticidal activity (Vaeck *et al.*, 1987).

BTI is widely used as a larvicide against mosquito larvae, where it is also considered an environmentally friendly method of mosquito control.

7.8 Entomopathogenic Nematodes

Entomopathogenic nematodes (EPNs) can also serve as important biocontrol agents for

a number of insect pests (Divya and Sankar, 2009). Seventy-three described EPN species have been identified (64 species of *Steinernema*, eight species of *Heterorhabditis* and one species of *Neosteinernema*) from various insects or from the soil worldwide (Grewal *et al.*, 2001). Nematode infection usually occurs in the haemocoel, but some groups such as the Phaenopsitylanchidae (e.g. *Deladenus*) and Iotonchiidae (e.g. *Paraiotonchium*) may invade the sexual organs, causing infertility debilitation, castration or death. Obligate parasitic nematodes of these sorts are relatively host specific, being associated with one or a small group of hosts, on the other hand steinernematids and heterorhabditids have broad host ranges.

The body of nematodes is non-segmented, covered with an elastic cuticle that is translucent and is usually elongated and cylindrical. Nematodes are multicellular animals that possess well-developed excretory, nervous, digestive, muscular and reproductive systems but lack circulatory and respiratory systems. The digestive system consists of a mouth, buccal cavity, intestine, rectum and anus. Nematode taxonomy is based primarily on the sexual characteristics of adults; consequently, immature stages are difficult to identify without molecular techniques.

EPNs are cosmopolitan in distribution and occupy nearly in all habitats. Their interactions with insects range from phoresy to parasitism. Some nematodes, such as *Deladenus* (*Beddingia*) *siricidicola* (Bedding), have complex life histories with both free-living and parasitic life cycles. Whereas, commercially reared insect-parasitic nematodes (*Steinernema* and *Heterorhabditis* spp.) have simple life cycles (van Driesche *et al.*, 2008).

The dauer or infective juvenile stage is the only free-living life stage for commercially reared nematode families (Steinernematidae and Heterorhabditidae). The dauer stage is the third juvenile stage which infects new hosts. This is the life stage found in commercial nematode products. This stage seeks a host and enters it via natural openings or through thin sections of cuticle. Within a few hours of host penetration, infective juveniles release symbiotic bacteria, then moult to the fourth stage and later to the adult. In the genus *Steinernema*, adults mate and females produce eggs, which hatch and develop through to an adult, producing eggs. These eggs usually develop into infective juveniles. There are usually three generations inside a single host. In the genus *Heterorhabditis*, infective juveniles develop into hermaphrodites that produce eggs. The next generation has three sexes: males, true females and hermaphrodites. The rest of the life cycle is same as for *Steinernema* (van Driesche *et al.*, 2008).

Host-finding by nematodes may be an active process in which nematodes move towards and recognize hosts using cues such as bacterial and carbon dioxide gradients and host faecal components (Grewal *et al.*, 1993), or compounds released from plant roots in response to root herbivory (Rasmann *et al.*, 2005). Nematode species vary in their host-searching strategies, some being ambush predators and others active hunters (Kaya *et al.*, 1993). For steinernematid and heterorhabditid nematodes, host penetration is an active process in which juveniles directly enter the mouth, anus or spiracles or use proteases to penetrate the integument. Nematode infection takes place in the haemocoel. Infection produces relatively few external signs prior to death. The internal effects of infection, however, may be profound. Sterility is induced by several groups of nematodes, including *Deladenus siricidicola*, the species which is responsible for suppressing wood wasps in Australia (van Driesche *et al.*, 2008).

Of the nine families of parasitic nematodes infecting insects, only the Steinernematidae and Heterorhabditidae can be reared economically for commercial use. These families can be reared easily if provided with their symbiotic bacteria and a non-living medium. Steinernematidae and Heterorhabditidae can kill their host in 2–3 days, a much shorter time than for other groups of nematodes. This occurs because these nematodes have symbiotic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.)

that kill the host by septicaemia (Burnell and Stock, 2000). Infective juvenile nematodes reach the haemocoel by penetrating the midgut wall or the host integument. *Xenorhabdus* spp. or *Photorhabdis* spp. are released into the host haemocoel by the nematode by defaecation and then kill the host (van Driesche *et al.*, 2008).

Nematodes feed on symbiotic bacteria and mature to adults. Infective juvenile nematodes after several generations, exit the decomposing host cadaver (Gaugler and Kaya, 1990; Kaya, 1990; Tanada and Kaya, 1993).

All nematodes can be reared in living hosts, for example, heterorhabditid and steinernematid nematodes, the groups of greatest commercial interest, may be reared in larvae of the greater wax moth (*G. mellonella* L.). Methods for rearing the insect host by nematode infection, harvesting and storing the juvenile nematodes of these families have been described (Woodring and Kaya, 1988). Nematodes are harvested by allowing them to swim away from the host cadaver into a collection device. For commercial production of heterorhabditid and steinernematid nematodes, non-living media can be used in large-scale, automated systems. Glaser *et al.* (1940) were the first to attempt large-scale rearing of such nematodes on non-living media. Such media must: (i) use sterile ingredients to avoid unwanted bacterial contamination; (ii) retain the nematodes' specific symbiotic bacterium (such as *Xenorhabdus* spp. and *Photorhabdus* spp.); and (iii) provide all the necessary nutrients for growth (Lunau *et al.*, 1993).

Historically, there were three challenges to the development of large-scale efficient nematode rearing: (i) using liquid rather than solid culture media (Friedman, 1990); (ii) identifying culture conditions that promoted high yield; and (iii) identifying inexpensive nutrients. Effective media are now known, the compositions of which are trade secrets of the producers. To support rearing in liquid medium in large tanks, it is necessary to add oxygen and to take into account the susceptibility of nematodes to damage from shearing caused by stirring.

7.9 Insect Repellents

These are chemicals that prevent insect damage of plants by rendering them unattractive, unpalatable or offensive, and include a wide range of chemicals such as volatile compounds that are active in vapour form (e.g. DEET) or form persistent chemicals such as Bordeaux mixture, which acts as feeding deterrent (Metcalf and Luckmann, 1994). Literally, a repellent has been defined as a chemical that causes insects to orient away from their source. There are also insect-repellent products available based on sound production, particularly ultrasound (high-frequency sounds that are inaudible to humans). These electronic devices have been shown to have no effect as a mosquito repellent by studies done by the EPA and many universities. Common insect repellents include: (i) azadiractin from the neem tree (*Azadiracta indica*) against locusts; (ii) 6-methoxy-benzoxazolinone (MBOA) used in maize varieties against maize borer (*Ostrinia nubilalis*); (iii) 4-6-dinitro-o-cresol (creosote) against chinch bugs (*Blissus leucopterus*); (iv) essential oil of the lemon eucalyptus (*Corymbia citriodora*) and its active compound p-menthane-3,8-diol (PMD), icaridin (also known as picaridin, Bayrepel, and KBR 3023), nepetalactone (also known as 'catnip oil'), citronella oil, *Achillea alpina* oil against mosquitoes; (v) beautyberry (*Callicarpa Americana*; Janice, 2001), castor oil (*Ricinus communis*) and cinnamon leaf oil against mosquito larvae; (vi) clove oil against mosquitoes; (vii) eucalyptus oil against mosquitoes, flies and dust mites; (viii) fennel oil (*Foeniculum vulgare*) against mosquitos; (ix) garlic (*Allium sativum*) against rice weevils and wheat flour beetles; (x) geranium oil (*Pelargonium graveolens*), lavender against numerous insects); (xi) lemongrass oil (*Cymbopogon* sp.) against mosquitos; (xii) marigolds (*Tagetes* sp.), marjoram against the spider mites (*T. urticae* and *Eutetranychus orientalis*); (xiii) neem oil against mosquitoes, their larvae and a plethora of other insects including those that cause problems in agriculture; (xiv) oleic acid against bees and ants (acts by

simulating the 'smell of death' produced by their decomposing corpses); (xv) peppermint (*Mentha* × *piperita*) against mosquitoes; (xvi) pennyroyal (*Mentha pulegium*) against mosquitoes and fleas (but very toxic to pets); (xvii) pyrethrum (from *Chrysanthemum* spp., particularly *Chrysanthemum cinerariifolium* and *Chrysanthemum coccineum*), and rosemary (*Rosmarinus officinalis*) against mosquitoes; (xviii) Spanish flag (*Lantana camara*) against the tea mosquito (*Helopeltis theivora*); (xix) *Solanum villosum* berry juice against *Stegomyia aegypti* mosquitoes); and (xx) tea tree oil and thyme (*Thymus* spp.) against mosquitoes.

7.10 Conclusion

In conclusion, biopesticides are environmentally friendly and offer a vital component for sustainable agriculture without the type of environmental hazards associated with chemical pesticides.

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8 Entomopathogenic Nematodes as Tools in Integrated Pest Management

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8.1 Introduction

Entomopathogenic nematodes (EPNs) are attractive alternatives to chemical insecticides, particularly for soil-inhabiting pests. Two genera, *Heterorhabditis* (Strongyloides: Heterorhabditidae) and *Steinernema* (Strongyloides: Steinernematidae), have mainly been used in biological control. Over 70 species of *Steinernema* and 15 of *Heterorhabditis* have been discovered worldwide. Due to the ease of nematode mass production, many products have been developed and are sold as biological insecticides. EPNs are well suited for pest control in integrated pest management (IPM) systems because they are safe, can easily be applied using sprinkling cans and conventional spray equipment, and are compatible with many agrochemicals. Application via irrigation has further improved grower acceptance. Currently, nematodes are most widely used for the control of citrus root weevil (*Diaprepes* spp.) in Florida, black vine weevil (*Otiorhynchus* spp.) and fungus gnats (*Bradysia* spp.) in Europe, mushroom sciarid fly (*Lycoriella* spp.) in Europe and North America, and white grubs in turfgrass in North America and Europe. In fact, EPNs replaced the then most widely used insecticide, aldrin, as the most effective control measure for black vine weevil in greenhouse and nursery industries in Europe when they were first introduced. They were also the only curative control method available for hunting billbug (*Sphenophorus* spp.) on golf courses in Japan and for black vine weevil and cranberry girdler (*Chrysoteuchia topiaria*) in cranberry bogs in North America until the introduction of preventive-use insecticides such as imidacloprid and halofenozide. At present, they are also the only method of controlling turfgrass pests including white grubs on municipal properties in cities across Canada. Although EPNs possess a broad host range, species and strains differ in their effectiveness against specific insect pests. Much research has been carried out on these nematodes, and several comprehensive reviews have been published. These nematodes have also been the subject of several notable books. George O. Poinar Jr wrote the first book on this subject, *Entomogenus Nematodes*, in 1975. This was followed by another volume by the same author, *Nematodes for Biological Control of Insects*, in 1979. Randy Gaugler and Harry K. Kaya edited *Entomopathogenic Nematodes in Biological Control* in 1990, and Robin A. Bedding, Raymond J. Akhurst and Harry K. Kaya edited *Nematodes and the Biological Control of Insects* in 1993. Randy Gaugler edited another volume, *Entomopathogenic Nematology*, in 2002 and

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the latest volume, *Nematodes as Biocontrol Agents*, was edited by Parwinder S. Grewal, Ralf Udo Ehlers and David I. Shapiro-Ilan in 2005. In this chapter, more recent developments in our understanding of the biology, safety, production, formulation, quality, application strategy and technology, and efficacy of EPNs in different ecosystems are briefly reviewed. Recent research on EPN compatibility with agrochemicals and other biological control agents, interactions with plant-parasitic nematodes, conservation approaches and genetic improvements are also summarized. Finally, a comprehensive list of insect pest species that have been targeted for control with specific EPN species under laboratory, greenhouse, and field conditions is provided.

8.2 Biology

EPNs are interesting parasites that have a mutualistic association with entomopathogenic bacteria (EPB): *Photorhabdus* spp. for *Heterorhabditis* and *Xenorhabdus* spp. for *Steinernema*. This partnership with bacteria enables the nematodes to exploit a diverse array of insects as hosts. Nematode infective juveniles (IJs), the only stage that is found in the soil, seek out and penetrate a suitable insect host through natural body openings and cuticle. Upon reaching the haemocoel, IJs release symbiotic bacteria, which multiply rapidly, killing the host within 2–3 days. Insects killed by the nematodes are flaccid, do not give off a foul smell and have conspicuous colours. For example, insects killed by *Steinernema carpocapsae* are yellow, while those killed by *Heterorhabditis bacteriophora* are reddish brown. After the death of the host, the nematodes feed on the bacteria (Poinar and Thomas, 1966) and insect body contents, and reproduce, completing one to three generations. When host nutrients are depleted, the next-generation IJs are produced, which exit the carcass to seek out new insect hosts and continue their life cycle anew (Fig. 8.1).

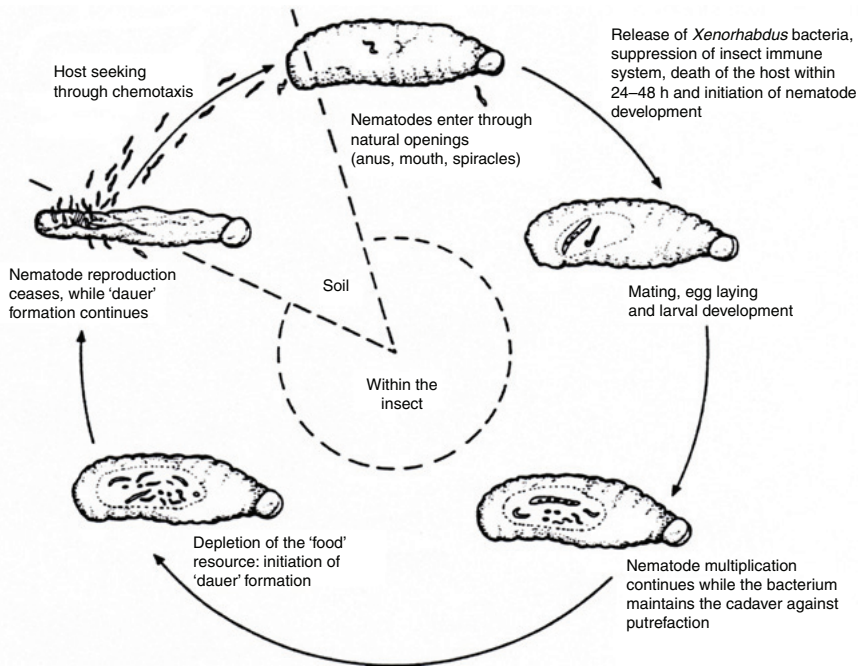

Fig. 8.1. Generalized life cycle of *Steinernema* nematodes. Note that all nematode stages occur in the host insect except for the infective juveniles (IJs) which exit the host cadaver to seek out new hosts in the soil. The *Heterorhabditis* life cycle is similar except that the IJs entering a new host release *Photorhabdus* bacteria and produce hermaphrodites.

The IJs serve several important functions in the life cycle of EPN–EPB symbiotic complexes. The IJs retain and nourish the EPB; survive under environmental conditions detrimental to other life stages; disperse, find, select and invade hosts; and finally transmit EPB into the insect haemocoel. The natural habitat for EPNs is the soil, which is a difficult environment for the IJs to persist in and locate insect hosts. Nevertheless, EPN IJs have been isolated from soils throughout the world, in ecosystems ranging from sub-Arctic to arid, and from temperate to tropical climates (Poinar, 1990; Hominick, 2002). This feat has been accomplished by EPN IJs by the evolution of diverse survival strategies and mechanisms to resist environmental stresses (see Grewal *et al.*, 2011, for a detailed treatment of this subject).

Recent research indicates that EPN IJs and EPB survive cooperatively, conserving energy while searching for their host (An and Grewal, 2010). While IJs do not feed in the soil environment and have an overall reduced metabolism, the symbiotic bacteria persist in the gut of the non-feeding IJs by lowering their metabolism through cellular acidification and by shedding mobility (An and Grewal, 2010). Such a cooperative endurance strategy maximizes the survival of this symbiotic couple until an insect host is found for both. Once the bacteria are released into the haemocoel by the IJs, the bacteria initiate their multiplication and produce an arsenal of virulence factors (ffrench-Constant and Bowen, 2000; ffrench-Constant and Waterfield, 2006; Goodrich-Blair and Clarke, 2007; An *et al.*, 2009) ensuring rapid insect mortality. However, Eleftherianos *et al.* (2010) showed that both the nematodes and the bacteria contributed separately to suppression of the insect immune response confirming an earlier report by Burman (1982) that axenic EPNs produced a toxin. Bioconversion of the insect cadaver by bacterial exoenzymes allows the bacteria to multiply and serve as food for the nematodes. The bacteria also produce secondary metabolites to prevent invasion of the insect cadaver by competing soil microbes (Webster *et al.*, 2002), enabling the nematodes and bacteria to reassociate in a protected niche. Indeed, phylogenetic congruence has been shown between *Heterorhabditis* and *Photorhabdus* spp., indicating significant coevolution of the two organisms at both species and strain levels (Maneesakorn *et al.*, 2011).

Recent research also suggests that *Photorhabdus* and *Xenorhabdus* bacteria use vastly different molecular mechanisms of pathogenicity, even in the same insect host (An and Grewal, 2010). For example, in *Photorhabdus temperata*, a *lysR* gene encoding a transcriptional activator is induced, while the genes *yijC* and *rseA* encoding transcriptional repressors are induced in *Xenorhabdus koppenhoeferi* in European chafer, *Rhizotrogus majalis*. The lipopolysaccharide synthesis gene *lpsE* is induced in *X. koppenhoeferi* but not in *P. temperata*. Except for the *tcaC* and haemolysin-related genes, other virulence genes are different between the two bacteria. Genes involved in the tricarboxylic acid cycle are induced in *P. temperata*, whereas those involved in the glyoxylate pathway are induced in *X. koppenhoeferi*, suggesting differences in metabolism between the two bacteria in the same insect host. Upregulation of genes encoding different types of nutrient-uptake systems further emphasize the differences in nutritional requirements of the two bacteria. *P. temperata* displays upregulation of genes encoding the siderophore-dependent iron-uptake system, while *X. koppenhoeferi* upregulates genes encoding a siderophore-independent iron-uptake system. *P. temperata* induces genes for amino acid acquisition, but *X. koppenhoeferi* up-regulates the *malF* gene encoding a maltose uptake system. These differences highlight how organisms with apparently similar biology can evolve different molecular mechanisms for the same goal.

Another major advance in the field of entomopathogenic nematology has been the recognition of dichotomy in the host-finding behaviour of EPN species. Moyle and Kaya (1981) were the first to note differences in vertical and horizontal dispersal of different

nematode species. They noted that *S. carpocapsae* did not move much from the site of application, whereas *Steinernema glaseri* moved long distances laterally. This was confirmed by several subsequent studies. However, it was not until Ishibashi and Kondo (1990) described the nictation behaviour of *S. carpocapsae* IJs that Gaugler and Campbell (1991) proposed that the ambushing type of host-finding behaviour may be a reason for the limited movement of some EPN species in the soil. A series of studies conducted in Gaugler's laboratory then described the ambushing and cruising types of host-finding behaviours of different nematode species. Lewis *et al.* (1992) showed differences in the response of *S. carpocapsae* and *S. glaseri* to long-range volatile cues, while Campbell and Gaugler (1993) characterized the nictation behaviour in several species. Grewal *et al.* (1994b) demonstrated that *Steinernema feltiae* and *Steinernema riobrave* use an intermediate type of strategy in the middle of the continuum between the two extreme modes of foraging behaviour, ambushing and cruising exhibited by *S. carpocapsae* and *H. bacteriophora*, respectively. This research helped to select the most effective nematode species against target insect pests by matching nematode host-finding behaviour to the life-history parameters of target pest species (Gaugler *et al.*, 1997). It was shown that nematode species that use the ambushing type of foraging behaviour are better adapted to finding and parasitizing hosts that are highly mobile and remain on the soil surface, while nematode species that use the cruising type of foraging behaviour are more adapted to finding and parasitizing sedentary hosts that feed deep in the soil.

8.3 Safety

Although the laboratory host-range of entomopathogenic nematodes is quite broad (Poinar, 1975, 1979), they have been shown to be safe to humans, animals and non-target invertebrates. Initially, Poinar (1975) reported on the safety of nematodes to mammals, Kaya *et al.* (1982) to the honeybee and Georgis *et al.* (1991) to soil invertebrates. Many studies have been conducted on the non-target effects of EPNs since the 1980s, and at least two comprehensive reviews on this subject have been published (Akhurst and Smith, 2002; Ehlers, 2005). Overall, EPNs have been found to be safe to humans, pets and other animals, and to non-target invertebrates. They also have no significant negative effect on soil microbial biomass, soil respiration and nitrogen pools (de Nardo *et al.*, 2006). Based on the available safety data, EPNs were exempted from registration requirements by the US Environmental Protection Agency (EPA). Ironically, a strain of bacteria closely related to the symbiotic bacteria of heterorhabditid nematodes was isolated from human wounds independently in the USA and Australia (Akhurst and Smith, 2002). These strains were later identified as *Photorhabdus asymbiotica* and were considered opportunistic human pathogens (Costa *et al.*, 2008; Kuwata *et al.*, 2008). Astonishingly, *P. asymbiotica* has recently been found associated with *Heterorhabditis indica* (Costa *et al.*, 2008; Kuwata *et al.*, 2008) and a newly described nematode species, *Heterorhabditis gerrardi* (Plichta *et al.*, 2009), which raises concerns about the safety of these nematode–bacteria complexes. However, *P. asymbiotica* has never been reported to be associated with *H. bacteriophora* and was found not to support the recovery and growth of *H. bacteriophora* (Gerrard *et al.*, 2006). The comparative genomics of *Photorhabdus luminescens* and *P. asymbiotica* has also revealed major genomic differences between strains of *Photorhabdus* exhibiting virulence against insects or humans (Tounsi *et al.*, 2006). Clearly, more research is needed on these bacteria, but caution should be exercised to avoid contact between these bacteria and human wounds.

8.4 Production

Rudolf Glaser was the first to establish a culture of the EPN *S. glaseri* and to conduct

field trials for the control of the introduced Japanese beetle (*Popillia japonica*) in New Jersey (Glaser, 1931). Although mass production in artificial media was attempted and large-scale field releases of *S. glaseri* were carried out, the Japanese beetle control programme failed due to the lack of knowledge about the symbiotic relationship between nematodes and bacteria (Gaugler *et al.*, 1992). The remarkable discovery of the symbiotic relationship between *Steinernema* and the bacterium *Achromobacter nematophilus* was made by Poinar and Thomas (1966). This bacterium was later named *Xenorhabdus nematophilus*. Another nematode genus, *Heterorhabditis*, with a biology similar to that of *Steinernema*, was described by Poinar (1976). The bacterial symbiont of this nematode was first described as *Xenorhabdus luminescens* but was later transferred to a new genus, *Photorhabdus*. It is now understood that all *Steinernema* spp. have mutualistic symbioses with *Xenorhabdus* spp., and all *Heterorhabditis* spp. with *Photorhabdus* spp. (Boemare, 2002; Griffin *et al.*, 2005).

Indeed, the discovery of symbiosis between EPNs and bacteria was a major turning point in the development of nematodes as commercial biological control agents. Exploiting the discovery of the symbiotic relationship between nematodes and bacteria, Robin Bedding was the first to establish a successful mass-production system, which has come to be known as a solid culture due to his clever use of a polyether polyurethane sponge as a three-dimensional support structure allowing nematodes to move through the matrix and provide air exchange (Bedding, 1981, 1984a). He demonstrated that the nematodes could be mass produced on symbiotic bacteria by impregnating the sponge with an artificial diet. This led to the formation of the first commercial company, Biotech Australia, selling nematodes for control of black vine weevil in Australia and Europe. The first flask-scale liquid culture of EPNs was developed by Stoll (1952) and the first attempt to use bioreactors was described by Pace *et al.* (1986). However, the first commercial production of EPNs in liquid culture was established by a team of researchers led by Milton Friedman in 1990 at Biosys, Inc. in Palo Alto, California, USA. This was soon followed by MicroBio, a company based in Littlehampton, UK, which established liquid production of *S. feltiae*. Surrey and Davies (1986) were the first to report on the liquid culture of heterorhabditids, but Ehlers *et al.* (1998) reported the first successful commercial-scale liquid production of heterorhabditids. More detailed treatment of this topic can be found in Friedman (1990) and Ehlers and Shapiro-Ilan (2005).

8.5 Formulation

Although EPN IJs can be stored in water for several months in refrigerated bubbled tanks, the high cost and difficulties of maintaining quality precludes the routine use of this method. Settling of nematodes, high oxygen demand, the sensitivity of some species to low temperature, susceptibility to microbial contamination and the effect of antimicrobial agents on nematode longevity are some of the major factors influencing nematode quality during storage in water (Grewal, 2002). Therefore, nematodes are formulated to improve their storage stability. Although the overall concept of nematode formulations is similar to traditional pesticide formulations, nematodes present unique challenges. High oxygen and moisture requirements of concentrated nematodes, sensitivity to temperature extremes and the behaviour of IJs limit the choice of the formulation method and ingredients. Major goals of developing nematode formulations include maintenance of quality, enhancement of storage stability, improvements in ease of transport and use, reductions in transport costs and enhancement of nematode survival during and after application.

Two distinct approaches have been used to formulate nematodes for storage and transport. In the first approach, the nematodes are placed in inert carriers that allow free gas exchange and movement of nematodes; in the second, functional

ingredients are added to reduce nematode activity and metabolism. Although the placement of nematodes in inert carriers such as sponges or vermiculite provides a convenient means of shipping small quantities of nematodes, the high activity of nematodes rapidly depletes their stored energy reserves. Sometimes, the nematodes even escape from the inert carriers and dry out. Therefore, formulations have been developed in which the mobility and metabolism of nematodes is minimized by physical trapping, the inclusion of metabolic inhibitors or by induction of partial anhydrobiosis (i.e. life without water).

Inert carriers such as polyether/polyurethane sponges and vermiculite are widely used for the storage and transport of small quantities of nematodes throughout the nematode industry. These formulations are easy and relatively cheap to produce but require constant refrigeration as the nematodes remain active, freely moving in or on the substrates. The shelf life of these formulations under refrigeration (2–10°C) varies from 1 month to 3–4 months depending on the nematode species (Grewal, 2002). The strict refrigeration requirement, even during transport, makes these formulations very expensive to the end user.

Active carrier formulations include functional ingredients that physically trap nematodes to reduce their movement, use metabolic inhibitors or reduce nematode activity and metabolism by the induction of partial anhydrobiosis. The nematodes are physically trapped in alginate and flowable gel formulations that contain sufficient moisture to prevent induction of nematode anhydrobiosis. In one formulation, sheets of calcium alginate spread over plastic screens have been used to trap the nematodes (Georgis, 1990). Trapping of nematodes in alginate gels allows storage at room temperature. For example, in one alginate gel formulation, *S. carpocapsae* could be stored for 3–4 months at 25°C and *S. feltiae* for 2 weeks to 1 month (Grewal, 2002). In another formulation, the nematodes were mixed in a viscous flowable gel or paste to reduce nematode activity (Georgis, 1990), but the room temperature storage stability in this medium was lower than the alginate formulation. Nematodes have also been formulated in various heteropolysaccharides (agarose, carbopol, carrageenan, dextran, guar gum and gellan gum) surrounded by a paste of hydrogenated oil. Storage of *S. carpocapsae* for up to 35 days at room temperature was reported for this hydrogenated oil formulation (Chang and Gehret, 1995). A liquid concentrate was developed for the transport of nematodes in bulk tanks, containing a proprietary metabolic inhibitor to reduce nematode oxygen demand (Grewal, 1998).

Simons and Poinar (1973) reported that, if desiccated slowly, IJs of *S. carpocapsae* can enter into partial anhydrobiosis. Based on these findings, Bedding (1988) developed a 'clay sandwich' formulation in which nematodes were placed in layers of clay to remove any surface water and induce partial anhydrobiosis. Scientists at Biosys, Inc. (Palo Alto, California) developed an alginate formulation in which sheets of calcium alginate spread over plastic screens were used to trap nematodes (Georgis, 1990). Bedding and Butler (1994) reported a formulation in which the nematode slurry was mixed with a powder of anhydrous polyacrylamide to achieve a water activity (A_w) of between 0.800 and 0.995. The nematodes were partially desiccated, but survival at room temperature was low. A composition of 2–3 g of polyacrylate with proprietary additives (Nemagel) to 250 ml of nematode slurry containing 40 million *S. feltiae* resulted in 2 years' survival at 4°C (Hokkanen and Menzler-Hokkanen, 2002). At room temperature, 1 year's survival was recorded in 25 ml bags with 2 million *S. feltiae*. The A_w in this formulation was much higher (>0.995). In a slightly different formulation where nematode slurry (concentrated nematodes) was mixed in attapulgite or bentonite clay, Strauch *et al.* (2000) reported that *H. bacteriophora* (hybrid strain) and *H. indica* (LN2 strain) only survived for 2 weeks and 1 week, respectively, at 25°C. At 5°C, the survival of *H. bacteriophora* was superior in sponge compared with clay, but that of *H. indica*

was superior in clay compared with the sponge at 15°C.

Capinera and Hibbard (1987) were the first to develop a granular formulation in which nematodes were partially encapsulated in lucerne meal and wheat flour. Later, Connick *et al.* (1993) described an extruded or formed granule in which nematodes were distributed throughout a wheat gluten matrix. This 'Pesta' formulation included a filler and humectant to enhance nematode survival. The process involved drying of granules to lower moisture to prevent nematode migration and reduce risk of contamination. However, granules rapidly dry out during storage resulting in poor nematode survival. A major leap in the development of nematode formulations was reported by Silver *et al.* (1995) who developed the first water-dispersible granular formulation, in which the nematodes were encased in 10–20 mm diameter granules consisting of a mixture of various types of silica, clay, cellulose, lignin and starches. The granular matrix allows the nematodes access to oxygen during storage and transport. At optimum temperature, the nematodes enter into a partial anhydrobiotic state due to the slow removal of body water by the substrate. The induction of partial anhydrobiosis is usually evident within 4–7 days by a three- to fourfold reduction in oxygen consumption by the nematodes following an initial increase (Grewal, 2000a,b). This formulation offered several advantages over other formulations. This was the first commercial formulation that enabled storage of *S. carpocapsae* for over 6 months at 25°C at a nematode concentration greater than 300,000/g (Grewal, 2000a). This shelf life represents an extension of IJ longevity by 3 months compared with nematodes stored in water (Grewal, 2000a,b). The water-dispersible granules also enhanced nematode tolerance to temperature extremes (Grewal and Jagdale, 2002), enabling: (i) easier and less expensive transport; (ii) improved ease of use of the nematodes by eliminating time and labour-intensive preparation steps; (iii) a decreased container size and coverage ratio; and (iv) reduced disposal material (i.e. screens and containers). However, this formulation was found to be prone to microbial contamination when stored at room temperature. Therefore, antimicrobial and antifungal agents are often added to suppress the growth of contaminating microbes. A detailed discussion of the factors affecting the survival of EPNs in formulations can be found in Grewal (2002) and Grewal and Peters (2005).

8.6 Quality

Methods have been developed for the rapid quality assessment of commercially produced nematodes. Miller (1989) reported the development of the first quality-control method to assess the virulence of commercially produced *S. carpocapsae*. This method is called the one-on-one *Galleria mellonella* bioassay (Converse and Miller, 1999). Grewal *et al.* (1999a) developed the sand-well method, which is suitable for routine quality assessment of most EPN species at low concentrations. Quality-control methods have been reviewed extensively by Grewal (2002) and Grewal and Peters (2005). Additional methods that serve as indicators of nematode infectiousness, pathogenicity or general quality have also been developed. A detailed listing of the different methods along with the detailed procedure for each method can be found in Grunder *et al.* (2005). Gaugler *et al.* (2000) were the first to assess the quality of commercially produced nematodes, raising awareness about the importance of effective quality control during commercialization of EPNs.

8.7 Application Strategies and Technology

EPNs are most widely used as inundative biological control agents for insect pests. They have proven most successful against insects in soil or cryptic habitats, where there is protection against environmental extremes including desiccation, UV

radiation and temperature. Nevertheless, applications against foliar pests have also been successful in certain circumstances (see section 8.10). Although nematodes are mostly applied as curative treatments, prophylactic application to protect seeds, seedlings and even trees from insect attacks have been advocated (see Grewal and Georgis, 1999, for a review). Delivery of EPNs directly through commercial plant-growing media for prophylactic control of pests in the potted-plant industry is also being explored (Deol *et al.*, 2006, 2011).

Nematodes can conveniently be applied using sprinkling cans and most conventional liquid pesticide, fertilizer or irrigation equipment. However, nematodes are sensitive to sheer and heat stress, require oxygen and will settle out of the spray solution. EPNs do not tolerate temperatures exceeding 30°C and require dissolved oxygen in the spray tank. With a density of about 1.05 g/cm^3, IJs are heavier than water and quickly settle. Larger nematodes settle more quickly than small nematode species (Wright *et al.*, 2005). If the tanks are agitated through excessive sparging (recirculation of the spray mix), or if the temperature in the tank rises above 30°C, the nematodes can be damaged. In addition, the shear forces experienced by the nematodes in most application equipment can be detrimental. Nilsson and Gripwall (1999) found that the survival of *S. feltiae* decreased by approximately 10% during a 20 min pumping period using a piston pump. They suggested that the probable reason for the viability decline was mechanical stress from the pump and nozzles, but might also have been caused by the rise in temperature of the liquid. In fact, lower-capacity pumps, such as a diaphragm or roller pump, are better suited for EPNs than a high-capacity centrifugal pump, which can contribute significant heat to the spray liquid (Fife *et al.*, 2007). Laczynski *et al.* (2007) reported that hydraulic agitation was more detrimental to nematodes than mechanical agitation. They also found that a lower initial temperature of the spray liquid yielded higher nematode viability compared with a higher temperature after hydraulic mixing. Nematode concentration had no effect, but air injection increased nematode viability. Brusselman *et al.* (2010) tested hydraulic, pneumatic and mechanical agitation systems in spray tanks for their effect on *S. carpocapsae* viability. Hydraulic agitation was tested using a centrifugal and a diaphragm pump. Only hydraulic agitation using the centrifugal pump damaged the nematodes, reducing their viability to 19% and their infectivity to zero. The temperature in the tank rose from 21.7 to 45.4°C, causing nematode mortality. The pneumatic agitation was ineffective in uniformly distributing the nematodes in the tank. They recommended the use of mechanical and hydraulic agitation using a diaphragm pump for the application of *S. carpocapsae*.

To reduce shear stress on EPNs, it is recommended that filters and sieves in nozzles should be at least 300 μm wide (50 mesh) or should be removed before nematode application. Nozzle apertures of over 500 μm are recommended for nematode application. Nematode species can also differ in shear sensitivity; IJs of *S. carpocapsae* are able to withstand greater pressure differentials (Fife *et al.*, 2003) and more intensive hydrodynamic conditions (Fife *et al.*, 2004) than those of *H. bacteriophora* and *Heterorhabditis megidis*. Fife *et al.* (2005) evaluated the flow characteristics of fan- and cone-type nozzles with respect to EPN damage. They found that the reduced flow area of the narrow, elliptic exit orifice of a flat-fan nozzle generated an extensional flow regime, where the tensile stresses that developed were large enough to cause nematode damage. The high rotational flow component within a cone nozzle did not produce hydrodynamic conditions detrimental to IJs. Fife *et al.* (2006) then used computational fluid dynamics to predict the damage to EPNs during passage through a hydraulic nozzle. Overall, it was concluded that common 01-type hydraulic nozzles are acceptable for EPN spray application provided the manufacturer's recommendations are followed. However, larger-capacity hydraulic nozzles are particularly

recommended for soil applications where a high volume of water is required.

Irrigation systems can also be used for applying most EPN species; however, high-pressure recycling pumping systems are not good delivery systems. Volumes of 0.08–0.24 L/m^2 (813–2439 L/ha) are recommended on most labels. Reed *et al.* (1986) were the first to apply nematodes through trickle irrigation, Wright *et al.* (1993) through centre-pivot irrigation, Cabanillas and Raulston (1996a,b) through furrow irrigation and Conner *et al.* (1998) through drip irrigation. Ellsbury *et al.* (1996) applied *S. carpocapsae* to maize by a lateral-move irrigation system and observed a threefold greater concentration of EPNs at the base of the plants by stem flow compared with the overall ground level. Successful subsurface application of *S. feltiae* against the billbug *Sphenophorus parvulus* in turfgrass with an adapted seed driller was reported by Shetlar *et al.* (1993). Peters and Backes (2003) discovered that adding 0.1% carboxymethycellulose decreased the sedimentation speed of nematodes, thus reducing nematode settling in irrigation systems as a result of the increased viscosity of the liquid. Overall, if used properly, irrigation systems can produce excellent and cost-effective EPN application in diverse cropping systems.

8.7.1 Soil application

A soil drench application rate of 1 billion nematodes per acre (= 2.5 billion/ha) is generally recommended to control most soil insects. For smaller areas, the recommended application rate is 250,000 nematodes/m^2 (Grewal and Peters, 2005). EPN IJs only fit into relatively large spray droplets that are not prone to drift (Lello *et al.*, 1996). Therefore, the application technology for soil fertilizers or irrigation rather than chemical pesticides, which is usually aimed at covering the highest possible proportion of the above-ground plant parts, is probably better suited for the application of nematodes to soil.

EPNs require moist soil for optimum activity and most species do not kill insects if soil temperatures are below 10°C (Grewal *et al.*, 1994a). They are also extremely sensitive to heat and sunlight, and will perish in a matter of minutes when exposed to full sun. Therefore, EPNs should be applied in the early morning or late in the day to prevent exposure to sunlight. The soil may also need to be irrigated before treatment if it is too dry, and irrigated again with at least 1.25 cm of water immediately after application to rinse nematodes from the foliage and move them into the soil (Shetlar *et al.*, 1988). Many studies have shown that post-application irrigation can improve EPN performance substantially (Georgis and Gaugler, 1991; Curran, 1992, Downing, 1994, Selvan *et al.*, 1994; Boselli *et al.*, 1997). Nematodes require a thin film of water for movement but are not capable of movement under flooded conditions. Thus, maintenance of optimum soil moisture for 2–4 weeks after the initial application can also substantially improve nematode performance and can enable secondary cycling of nematode infections (Grewal *et al.*, 2004). In general, nematode activity and survival is lower in heavy clay soils than in sandy loam soils. Soil temperature during and after application can also affect EPN efficacy. Warmer temperatures usually reduce nematode survival, while cooler temperatures reduce activity and infectivity. Soil temperatures between 10 and 30°C are favourable for application of most nematode species (Grewal *et al.*, 1994a). If the soil temperature is above 30°C, a pre-application irrigation is usually recommended to reduce the temperature prior to nematode application.

8.7.2 Prophylactic application

Nematodes can also be successfully applied prophylactically to protect plant-propagation materials from insect pest attack. Bedding and Miller (1981b) obtained 99% control of the blackcurrant borer *Synanthedon tipuliformis* with *S. feltiae* (=*N. bibionis*) in cuttings. Bari (1992)

developed a method to soak plant cuttings in nematode suspension to control the artichoke plume moth (*Platyptilia carduidactyla*), while Pye and Pye (1985) proposed a root dip method to economize on nematode application rates. Susurluk and Ehlers (2008a) found increased attachment of *H. bacteriophora* to strawberry roots when the roots were dipped in nematode suspension containing carboxymethylcellulose, while Deol *et al.* (2006, 2011) have explored the potential for prophylactic application of EPNs to growing medium used in the greenhouse and potted-plant industry.

8.7.3 Trap application

EPNs are also suitable for use in traps designed to lure and kill insects. Traps with nematodes have been tested against: (i) immature and/or adult stages of hemimetabolous insects including grasshoppers (*Melanoplus* spp.) (Capinera and Hibbard, 1987), the tawny mole cricket (*Scapteriscus vicinus*) (Georgis *et al.*, 1989; Parkman and Frank, 1993) and the German cockroach (*Blattella germanica*) (Appel *et al.*, 1993; Manweiler *et al.*, 1993); (ii) adults of holometabolus insects such as the house fly (*Musca domestica*) (Renn *et al.*, 1985; Renn, 1990), the banana weevil (*Cosmopolites sordidus*) (Schmitt *et al.*, 1992; Treverrow and Bedding, 1993) and yellowjackets (*Vesupa* spp.) (Poinar and Ennik, 1972; Wojcik and Georgis, 1988); and (iii) the larval stages of the holometabolous insect, the black cutworm (*Agrotis ipsilon*) (Capinera *et al.*, 1988; Georgis *et al.*, 1989). The traps may contain an attractive food source for the target insect, or a food arrestant and a sex pheromone. The traps may also serve as harborage or pupation sites, or mating and oviposition sites. Sound traps have been used to attract flying adult mole crickets to a source of *Steinernema scapterisci* nematodes (Parkman *et al.*, 1993), and traps containing a feeding lure and sex pheromone to attract and infect adult Japanese beetles with *S. glaseri* (Lacey *et al.*, 1993).

8.7.4 Foliar application

Foliar applications of EPNs have also proven successful, especially under controlled environments. Greenhouse trials have shown that *S. feltiae* can give up to 80% control of the leaf miners *Liriomyza bryoniae*, *Liriomyza huidobrensis*, *Liriomyza trifolii* and *Chromatomyia syngensiae* on vegetables (lettuce, tomato) and thrips and white flies on ornamentals (Hara *et al.*, 1993; Broadbent and Olthof, 1995; Williams and MacDonald, 1995; Bennison *et al.*, 1998; Williams and Walters, 2000; Cuthbertson *et al.*, 2003, 2007, 2008). Qiu *et al.* (2008) found that adjuvants, Triton X-100 and horticultural oil greatly enhanced *S. feltiae* efficacy against *Bemisia tabaci* nymphs on several crops in greenhouse conditions. The combination of *S. feltiae* and 1% horticultural oil increased the mortality of the second-instar nymphs to 85% on collard and 90% on hibiscus from about 18% when nematodes were applied alone. Similarly, the combination of *S. feltiae* and Triton X-100 resulted in 83% nymph mortality on collard and 89% on hibiscus.

Under more exposed field conditions, results have been more variable (Begley, 1990; Glazer *et al.*, 1992), although the potential success of EPN against early season apple pests has been reported, for example (Bélair *et al.*, 1998). A major problem in these exposed situations is inactivation of the EPNs due to rapid desiccation, UV and high temperature. The addition of various adjuvants containing antidesiccant or UV protective action to the spray mixture can improve nematode survival on exposed foliage (MacVean *et al.*, 1982; Glazer *et al.*, 1992; Nickle and Shapiro, 1994; Broadbent and Olthof, 1995; Baur *et al.*, 1997b; Mason *et al.*, 1998b; Navon *et al.*, 2002). Schroer and Ehlers (2005) developed a formulation of a surfactant suitable for emulsification of a heavy plant oil and a polymer, with a feature to increase the viscosity at low concentrations, which increased EPN efficacy and decreased EPN runoff from the foliage. Navaneethan *et al.* (2010) reported that efficacy of *S. feltiae* against the

diapausing codling moth larvae (*Cydia pomonella*) could be enhanced using a surfactant-polymer formulation. Above-ground nematode application followed by a sprayable gel such as Barricade can provide some enhancement of control of the lesser peachtree borer (*Synanthedon pictipes*) on peach limbs (Shapiro-Ilan *et al.*, 2010b).

In most trials on EPN foliar application, standard hydraulic application equipment has been used. Mistblowers (Matthews, 2000) have also been used to spray EPNs against thrips and leaf miners on ornamentals in commercial greenhouses (Wright *et al.*, 2005). However, standard spray systems that are designed for chemical application do not perform efficiently when applying particulate materials such as EPN IJs (Lello *et al.*, 1996; Mason *et al.,* 1998a; 1999). Flat-fan and full-cone hydraulic nozzles produce a wide range of droplet sizes, many of which are too small to carry IJs and therefore have a high water-to-nematode ratio. Although conventional discs are designed to produce very small droplets, most of which are too small to carry nematode IJs, studies have shown that the deposition of nematodes was higher at slower rotational disc speeds, which produced larger droplets (Mason *et al.*, 1998a, 1999). Piggott *et al.* (2003) developed a prototype spinning disc with improved efficiency for the application of EPN IJs. However, more research is needed to improve and adapt this spinning disc technology for the application of EPNs and other biological control agents.

8.7.5 Slow-release application

Kaya and Nelsen (1985) suggested that nematodes could be applied to soil in alginate gels for increased persistence, and this concept was later commercialized in the application of nematodes to tree trunks. A slow-release formulation using an absorbent gel was used to apply EPNs to citrus plants (Georgis, 1990) and a similar formulation (applied in tea bags) has been used in oilseed rape (Menzler-Hokkanen and Hokkanen, 2004). Infected insect cadavers can also serve as slow-release systems for nematodes (Jansson and Lecrone, 1994). Shapiro-Ilan *et al.* (2001, 2003) improved this method by formulating nematode-infected cadavers for application by coating them with powdered starch to reduce their stickiness and to prevent rupturing during storage and shipping. Ansari *et al.* (2009) reported that kaolin and starch mixtures were better than starch alone for coating infected insect cadavers for application against white grubs in turfgrass. del Valle *et al.* (2009) also tested a commercial calcareous powder, talc powder and gelatin capsules and found that these coatings did not interfere with nematode emergence or infectivity. They also found that ant predation was reduced on the cadavers enclosed in the gelatin capsules. Shapiro-Ilan *et al.* (2010a) wrapped nematode-infected mealworm beetle (*Tenebrio molitor*) cadavers in masking tape using an automatic packaging machine and applied the tape directly in the field. Cadavers held in the tape were more resistant to rupture than those without the tape and provided effective control of the citrus root weevil (*Diaprepes abbreviatus*) and the small hive beetle (*Aethina tumida*). Zhu *et al.* (2011) have developed a new prototype machine for automatic delivery of nematode-infected *G. mellonella* cadavers, while Deol *et al.* (2011) have shown that *S. carpocapsae*-infected *G. mellonella* cadavers can enhance the survival of nematodes in commercial potting media during storage, thus opening a new door for the prophylactic use of EPNs through pre-treated media.

8.8 Compatibility with Agrochemicals and other Biological Control Agents

EPN IJs can tolerate short-term exposure (2–24 h) to many chemical and biological insecticides, fungicides, herbicides, fertilizers and growth regulators and are therefore well suited for IPM systems. Prakasa Rao (1975) was the first to show that *S. carpocapsae* is compatible with certain insecticides and thus can be tank

mixed to save application costs. The compatibility of different EPN species with diverse agrochemicals has since been determined, and this subject has been comprehensively reviewed by Koppenhöfer and Grewal (2005) who provided detailed lists of agrochemicals that have been evaluated for compatibility with different EPN species. The actual concentration of the chemical to which the nematodes will be exposed can vary depending on the application volume and spray system used (Alumai and Grewal, 2004). Incompatibility between agrochemicals and EPNs can be managed by choosing an appropriate time interval between applications (Grewal *et al.*, 1998), the length of which may depend on the persistence of the chemical in the target substrate. Although specific information on appropriate application intervals is limited, it is usually recommended to wait for 1 and 2 weeks after the application of chemical insecticides and chemical nematicides, respectively, before applying the nematodes. Because some chemicals used as inert ingredients or adjuvants in formulations can be toxic to nematodes (Krishnayya and Grewal, 2002), the compatibility of each formulation with the specific nematode species should be evaluated. Thus, it is recommended that the nematode product label be checked carefully or the producer/supplier consulted before tank mixing the nematodes with other chemicals.

In addition to savings on application costs, EPNs may be combined with specific agricultural chemicals or biological control agents to achieve better control of the target pest through additive or, preferably, synergistic effects on pest mortality. Synergism between neonicotinoid insecticides, such as imidacloprid, and EPNs against white grubs has been well documented (Koppenhöfer and Kaya, 1998; Koppenhöfer *et al.*, 2000b). Imidacloprid reduces the grub's defensive behaviour, enhancing nematode attachment and penetration (Koppenhöfer *et al.*, 2000a). Furthermore, there is no negative effect of imidacloprid on the reproduction of EPNs in the treated grubs (Koppenhöfer *et al.*, 2002). Koppenhöfer and Fuzy (2008) reported that the anthranilic diamide insecticide chlorantraniliprole and *H. bacteriophora* can produce either additive or syngersistic effects on the mortality of the oriental beetle (*Anomala orientalis*). Similarly, Cuthbertson *et al.* (2008) found that the insecticide thiacloprid in combination with *S. carpocapsae* produced significantly higher mortality of the sweetpotato whitefly, *B. tabaci*, than using the chemical alone. Reis-Menini *et al.* (2008) showed that *S. glaseri* combined with a one-eighth or one-sixteenth the commercial dose of an organophosphate acaricide was significantly more effective than the nematode alone against cattle ticks (*Rhipicephalus microplus*).

EPNs have also been found to be compatible with many insect pathogens that are used in biological pest control. Combinations of EPNs with other nematode species and fungi and viruses generally result in additive effects on pest mortality, whereas nematode and bacteria combinations may produce antagonistic to syngergistic effects (see Koppenhöfer and Grewal, 2005, for a full review). More recently, Acevedo *et al.* (2007) found that *H. bacteriophora* and the entomopathogenic fungus *Metarhizium anisopliae* could be combined for more rapid mortality of the sugarcane borer *Diatraea saccharalis* but only at the expense of reduced nematode IJ production in the borer larvae. Anbesse *et al.* (2008) discovered that *H. bacteriophora* and *Steinernema yirgalemense* and *Metarhizium anisopliae* acted both additively and syngergistically against the barley chafer grub (*Coptognathus curtipennis*). Schulte *et al.* (2009) reported that a single combined application of *Beauveria bassiana* and *S. carpocapsae* to tree trunks resulted in 100% mortality of the bark-feeding moth (*Indarbela dea*), while two applications of the nematode alone were needed to achieve the same result. Ansari *et al.* (2010) reported that *Steinernema kraussei* and *M. anisopliae* showed syngergistic activity against the overwintering larvae of the black vine weevil (*Otiorhynchus sulcatus*).

Interactions between EPNs and insect parasitoids and predators have also been studied. Most studies indicate greater mortality of the target insect pest, but usually some negative effect on the development and survival of the parasitoid in the EPN-infected host is observed (see Koppenhöfer and Grewal, 2005, for a review). In a Petri dish bioassay, Rojht *et al.* (2009) showed that *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* caused significant mortality to the larvae of the two-spotted lady beetle (*Adalia bipunctata*), and the common green lacewing (*Chrysoperla carnea*). Everard *et al.* (2009) studied competition and intraguild predation between the braconid parasitoid *Bracon hylobii* and *Heterorhabditis downesi*, and found that the parasitoid avoided egg laying on larvae of the large pine weevil (*Hylobius abietis*) that had been exposed to nematodes 1–7 days previously. However, when the nematodes were applied directly to the parasitoid feeding on the weevil larvae, they parasitized the parasitoid larvae, reducing cocoon formation and eclosion. Mbata and Shapiro-Ilan (2010) found that a combination of *H. indica* and the braconid parasitoid *Habrobracon hebetor* increased mortality of the Indianmeal moth (*Plodia interpunctella*), in the laboratory. However, the nematodes parasitized the larvae of the parasitoid as well. Therefore, caution is recommended when combining the use of parasitoids and EPNs.

Soil fumigants such as methyl bromide and formaldehyde can have a significant negative affect on biocontrol organisms, mainly because of their broad-spectrum activity. Interest in alternative soil fumigants, including those of biological origin, has increased in recent years due to the impending ban on methyl bromide. Henderson *et al.* (2009) evaluated the soil incorporation of mustard *Brassica carinata* seed meal (as a biological fumigant), both in controlling the plant-parasitic nematode *Meloidogyne chitwoodi*, and on the biological control exerted by *S. feltiae* and *S. riobrave* on *M. chitwoodi* and Colorado potato beetle (*Leptinotarsa decemlineata*). Both the mustard seed meal and *Steinernema* spp., applied singly, reduced *M. chitwoodi* populations and increased marketable potato yield, but the combination of the two bioagents had a negative interaction. The mustard seed meal disrupted both the suppression of *M. chitwoodi* and biocontrol of *L. decemlineata* by the two *Steinernema* spp.

8.9 Efficacy and Commercial Use

Since the first field application of *S. glaseri* against grubs of the Japanese beetle (*P. japonica*) in New Jersey, USA (Glaser and Farrell, 1935), EPNs have been evaluated for their potential to control over 198 pest species representing 12 orders in the class Insecta and over 17 species representing one order in the class Arachnida. Table 8.1 lists the target pest species by order and the nematode species that have been evaluated against each pest, along with the pest life stage and conditions under which the evaluations occurred. Pest species in the order Coleoptera have received by far the most attention followed by those in the orders Lepidoptera and Diptera. Within the Coleoptera, white grubs (Scarabeidae) and weevils (Curculionidae) have received the most attention from researchers. While a complete listing of the studies conducted on the laboratory and field evaluations of nematodes on diverse insect and arachnid pest species is provided in Table 8.1, below is a brief discussion of the progress and hurdles in the commercial use of EPNs for pest control in various ecosystems/habitats.

8.9.1 Controlled environments

Controlled environments such as greenhouses or glasshouses, mushroom facilities and interiorscapes offer excellent conditions for the use of EPNs for pest control mainly because of moderate temperature, high relative humidity, appropriate soil moisture and partial protection from UV radiation. Although IPM is a common practice in greenhouses, it is often difficult to establish due to a

Table 8.1. Target insect and arachnid pests of EPNs and their life stage and site of evaluation.

Target insect	Life stage	Nematode species	Site	Reference(s)
Coleoptera: white grubs				
Amphimallon solstitiale	Grubs	*Steinernema glaseri*	Laboratory	Peters *et al.* (2002)
Anomala cuprea	Grubs	*Steinernema kushidai*	Laboratory	Fujii *et al.* (1993)
Anomala orientalis	Grubs	*Heterorhabditis bacteriophora*	Laboratory	Grewal *et al.* (2002); Koppenhöfer and Fuzy (2003a, 2008); Koppenhöfer *et al.* (2004)
		Heterorhabditis megidis	Laboratory	Grewal *et al.* (2002)
		Heterorhabditis zealandica	Laboratory	Grewal *et al.* (2002)
		Heterorhabditis sp.	Laboratory	Lee *et al.* (2002); Koppenhöfer and Fuzy (2003a)
		Steinernema carpocapsae	Field	Lee *et al.* (2002)
		S. glaseri	Field	Koppenhöfer *et al.* (1999, 2004); Lee *et al.* (2002); Koppenhöfer and Fuzy (2003a)
		Steinernema longicaudatum	Field	Lee *et al.* (2002)
		Steinernema scarabaei	Field	Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004)
Anomala orientalis	Grubs	*H. bacteriophora*	Field	Nielsen and Cowles (1998); Koppenhöfer *et al.* (2002); Koppenhöfer and Fuzy (2003a,b,c, 2008); Grewal *et al.* (2004)
		Heterorhabditis sp.	Field	Lee *et al.* (2002)
		S. carpocapsae	Field	Lee *et al.* (2002)
		S. glaseri	Field	Yeh and Alm (1995); Koppenhöfer *et al.* (1999); Lee *et al.* (2002)
		S. longicaudum	Field	Lee *et al.* (2002)
		S. scarabaei	Field	Koppenhöfer and Fuzy (2003a)
Antitrogus consanguineus	Pre-pupae, pupae	*Heterorhabditis* spp.	Laboratory	Akhurst *et al.* (1992)
Aphodius contaminates	Grubs	*H. bacteriophora*	Field	Sulistyanto and Ehlers (1996)
		H. megidis	Field	Sulistyanto and Ehlers (1996)
Ataenius spretulus	Grubs	*H. bacteriophora*	Laboratory	Koppenhöfer *et al.* (2004)
		S. carpocapsae	Field	Alm *et al.* (1992)
		S. glaseri	Laboratory	Koppenhöfer *et al.* (2004)
			Field	Alm *et al.* (1992)
		S. scarabaei	Laboratory	Koppenhöfer *et al.* (2004)
Coptognathus curtipennis	Grubs	*H. bacteriophora*	Laboratory	Anbesse *et al.* (2008)
		Steinernema yirgalemense	Laboratory	Anbesse *et al.* (2008)
Cotinus nitida	Grubs	*H. bacteriophora*	Laboratory	Wang *et al.* (1994); Townsend *et al.* (1998); Koppenhöfer *et al.* (2004)
		S. carpocapsae	Laboratory	Wang *et al.* (1994); Townsend *et al.* (1998)
		S. feltiae	Laboratory	Wang *et al.* (1994); Townsend *et al.* (1998)

Continued

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
		S. glaseri	Laboratory	Wang *et al.* (1994); Townsend *et al.* (1998); Koppenhöfer *et al.* (2004)
		S. scarabaei	Laboratory	Koppenhöfer *et al.* (2004)
Costelytra zealandica	Grubs	*H. bacteriophora*	Laboratory	Kain *et al.* (1982)
		S. glaseri	Laboratory	Kain *et al.* (1982)
Cyclocephala borealis	Grubs	*H. bacteriophora*	Laboratory	Grewal *et al.* (2002); Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004); An and Grewal (2007)
			Field	Koppenhöfer and Fuzy (2003a); Grewal *et al.* (2004)
		Heterorhabditis indica	Laboratory	Grewal *et al.* (2002)
		Heterorhabditis marelatus	Laboratory	Grewal *et al.* (2002)
		H. megidis	Laboratory	Grewal *et al.* (2002)
		H. zealandica	Laboratory	Grewal *et al.* (2002)
			Field	Grewal *et al.* (2004)
		Heterorhabditis sp.	Laboratory	Koppenhöfer and Fuzy (2003a)
		S. glaseri	Laboratory	Koppenhöfer and Fuzy (2003a)
			Field	Grewal *et al.* (2004)
		Steinernema kraussei	Field	Grewal *et al.* (2004)
		S. scarabaei	Laboratory	Koppenhöfer and Fuzy (2003a); An and Grewal (2007)
			Field	Koppenhöfer and Fuzy (2003a)
Cyclocephala hirta	Grubs	*H. bacteriophora*	Laboratory	Converse and Grewal (1998); Koppenhöfer *et al.* (2000b)
			Field	Koppenhöfer *et al.* (1999, 2000a, b)
		H. megidis	Laboratory	Converse and Grewal (1998)
		Heterorhabditis sp.	Laboratory	Converse and Grewal (1998)
		S. carpocapsae	Laboratory	Converse and Grewal (1998)
		S. feltiae	Laboratory	Converse and Grewal (1998)
		S. glaseri	Laboratory	Converse and Grewal (1998)
			Field	Koppenhöfer *et al.* (2000a)
		S. kushidai	Laboratory	Converse and Grewal (1998); Koppenhöfer *et al.* (2000b)
			Field	Koppenhöfer *et al.* (2000b)
		S. riobravae	Laboratory	Converse and Grewal (1998)
		Steinernema scapterisci	Laboratory	Converse and Grewal (1998)
Cyclocephala lurida	Grubs	*H. bacteriophora*	Laboratory	Koppenhöfer *et al.* (2004)
		S. glaseri	Laboratory	Koppenhöfer *et al.* (2004)
		S. scarabaei	Laboratory	Koppenhöfer *et al.* (2004)
Cyclocephala pasadenae	Grubs	*H. bacteriophora*	Laboratory	Koppenhöfer *et al.* (2004)
			Field	Koppenhöfer *et al.* (1999)
		S. glaseri	Laboratory	Koppenhöfer *et al.* (2004)
		S. scarabaei	Laboratory	Koppenhöfer *et al.* (2004)
Holotrichia consanguinea	Grubs	*S. glaseri*	Field	Vyas and Yadav (1993)

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Hoplia philanthus	Grubs	*H. bacteriophora*	Field	Weber and Henderson (1998); Ansari *et al.* (2009)
		H. megidis	Laboratory	Ansari *et al.* (2003)
		S. feltiae	Laboratory	Ansari *et al.* (2003)
		S. glaseri	Laboratory	Ansari *et al.* (2003)
Lepidiota crinita	Grubs	*Heterorhabditis* spp.	Laboratory	Akhurst *et al.* (1992)
Lepidiota nagatoria	Grubs	*Heterorhabditis* spp.	Laboratory	Akhurst *et al.* (1992)
Lepidiota picticollis	Grubs	*Heterorhabditis* spp.	Laboratory	Akhurst *et al.* (1992)
Lichnanthe vulpine	Larvae	*H. bacteriophora*	Laboratory/field	Weber and Henderson (1998)
		S. feltiae	Field	Dapsis (1991)
Maladera castanea	Grubs	*H. bacteriophora*	Laboratory	Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004)
			Field	Koppenhöfer and Fuzy (2003b)
		Heterorhabditis sp.	Laboratory	Koppenhöfer and Fuzy (2003a)
		S. glaseri	Laboratory	Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004)
		S. scarabaei	Laboratory	Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004)
			Field	Koppenhöfer and Fuzy (2003b)
Maladera matrida	Grubs	*H. bacteriophora*	Laboratory/field	Glazer and Gol'berg (1989, 1993)
		S. carpocapsae	Laboratory/field	Glazer and Gol'berg (1989, 1993)
Melolontha melolontha	Grubs	*H. bacteriophora*	Laboratory	Selvan *et al.* (1993); Peters (2000); Berner and Schnetter (2001)
		H. marelatus	Laboratory	Peters and Keller (2000); Berner and Schnetter (2001)
		H. megidis	Laboratory	Peters (2000); Peters and Keller (2000)
		Steinernema arenaria	Laboratory	Berner and Schnetter (2001)
		S. feltiae	Laboratory	Selvan *et al.* (1993); Berner and Schnetter (2001)
		S. glaseri	Laboratory	Selvan *et al.* (1993); Peters (2000); Berner and Schnetter (2001); Peters *et al.* (2002)
		S. riobrave	Laboratory	Selvan *et al.* (1993)
		Steinernema sp.	Laboratory	Berner and Schnetter (2001)
Phyllopertha horticola	Grubs	*H. bacteriophora*	Field	Smits *et al.* (1994); Sulistyanto and Ehlers (1996); Ehlers and Peters (1998)
		H. megidis	Field	Smits *et al.* (1994); Sulistyanto and Ehlers (1996); Ehlers and Peters (1998)
		S. glaseri	Laboratory/field	Smits *et al.* (1994)

Continued

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Phyllophaga anxia	Grubs	*H. bacteriophora*	Field	Dapsis (1991, 1993); Dittl (1996); Weber and Henderson (1998); Liesch and Williamson (2010)
	Grubs	*S. carpocpsae*	Field	Dapsis (1991)
	Grubs	*S. feltiae*	Field	Dapsis (1991)
	Grubs	*S. glaseri*	Field	Dapsis (1993); Dittl (1996)
Phyllophaga congrua	Grubs	*H. bacteriophora*	Laboratory	Koppenhöfer *et al.* (2004)
		S. glaseri	Laboratory	Koppenhöfer *et al.* (2004)
		S. scarabaei	Laboratory	Koppenhöfer *et al.* (2004)
Phyllophaga crinita	Grubs	*H. bacteriophora*	Laboratory	Koppenhöfer *et al.* (2004)
		S. glaseri	Laboratory	Koppenhöfer *et al.* (2004)
		S. scarabaei	Laboratory	Koppenhöfer *et al.* (2004)
Phyllophaga georgiana	Grubs	*H. bacteriophora*	Laboratory	Koppenhöfer *et al.* (2004)
		S. glaseri	Laboratory	Koppenhöfer *et al.* (2004)
		S. scarabaei	Laboratory	Koppenhöfer *et al.* (2004)
Phyllophaga hirticula	Grubs	*H. bacteriophora*	Laboratory/field	Forschler and Gardner (1991)
		S. carpocapsae	Laboratory/field	Forschler and Gardner (1991)
Phyllophaga spp.	Grubs	*H. bacteriophora*	Field	Kard *et al.* (1988)
		S. carpocapsae	Field	Kard *et al.* (1988)
Popillia japonica	Grubs	*H. bacteriophora*	Laboratory	Wright *et al.* (1988); Selvan *et al.* (1994); Yeh and Alm (1995); Simard *et al.* (2001); Grewal *et al.* (2002); Cappaert and Koppenhöfer (2003); Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004); An and Grewal (2007)
			Field	Georgis and Gaugler (1991); Selvan *et al.* (1993); Nielsen and Cowles (1998); Koppenhöfer *et al.* (2000a,b); Mannion *et al.* (2001); Koppenhöfer and Fuzy (2003a); Grewal *et al.* (2004)
		H. indica	Laboratory	Grewal *et al.* (2002)
		H. marelatus	Laboratory	Mannion *et al.* (2000); Grewal *et al.* (2002)
			Field	Mannion *et al.* (2001)
		H. megidis	Laboratory	Grewal *et al.* (2002)
		H. zealandica	Laboratory	Grewal *et al.* (2002)
			Field	Grewal *et al.* (2004)
		Heterorhabditis sp.	Laboratory/pot	Wright *et al.* (1988); Koppenhöfer and Fuzy (2003a)
		Steinernema anomali	Laboratory	Simard *et al.* (2001)
		S. carpocapsae	Laboratory	Wang *et al.* (1994); Yeh and Alm (1995)
			Field	Georgis and Gaugler (1991); Alm *et al.* (1992)
		S. feltiae	Laboratory/pot	Yeh and Alm (1995)
			Field	Alm *et al.* (1992)
		S. glaseri	Laboratory/pot	Wright *et al.* (1988); Selvan *et al.* (1994); Wang *et al.* (1994); Simard *et al.* (2001); Koppenhöfer and Fuzy (2003a)

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
		S. glaseri	Field	Selvan *et al.* (1993, 1994); Yeh and Alm (1995); Koppenhöfer *et al.* (2000a); Grewal *et al.* (2004)
		S. kraussei	Field	Grewal *et al.* (2004)
		S. kushidai	Field	Koppenhöfer *et al.* (2000b)
		S. riobrave	Laboratory	Selvan *et al.* (1994)
		S. scapterisci	Laboratory	Townsend *et al.* (1998)
		S. scarabaei	Laboratory	Cappaert and Koppenhöfer (2003); Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004); An and Grewal (2007)
			Field	Koppenhöfer and Fuzy (2003a)
Rhizotrogus majalis	Grubs	*H. bacteriophora*	Laboratory	Wright *et al.* (1988); Townsend *et al.* (1994); Yeh and Alm (1995); Nielsen and Cowles (1998); Simard *et al.* (2001); Grewal *et al.* (2002); Cappaert and Koppenhöfer (2003); Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004); An and Grewal (2007)
			Field	Cappaert and Koppenhöfer (2003)
		H. megidis	Laboratory	Grewal *et al.* (2002)
		H. zealandica	Laboratory	Grewal *et al.* (2002)
		S. carpocapsae	Laboratory	Townsend *et al.* (1994); Yeh and Alm (1995)
		S. feltiae	Laboratory	Wright *et al.* (1988); Townsend *et al.* (1994)
		S. glaseri	Laboratory	Wright *et al.* (1988); Townsend *et al.* (1994); Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004)
		S. scarabaei	Laboratory	Cappaert and Koppenhöfer (2003); Koppenhöfer and Fuzy (2003a); Koppenhöfer *et al.* (2004); An and Grewal (2007)
			Field	Cappaert and Koppenhöfer (2003)
Tomarus subtropicus	Grubs	*H. bacteriophora*	Field	Kostromytska and Buss (2008)
		S. glaseri	Field	Kostromytska and Buss (2008)
		S. scarabaei	Field	Kostromytska and Buss (2008)
Coleoptera: other beetles				
Acalymma vittatum	Larvae, pupae	*S. carpocapsae*	Laboratory/field	Ellers-Kirk *et al.* (2000)
	Larvae, pupae	*S. feltiae*	Laboratory/field	Ellers-Kirk *et al.* (2000)
Aethina tumida	Larvae, pupae	*H. indica*	Laboratory/field	Ellis *et al.* (2010); Shapiro-Ilan *et al.* (2010a)

Continued

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
	Larvae, pupae	*S. riobrave*	Laboratory/field	Ellis *et al.* (2010)
Agelastica alni	Larvae	*H. megidis*	Pot	Tomalak (2004)
		S. feltiae	Pot	Tomalak (2004)
Altica quercetorum	Larvae	*H. megidis*	Pot	Tomalak (2004)
		S. feltiae	Pot	Tomalak (2004)
Anoplophora glabripennis	Larvae	*S. feltiae*	Field	Qin *et al.* (1988)
Arbela dea	Larvae	*S. carpocapsae*	Field	Xu and Yang (1992)
Aristobia testudo	Larvae	*S. carpocapsae*	Field	Xu *et al.* (1995); Han *et al.* (1996)
Carpophilus hemipterus	Larvae	*H. bacteriophora*	Laboratory/pot	Glazer *et al.* (1999)
		Heterorhabditis sp.	Laboratory/pot	Glazer *et al.* (1999)
		S. carpocapsae	Laboratory	Vega *et al.* (1994)
		S. feltiae	Laboratory	Vega *et al.* (1994)
		S. glaseri	Laboratory	Vega *et al.* (1994)
		S. riobrave	Laboratory/pot	Vega *et al.* (1994); Glazer *et al.* (1999)
Carpophilus humeralis	Larvae	*H. bacteriophora*	Laboratory/pot	Glazer *et al.* (1999)
		Heterorhabditis sp.	Laboratory/pot	Glazer *et al.* (1999, 2007)
		S. riobrave	Laboratory/pot	Glazer *et al.* (1999)
Diabrotica virgifera virgifera	Larvae	*H. bacteriophora*	Field	Jackson (1996); Hiltpold *et al.* (2010); Toepfer *et al.* (2010)
		H. megidis	Field	Toepfer *et al.* (2010);
		S. carpocapsae	Field	Rohrbach (1969); Munson and Helms (1970); Thurston and Yule (1990); Georgis *et al.* (1991); Wright *et al.* (1993); Jackson and Hesler (1995); Ellsbury *et al.* (1996); Jackson (1996); Journey and Ostlie (2000)
		S. feltiae	Field	Toepfer *et al.* (2010)
Dorysthenes hydropicus	Larvae	*H. bacteriophora*	Laboratory	Xu *et al.* (2010b)
		S. carpocapsae	Laboratory	Xu *et al.* (2010b)
		S. scapterisci	Laboratory	Xu *et al.* (2010b)
Leptinotarsa decemlineata	Larvae	*H. bacteriophora*	Field	Wright *et al.* (1987); Berry *et al.* (1997a)
		H. marelatus	Field	Berry *et al.* (1997b)
		S. carpocapsae	Laboratory/field	Welch (1958); Welch and Briand (1961b); Toba *et al.* (1983); Wright *et al.* (1987); Bélair and Boivin (1995); Stewart *et al.* (1998)
		S. feltiae	Field	Laznik *et al.* (2010a)
Longitarsus ferrugineus	Larvae	*H. bacteriophora*	Field	Grewal and Georgis (1999)
		S. carpocapsae	Field	Grewal and Georgis (1999)
Longitarsus waterhousei	Larvae	*H. bacteriophora*	Field	Grewal and Georgis (1999)
		S. carpocapsae	Field	Grewal and Georgis (1999)
Phyllotreta striolata	Larvae, pupae	*H. indica*	Laboratory	Trdan *et al.* (2008); Xu *et al.* (2010a)
		Heterorhabditis sp.	Laboratory	Xu *et al.* (2010a)
		S. carpocapsae	Laboratory	Hou *et al.* (2001); Kakizaki (2004); Trdan *et al.* (2008); Xu *et al.* (2010a)
		S. feltiae	Laboratory	Wei *et al.* (1992); Trdan *et al.* (2008)

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
		S. glaseri	Laboratory	Xu *et al.* (2010a)
		S. longicaudum	Laboratory	Xu *et al.* (2010a)
		S. pakistanensise	Laboratory	Xu *et al.* (2010a)
		S. scaptersici	Laboratory	Xu *et al.* (2010a)
Pyrrhalta viburni	Larvae, pupae	*H. bacteriophora*	Laboratory	Weston and Desurmont (2008)
		S. carpocapsae	Laboratory	Weston and Desurmont (2008)
Rhabdopterus picipes	Larvae	*H. bacteriophora*	Laboratory/field	Stuart and Polavarapu (1997); Polavarapu *et al.* (2000)
		S. glaseri	Laboratory/field	Stuart and Polavarapu (1997)
Rhyzopertha dominica	Adults	*H. bacteriophora*	Laboratory	Athanassiou *et al.* (2010)
		S. carpocapsae	Laboratory	Athanassiou *et al.* (2010)
		S. feltiae	Laboratory	Athanassiou *et al.* (2010)
Scotlytus scolytus	Larvae	*S. carpocapsae*	Field	Finney and Walker (1979)
Saperda carcharias	Larvae	*H. megidis*	Field	Barani *et al.* (2000)
		S. feltiae	Field	Barani *et al.* (2000)
Tribolium confusum	Larvae	*H. bacteriophora*	Laboratory	Athanassiou *et al.* (2010)
		S. carpocapsae	Laboratory	Athanassiou *et al.* (2008, 2010)
		S. feltiae	Laboratory	Athanassiou *et al.* (2008, 2010)
Xanthogaleruca luteola	Larvae	*S. carpocapsae*	Pot/field	Kaya *et al.* (1981)
Coleoptera: weevils				
Anthonomus grandis	Larvae	*S. riobrave*	Field	Cabanillas (2003)
Bothynoderes punctiventris	Larvae	*H. bacteriophora*	Laboratory	Susurluk (2008)
		S. feltiae	Laboratory	Susurluk (2008)
		Steinernema weiseri	Laboratory	Susurluk (2008)
Conotrachelus nenuphar	Adults	*H. bacteriophora*	Laboratory	Kim and Alston (2008)
	Larvae	*H. bacteriophora*	Laboratory	Kim and Alston (2008)
		S. carpocapsae	Laboratory/field	Shapiro-Ilan *et al.* (2002a)
			Field	Bélair *et al.* (1998)
		S. feltiae	Laboratory/field	Shapiro-Ilan *et al.* (2002a); Kim and Alston (2008)
		S. riobrave	Laboratory/field	Shapiro-Ilan *et al.* (2002a, 2004)
	Adults	*S. carpocapsae*	Laboratory	Shapiro-Ilan *et al.* (2002a)
		S. feltiae	Laboratory	Shapiro-Ilan *et al.* (2002a); Kim and Alston (2008)
		S. riobrave	Laboratory	Shapiro-Ilan *et al.* (2002a)
	Pupae	*H. bacteriophora*	Laboratory	Kim and Alston (2008)
		S. feltiae	Laboratory	Kim and Alston (2008)
Conotrachelus psidii	Larvae	*Heterorhabditis baujardi*	Field	del Valle *et al.* (2008)
Cosmopolites sordidus	Adults	*S. carpocapsae*	Laboratory	Laumond *et al.* (1979)
	Larvae	*S. carpocapsae*	Laboratory/field	Figueroa (1990)
		S. feltiae	Laboratory/field	Figueroa (1990)
		S. glaseri	Laboratory/field	Figueroa (1990)
	Adults, larvae	*S. carpocapsae*	Field	Kermarrec and Mauléon (1989); Treverrow *et al.* (1991)
Curculio caryae	Larvae	*H. bacteriophora*	Laboratory/field	Nyczepir *et al.* (1992); Smith *et al.* (1993); Shapiro-Ilan (2001a)
		H. indica	Laboratory	Shapiro-Ilan (2001a)

Continued

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
		H. marelatus	Laboratory	Shapiro-Ilan (2001a)
		H. megidis	Laboratory	Shapiro-Ilan (2001a)
		H. zealandica	Laboratory	Shapiro-Ilan (2001a)
		S. carpocapsae	Laboratory/field	Smith *et al.* (1993); Shapiro-Ilan (2001a)
		S. feltiae	Laboratory	Smith *et al.* (1993); Shapiro-Ilan (2001a)
		S. glaseri	Laboratory	Shapiro-Ilan (2001a)
		S. riobrave	Laboratory	Shapiro-Ilan (2001a)
	Adults	*H. bacteriophora*	Laboratory	Shapiro-Ilan (2001b)
		S. carpocapsae	Laboratory	Shapiro-Ilan (2001b)
		S. feltiae	Laboratory	Shapiro-Ilan (2001b)
		S. riobrave	Laboratory	Shapiro-Ilan (2001b)
Cylas formicarius	Adults	*H. bacteriophora*	Field	Jansson *et al.* (1990, 1991, 1993); Mannion and Jansson (1992, 1993); Jansson and Lecrone (1994, 1997); Ekanayake *et al.* (2001)
		H. megidis		Jansson *et al.* (1990, 1991, 1993); Mannion and Jansson (1992, 1993); Jansson and Lecrone (1994, 1997); Ekanayake *et al.* (2001)
		Heterorhabditis sp.		Jansson *et al.* (1990, 1993); Mannion and Jansson (1992, 1993); Jansson and Lecrone (1994, 1997)
		S. carpocapsae		Jansson *et al.* (1990, 1993); Mannion and Jansson (1992, 1993); Jansson and Lecrone (1994, 1997)
		S. feltiae		Jansson *et al.* (1990, 1993); Mannion and Jansson (1992, 1993); Jansson and Lecrone (1994, 1997)
		S. glaseri		Jansson *et al.* (1990, 1993); Mannion and Jansson (1992, 1993); Jansson and Lecrone (1994, 1997)
Diaprepes abbreviates	Larvae	*H. bacteriophora*	Field	Downing *et al.* (1991); Schroeder (1992); Duncan and McCoy (1996); Duncan *et al.* (1996); McCoy *et al.* (2000)
		H. indica	Field	McCoy *et al.* (2000, 2002)
		S. carpocapsae	Field	Schroeder (1987, 1990, 1992, 1994); Downing *et al.* (1991); Bullock and Miller (1994); Duncan *et al.* (1996)
		S. glaseri	Field	Schroeder (1987)
		S. riobrave	Field	Schroeder (1994); Duncan and McCoy (1996); Duncan *et al.* (1996, 2001, 2003); Bullock *et al.* (1999); McCoy *et al.* (2000, 2002)

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Hylobius abietis	Larvae	*H. megidis*	Field	Brixey (2000); Collins (2003)
		S. carpocapsae		Brixey (2000); Collins (2003)
		S. feltiae		Brixey (2000); Collins (2003)
	Adults	*Heterorhabditis downesi*	Field	Everard *et al.* (2009)
		H. downesi	Laboratory	Girling *et al.* (2010)
		S. carpocapsae		Girling *et al.* (2010)
Listronotus maculicollis	Adults, larvae	*H. bacteriophora*	Field	Watschke *et al.* (1995); Shetlar (2002)
		S. carpocapsae	Field	Watschke *et al.* (1995); Shetlar (2002)
Listronotus oregonensis	Adults	*H. bacteriophora*	Field	Miklasiewicz *et al.* (2002)
		S. carpocapsae	Field	Bélair and Boivin (1985, 1995); Miklasiewicz *et al.* (2002)
		S. feltiae	Laboratory/field	Bélair and Boivin (1985); Boivin and Bélair (1989)
Odioporus longicollis	Larvae, pupae, adults	*S. carpocapsae*	Field	Xu *et al.* (1991)
Otiorhynchus lingustici	Larvae	*H. bacteriophora*	Field	Neumann and Shields (2008)
		S. carpocapsae	Field	Neumann and Shields (2008)
		S. feltiae	Field	Neumann and Shields (2008)
Otiorhynchus rugosostriatus	Larvae, adults	*H. bacteriophora*	Field	Simser and Roberts (1994; Booth *et al.* (2002)
		H. marelatus	Field	Booth *et al.* (2002)
		S. carpocapsae	Field	Simser and Roberts (1994); Booth *et al.* (2002)
Otiorhynchus sulcatus	Larvae, adults	*H. bacteriophora*	Field	Scherer (1987); Shanks and Agudelo-Silva (1990); Booth *et al.* (2002); Susurluk and Ehlers (2008a)
		H. marelatus	Laboratory	Berry *et al.* (1997b); Berry and Liu (1999)
		H. megidis	Pot/field	Georgis and Poinar (1984); Klinger (1988); van Tol (1993a,b, 1994, 1998); Backhaus (1994); Miduturi *et al.* (1994); Kakouli-Durate *et al.* (1997); Neubauer (1997); van Tol (1998); Berry and Liu (1999); Long *et al.* (2000); Fitters *et al.* (2001); Lola-Luz and Downes (2007); Haukeland and Lola-Luz (2010)
		H. zealandica	Field	Backhaus (1994)
		Heterorhabditis sp.	Field	Simons (1981); Curran (1992)
		S. carpocapsae	Pot/field	Simons (1981); Georgis and Poinar (1984); Shanks and Agudelo-Silva (1990); Hanula (1993); van Tol (1993b, 1998); Sampson (1994); Kakouli-Duarte *et al.* (1997); Booth *et al.* (2002); Willmott *et al.* (2002)

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Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
		S. feltiae	Pot	Stimmann *et al.* (1985); Hanula (1993); van Tol (1993b, 1998); Kakouli-Duarte *et al.* (1997)
		S. glaseri	Pot	Georgis and Poinar (1984); Kakouli-Duarte *et al.* (1997); Booth *et al.* (2002)
		S. kraussei	Bags/field	Long *et al.* (2000); Willmott *et al.* (2002); Ansari *et al.* (2010); Haukeland and Lola-Luz (2010)
Phyllobius utricae	Larvae	*H. bacteriophora*	Laboratory	Pollit *et al.* (1994)
		S. carpocapsae	Laboratory	Pollit *et al.* (1994)
		S. feltiae	Laboratory	Pollit *et al.* (1994)
Rhynchophorus ferrugineus	Larvae	*S. carpocapsae*	Field	Dembilio *et al.* (2010)
Sitophilus oryzae	Larvae	*S. carpocapsae*	Laboratory	Athanassiou *et al.* (2010)
		S. feltiae	Laboratory	Athanassiou *et al.* (2010); Laznik *et al.* (2010b)
Sphenophorus venatus vestitus	Larvae, adults	*S. carpocapsae*	Field	Smith (1994); Kinoshita and Yamanaka (1998)
Sphenophorus parvulus	Larvae, adults	*H. bacteriophora*	Field	Georgis and Poinar (1989, 1994); Klein (1990); Smith (1994); Shetlar (1995)
		S. carpocapsae	Field	Georgis and Poinar (1989, 1994); Klein (1990); Smith (1994); Shetlar (1995)
Temnorhinus mendicus	Larvae; pupae, adults	*Heterorhabditis* sp.	Field	Deseö (1987); Boselli *et al.* (1991, 1994, 1997); Curto *et al.* (1992, 1999)
		S. carpocapsae	Field	Deseö (1987); Boselli *et al.* (1991, 1994, 1997); Curto *et al.* (1992, 1999)
Lepidoptera				
Agrotis ipsilon	Larvae	*S. carpocapsae*	Laboratory/ greenhouse/ field	Capinera *et al.* (1988); Georgis and Poinar (1989); Levine and Oloumi-Sadeghi (1992); Watschke *et al.* (1995); Baur *et al.* (1997a); Shapiro *et al.* (1999); Kunkel and Grewal (2003); Kunkel *et al.* (2004); Richmond and Bigelow (2009)
Agrotis segetum	Larvae	*S. feltiae*	Laboratory/field	Lössbroek and Theunissen (1985)
Acrolepiopsis assectella	Larvae	*S. feltiae*	Laboratory/field	del Pino and Morton (2008)
Amyelois transitella	Larvae	*S. carpocapsae*	Field	Lindegren *et al.* (1987); Agudelo-Silva *et al.* (1995); Siegal *et al.* (2004)
Capnodis tenebrionis	Larvae	*H. bacteriophora*	Pots	Morton and del Pino (2008a)
		S. carpocapsae	Pots	de Altube *et al.* (2008); Morton and del Pino (2008a)
		S. feltiae	Pots	Morton and del Pino (2008a)
		S. feltiae	Field	Morton and del Pino (2008b)
		S. affine	Pots	Morton and Del Pino (2008a)
Carposnia nipponensis	Larvae	*S. carpocapsae*	Field	Wang (1990)

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Choristoneura occidentalis	Larvae	*S. carpocapsae*	Field	Kaya *et al.* (1981); Kaya and Reardon (1982)
Choristoneura rosaceanna	Larvae	*H. bacteriophora*	Pot	Bélair *et al.* (1999)
		S. carpocapsae	Pot	Bélair *et al.* (1999)
Chrysoteuchia topiaria	Larvae	*H. bacteriophora*	Field	Berry and Liu (1998); Henderson and Singhai (1999)
		H. marelatus	Laboratory/field	Berry and Liu (1998)
		H. megidis	Laboratory/field	Simard *et al.* (2002)
		S. carpocapsae	Laboratory/field	Dapsis (1993); Smith *et al.* (1993); Henderson and Singhai (1999); Simard *et al.* (2002)
		S. feltiae	Laboratory	Simard *et al.* (2002)
		S. glaseri	Laboratory	Simard *et al.* (2002)
		S. kraussei	Field	Henderson and Singhai (1999)
Cydia latiferreana	Larvae	*S. carpocapsae*	Laboratory/field	Chambers *et al.* (2010)
Cydia pomonella	Larvae	*H. zealandica*	Field	de Waal *et al.* (2011)
		S. carpocapsae	Field	Kaya *et al.* (1984); Sledzevskaya (1987); Nachtigall and Dickler (1992); Lacey and Unruh (1998); Lacey and Chauvin (1999); Unruh and Lacey (2001); Cossentine *et al.* (2002)
		S. feltiae	Field	Navaneethan *et al.* (2010)
Diatraea saccharalis	Larvae	*H. bacteriophora*	Laboratory	Acevedo *et al.* (2007)
Ephestia kuehniella	Larvae	*S. carpocapsae*	Laboratory	Athanassiou *et al.* (2008, 2010)
		S. feltiae	Laboratory	Athanassiou *et al.* (2008, 2010)
Earias insulana	Larvae	*S. feltiae*	Greenhouse	Glazer *et al.* (1992)
Euzophera semifuneralis		*H. bacteriophora*	Field	Kain and Agnello (1999)
		S. feltiae	Field	Kain and Agnello (1999)
Fumibotrys fumalis	Larvae	*S. carpocapsae*	Field	Grewal and Georgis (1999)
Helicoverpa zea	Larvae	*S. carpocapsae*	Field	Tanada and Reiner (1962); Bong and Sikorowski (1983); Bong (1986); Richter and Fuxa (1990); Purcell *et al.* (1992)
		S. riobrave	Field	Raulston *et al.* (1992); Cabanillas and Raulston (1994a,b, 1996a,b); Cabanillas *et al.* (1994); Feaster and Steinkraus (1996)
Heliothis armigera	Larvae	*S. carpocapsae*	Greenhouse	Samsook and Sikora (1981); Ishibashi (1987); Glazer and Navon (1990)
		S. feltiae	Greenhouse	Glazer and Navon (1990)
Heliothis virescens	Larvae	*S. riobrave*	Field	Bell (1995)
Hepialus californicus	Larvae	*H. marelatus*	Field	Strong *et al.* (1996)
Holcocerus insularis	Larvae, pupae	*S. carpocapsae*	Laboratory	Yang *et al.* (1989)
	Larvae	*S. feltiae*	Field	Qin *et al.* (1988)

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Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Hyphantria cunea	Larvae	*S. feltiae*	Field	Yamanaka *et al.* (1986)
Indarbela dea	Larvae	*S. carpocapsae*	Field	Schulte *et al.* (2009)
Lymantria dispar	Larvae	*S. feltiae*	Field	Shapiro *et al.* (1985); Reardon *et al.* (1986)
Macronoctua onusta	Larvae	*H. bacteriophora*	Field	Gill and Raupp (1997)
		S. carpocapsae	Field	Gill and Raupp (1997)
Operophtera spp.	Larvae	*H. megidis*	Field	Tomalak (2003)
		S. feltiae	Field	Tomalak (2003)
Paranthrene robiniae	Larvae	*S. carpocapsae*	Field	Kaya and Lindegren (1983)
Pectinophora gossypiella	Larvae	*H. bacteriophora*	Laboratory/field	Henneberry *et al.* (1996); Gouge *et al.* (1999)
	Larvae, pupae	*S. riobrave*	Laboratory/field	Henneberry *et al.* (1995, 1996); Gouge *et al.* (1997, 1999)
Pennisetia marginata	Larvae	*S. feltiae*	Field	Miller and Bedding (1982); Capinera *et al.* (1986)
Plodia interpunctella	Larvae	*H. indica*	Laboratory	Mbata and Shapiro-Ilan (2010)
Plutella xylostella	Larvae	*S. carpocapsae*	Laboratory/field	Baur *et al.* (1995, 1997a,b, 1998); Mason and Wright (1997); Schroer and Ehlers (2005)
		S. glaseri	Field	Vyas *et al.* (2000)
		S. thermophilum	Field	Ganguly and Somvanshi (2003)
Podosesia aureocincta	Larvae	*S. carpocapsae*	Field	Gill *et al.* (1994); Smith-Fiola *et al.* (1996)
		S. feltiae	Field	Gill *et al.* (1994); Smith-Fiola *et al.* (1996)
		S. glaser	Field	Smith-Fiola *et al.* (1996)
Pseudaletia unipuncta	Larvae	*H. bacteriophora*	Laboratory/field	Rosa and Simões (2004)
		S. carpocapsae	Laboratory/field	Rosa and Simões (2004)
Rhyacionia frustana	Larvae	*S. carpocapsae*	Field	Nash and Fox (1969); Smith-Fiola *et al.* (1996)
Spodoptera exigua	Larvae	*S. carpocapsae*	Laboratory/field	Samsook and Sikora (1981); Ishibashi (1987); Barbercheck and Kaya (1991); Gothama *et al.* (1996); Sezhian *et al.* (1996); Ansari *et al.* (2007)
Spodoptera fruigiperda	Larvae	*S. carpocapsae*	Laboratory	Richmond *et al.* (2004)
		H. indica	Field	Garcia *et al.* (2008)
		Steinernema sp.	Field	Garcia *et al.* (2008)
Spodoptera littura	Larvae	*S. carpocapsae*	Laboratory/field	Glazer *et al.* (1992); Shahidi Noghabi *et al.* (2006)
		S. feltiae	Laboratory/field	Glazer *et al.* (1992)
		S. siamkayai	Laboratory	Wetchayunt *et al.* (2009)
Synanthedon culiciformis	Larvae	*S. feltiae*	Field	Kaya and Brown (1986)
Synanthedon exitiosa	Larvae	*H. bacteriophora*	Field	Cossentine *et al.* (1990)
		S. carpocapsae	Laboratory/field	Gill *et al.* (1992); Cottrell and Shapiro-Ilan (2006)
		S. riobrave	Laboratory/field	Cottrell and Shapiro-Ilan (2006)
Synanthedon myopaeformis	Larvae	*Steinernema* sp.	Field	Kahounova and Mracek (1991)
Synanthedon pictipes	Larvae	*Hetrorhabditis* spp.	Laboratory	Cottrell *et al.* (2011)
		S. carpocapsae	Laboratory	Cottrell *et al.* (2011)
		S. carpocapsae	Field	Shapiro-Ilan *et al.* (2010b)

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
		S. riobrave	Laboratory	Cottrell *et al.* (2011)
Synanthedon resplendens	Larvae	*S. feltiae*	Field	Kaya and Brown (1986)
Synanthedon scitula	Larvae	*S. feltiae*	Field	Davidson *et al.* (1992)
Synanthedon tipuliformis	Larvae	*H. bacteriophora*	Laboratory/field	Bedding and Miller (1981)
		S. carpocapsae	Laboratory/field	Bedding and Miller (1981)
		S. feltiae	Laboratory/field	Bedding and Miller (1981); Miller and Bedding (1982)
		S. riobrave	Laboratory	Cottrell *et al.* (2011)
Thyridopterix ephemeraeformis	Larvae	*S. feltiae*	Field	Gill and Raupp (1994)
		S. carpocapsae	Field	Gill and Raupp (1994)
Trichoplusia ni	Pupae	*S. carpocapsae*	Laboratory	Henneberry *et al.* (1995)
		S. riobrave	Laboratory	Henneberry *et al.* 1995)
Vitacea polistiformis	Larvae	*H. bacteriophora*	Laboratory	Williams *et al.* (2002)
		H. zealandica	Laboratory	Williams *et al.* (2002)
		S. carpocapsae	Laboratory/field	All *et al.* (1980); Saunders and All (1985); Williams *et al.* (2002)
		S. feltiae	Laboratory	Williams *et al.* (2002)
		H. bacteriophora	Field	Williams *et al.* (2010)
		H. zealandica	Field	Williams *et al.* (2010)
Zeuzera pyrina	Larvae	*S. carpocapsae*	Field	Deseö and Rovesti (1992)
Homoptera: *Phylloxera*				
Daktuloshaira vitifoliae	Larvae	*H. bacteriophora*	Laboratory	English-Loeb *et al.* (1999)
		S. glaseri	Laboratory	English-Loeb *et al.* (1999)
Orthoptera: mole crickets				
Scapteriscus abbreviatus	Adults	*S. scaptersici*	Laboratory	Hudson and Nyuyen (1989a,b); Nguyen and Smart (1991)
Scapteriscus acletus	Adults	*S. scaptersici*	Laboratory	Hudson and Nguyen (1989a)
Scapteriscus borellii	Adults	*S. carpocapsae*	Field	Georgis and Poinar (1994)
	Adults, nymphs	*S. scaptersici*	Laboratory/field	Hudson and Nguyen (1989a,b); Nguyen and Smart (1991); Parkman and Frank (1992); Parkman *et al.* (1993, 1994, 1996)
Scapteriscus vicinus	Adults	*S. carpocapsae*	Laboratory/field	Georgis and Poinar (1994); Alves *et al.* (2009)
	Adults, nymphs	*S. scapterisci*	Laboratory/field	Hudson and Nguyen (1989a,b); Nguyen and Smart (1991); Parkman and Frank 1992); Parkman *et al.* (1993, 1994, 1996)
		S. riobravae	Field	Gorsuch (1995)
Hemiptera: Mealybugs				
Dysmicoccus texensis	Adults	*Heterorhabditis* sp.	Greenhouse/ field	Alves *et al.* (2009)
Dysmicoccus vaccinii	Adults	*H. bacteriophora*	Laboratory	Stuart *et al.* (1997)
		H. indica	Laboratory	Stuart *et al.* (1997)
		H. zealandica	Laboratory	Stuart *et al.* (1997)
Hemiptera: white flies				
Bemisia tabaci	Nymphs	*S. carpocapsae*	Laboratory	Cuthbertson *et al.* (2008)
		S. feltiae	Laboratory/ greenhouse	Cuthbertson *et al.* (2007); Qiu *et al.* (2008)

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Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Thysanoptera: thrips				
Frankliniella occidentalis	Adults, pupae	*H. bacteriophora*	Laboratory/ greenhouse	Chyzik *et al.* (1996); Premachandra *et al.* (2003a,b); Ebssa *et al.* (2004)
	Larvae	*H. indica*	Laboratory/ greenhouse	Ebssa *et al.* (2004)
		H. marelatus	Laboratory/ greenhouse	Ebssa *et al.* (2004)
		Steinernema abbasi	Laboratory/ greenhouse	Ebssa *et al.* (2004)
		Steinernema arenarium	Laboratory/ greenhouse	Ebssa *et al.* (2004)
		Steinernema bicornutum	Laboratory/ greenhouse	Ebssa *et al.* (2004)
		S. carpocapsae	Laboratory/ greenhouse	Helyer *et al.* (1995); Premchandra *et al.* (2003a,b); Ebssa *et al.* (2004)
		S. feltiae	Laboratory/ greenhouse	Tomalak (1994); Helyer *et al.* (1995); Bennison *et al.* (1998); Ebssa *et al.* (2001a,b, 2004); Wardlow *et al.* (2001); Premchandra *et al.* (2003a,b); Trdan *et al.* (2007)
		Steinernema sp.		Ebssa *et al.* (2004)
Thrips palmi	Adults, juveniles	*S. feltiae*	Laboratory	North *et al.* (2006)
Thrips tabaci	Adults, pupae	*H. indica*	Laboratory	Al-Siyabi *et al.* (2006)
Diptera: flies, leaf minors				
Anastrepha fraterculus	Larvae	*H. bacteriophora*	Pot	Barbosa-Negrisoli *et al.* (2009)
		S. riobrave	Pot	Barbosa-Negrisoli *et al.* (2009)
Anastrepha obliqua	Larvae	*S. carpocapsae*	Laboratory	Toledo *et al.* (2009)
Anastrepha suspense	Larvae	*H. bacteriophora*	Laboratory	Beavers and Calkins (1984)
		S. carpocapsae	Laboratory	Beavers and Calkins (1984)
Bactrocera zonata	Larvae	*H. bacteriophora*	Pot	Soliman (2007)
		S. riobrave	Pot	Soliman (2007)
Bradysia agrestis	Larvae	*S. carpocapsae*	Laboratory/ greenhouse	Kim *et al.* (2004)
Bradysia caprophila	Larvae	*S. carpocapsae*	Laboratory/ greenhouse	Lindquist and Piatkowski (1993); Lindquist *et al.* (1994); Harris *et al.* (1995)
		S. feltiae		Lindquist *et al.* (1994); Harris *et al.* (1995); Jagdale *et al.* (2004)
Bradysia difformis	Larvae	*H. bacteriophora*	Laboratory/ greenhouse	Jagdale *et al.* (2007)
		H. indica	Laboratory/ greenhouse	Jagdale *et al.* (2007)
		H. zealandica	Laboratory/ greenhouse	Jagdale *et al.* (2007)
		S. anomali	Laboratory/ greenhouse	Jagdale *et al.* (2007)
		S. feltiae	Laboratory/ greenhouse	Jagdale *et al.* (2007)
		S. riobrave	Laboratory/ greenhouse	Jagdale *et al.* (2007)

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Bradysia paupera	Larvae	*H. megidis*	Laboratory/ greenhouse	Gouge (1994); Gouge and Hague (1995a)
		S. feltiae		Gouge (1994); Gouge and Hague (1995a,b)
Ceratitis capitata	Larvae	*H. bacteriophora*	Laboratory	Soliman (2007); Malan and Manrakhan (2009); Moino *et al.* (2010)
		H. zealandica	Laboratory	Malan and Manrakhan (2009)
		S. carpocapsae	Laboratory	Moino *et al.* (2010)
		S. khoisanae	Laboratory	Malan and Manrakhan (2009)
		S. riobravae	Pot	Soliman (2007)
Chromatomyia syngensiae	Larvae	*S. feltiae*	Laboratory/ greenhouse	Williams and Walters (2000)
Delia radicum	Larvae	*H. bacteriophora*	Laboratory/field	Welch and Briand (1961a); Bracken (1990); Simser (1992); Schroeder *et al.* (1996)
		S. carpocapsae	Laboratory/field	Simser (1992); Schroeder *et al.* (1996)
		S. feltiae	Laboratory/field	Bracken (1990); Schroeder *et al.* (1996)
Liriomyza bryoniae	Larvae	*S. feltiae*	Laboratory/ greenhouse	Williams and Walters (2000)
Liriomyza huidobrensis	Larvae	*Heterorhabditis* sp.	Laboratory/ greenhouse	Williams and MacDonald (1995)
		Steinernema feltiae	Laboratory/ greenhouse	Williams and Walters (1994, 2000); Williams and MacDonald (1995); Head *et al.* (2000); Head and Walters (2003)
Liriomyza trifolii	Larvae	*Heterorhabditis* sp.	Laboratory/ greenhouse	Hara *et al.* (1993)
		Steinernema carpocapsae	Laboratory/ greenhouse	Harris *et al.* (1990); Olthof and Broadbent (1992); Hara *et al.* (1993); LeBeck *et al.* (1993)
		S. feltiae		Hara *et al.* (1993)
Lycoriella castanescens	Larvae	*H. bacteriophora*	Laboratory	Richardson (1987); Scheepmaker *et al.* (1998c)
		H. megidis	Laboratory	Scheepmaker *et al.* (1998c)
		S. carpocapsae		Gouge and Hague (1995a); Scheepmaker *et al.* (1998c)
		S. feltiae	Laboratory	Richardson (1987); Hay and Fenlon (1995); Hay and Richardson (1995); Scheepmaker *et al.* (1998c); Jess and Bingham (2004)
		Steinernema intermedium	Laboratory	Scheepmaker *et al.* (1998b)
		S. riobrave	Laboratory	Scheepmaker *et al.* (1998b)
		S. feltiae	Field	Richardson and Grewal (1991); Grewal and Richardson (1993); Grewal *et al.* (1993); Scheepmaker *et al.* (1997)
Lycoriella ingenua	Larvae	*Steinernema affinae*	Laboratory	Scheepmaker *et al.* (1998b)

Continued

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
		S. carpocapsae	Laboratory	Richardson (1987); Gouge and Hague (1995a); Cantelo *et al.* (1997); Scheepmaker *et al.* (1998b,c)
		S. feltiae	Laboratory	Nickle and Cantelo (1991); Tomalak and Lippa (1991); Tomalak (1994); Gouge and Hague (1995a); Hay and Richardson (1995); Jess and Bingham (2004)
		S. feltiae	Field	Olthof and Rinker (1990); Olthof *et al.* (1991); Grewal *et al.* (1993); Rinker *et al.* (1995); Jess and Kilpatrick (2000); Jess and Schweizer (2009)
		S. kraussei	Laboratory	Hay and Richardson (1995)
Megaselia halterata	Larvae	*H. bacteriophora*	Laboratory	Richardson (1987); Scheepmaker *et al.* (1998b,c)
		H. megidis	Laboratory	Scheepmaker *et al.* (1998b,c)
		S. affinae	Laboratory	Scheepmaker *et al.* (1998b)
		S. anomali	Laboratory	Scheepmaker *et al.* (1998b)
		S. carpocapsae	Laboratory	Richardson (1987); Cantelo *et al.* (1997); Scheepmaker *et al.* (1998b, c)
		S. feltiae	Laboratory	Long *et al.* (1998); Scheepmaker *et al.* (1998a, b, c); Jess and Bingham (2004)
		S. intermedium	Laboratory	Scheepmaker *et al.* (1998b)
		S. kraussei	Laboratory	Scheepmaker *et al.* (1998b)
		S. riobrave	Laboratory	Scheepmaker *et al.* (1998b)
		S. feltiae	Field	Richardson and Grewal (1991); Grewal and Richardson (1993); Grewal *et al.* (1993); Scheepmaker *et al.* (1997)
Musca domestica	Adults	*H. bacteriophora*	Field	Belton *et al.* (1987)
	Larvae	*S. feltiae*	Laboratory	Geden *et al.* (1986); Taylor *et al.* (1998)
			Field	Geden *et al.* (1986); Georgis *et al.* (1987)
Rhagoletis indifferens	Larvae	*S. carpocapsae*	Laboratory	Lindegren and Vail (1986); Lindegren *et al.* (1990); Gazit *et al.* (2000); Yee and Lacey (2003)
		S. feltiae	Laboratory	Yee and Lacey (2003)
		S. intermedium	Laboratory	Yee and Lacey (2003)
Scatella stagnalis	Larvae	*H. megidis*	Laboratory/field	Morton and del Pino (2003)
		S. arenarium	Laboratory/field	Morton and del Pino (2003)
		S. carpocapsae	Laboratory/field	Gouge (1994)
		S. feltiae	Laboratory/field	Gouge (1994); Lindquist *et al.* (1994); Vänninen *et al.* (1996); Morton and del Pino (2003)
Tipula oleracea	Larvae	*S. feltiae*	Laboratory	Peters and Ehlers (1994)
Tipula paludosa	Larvae	*S. feltiae*	Laboratory	Ehlers and Gerwien (1993); Peters and Ehlers (1994)

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Hymenoptera:sawflies				
Cephalacia falleni	Larvae	*S. feltiae*	Field	Bednarek and Mrácek (1986)
Cephalacia lariciphila	Larvae	*S. feltiae*	Field	Georgis and Hague (1988)
Hoplocampa testudinea	Larvae	*S. carpocapsae*	Field	Vincent and Bélair (1992); Bélair *et al.* (1998)
Hymenoptera: ants				
Acrmyrmex octospinosus	Adults, larvae, pupae	*S. carpocapsae*	Laboratory	Kermarrec (1975); Laumond *et al.* (1979); Bedding (1984b); Kermarrec *et al.* (1986)
Camponotus spp.	Adults, larvae	*S. carpocapsae*	Laboratory	Kermarrec (1975); Laumond *et al.* (1979); Bedding (1984)
Myrmica sp.	Workers	*S. carpocapsae*	Laboratory	Kermarrec (1975); Laumond *et al.* (1979); Bedding (1984)
Neotermes sp.	Adults, workers	*H. bacteriophora*	Field	Lenz and Runko (1992); Lenz *et al.* (2000)
Pogonomyrmex spp.	Adults	*S. carpocapsae*	Laboratory	Georgis (1987)
Postelectrotermes militaris	Adults, workers	*Heterorhabditis* spp.	Laboratory	Amarasinghe and Hominick (1993a,b)
		S. carpocapsae	Laboratory	Amarasinghe and Hominick (1993a,b)
Solenopsis germinate	Adults, workers	*H. bacteriophora*	Laboratory	Castellanos *et al.* (1997)
	Adults	*S. carpocapsae*	Laboratory	Kermarrec (1975); Laumond *et al.* (1979); Bedding (1984)
Solenopsis invicta	Adults, larvae	*H. bacteriophora*	Laboratory/field	Quattlebaum (1980); Drees *et al.* (1992)
Solenopsis invicta	Adults, larvae	*S. carpocapsae*	Laboratory/field	Kermarrec (1975); Poole (1976); Laumond *et al.* (1979); Quattlebaum (1980); Bedding (1984); Jouvenaz *et al.* (1990); Morris *et al.* (1990); Drees *et al.* (1992); Jouvenaz and Martin (1992); Zhang *et al.* (2010)
Solenopsis invicta	Adults, larvae	*S. feltiae*	Laboratory/field	Jouvenaz *et al.* (1990); Jouvenaz and Martin (1992)
		S. scapterisci	Laboratory	Zhang *et al.* (2010)
Solenopsis richteri	Adults, larvae	*S. carpocapsae*	Laboratory/field	Poole (1976); Quattlebaum (1980)
Hymenoptera: yellowjackets				
Vespula altropilosa	Workers	*S. carpocapsae*	Laboratory	Poinar and Ennick (1972)
Vespula germanica	Workers	*S. feltiae*	Laboratory	Guzman (1984)
Vespula pensylvanica	Workers	*H. bacteriophora*	Laboratory	Gambino (1984)
		S. carpocapsae	Laboratory	Poinar and Ennik (1972); Gambino (1984)
	Workers, adults	*S. feltiae*	Laboratory/field	Gambino (1984); Wojcik and Georgis (1987, 1988); Gambino *et al.* (1992)
		Steinernema sp.	Laboratory/field	Gambino (1984)
Vespula rufa	Workers	*S. carpocapsae*	Laboratory	Poinar and Ennik (1972)
Vespula sp.		*S. carpocapsae*	Laboratory	Poinar and Ennick (1972)

Continued

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Isoptera: termites				
Coptermes formosanus	Adults	*H. bacteriophora*	Field	Gouge (2005)
		S. carpocapsae	Laboratory/field	Reese (1971); Fujii (1975); Wu *et al.* (1991)
		S. feltiae	Laboratory	Wu *et al.* (1991)
Glyptotermes dilatatus	Workers	*Heterorhabditis* sp.	Laboratory/field	Danthanarayana (1983); Danthanarayana and Vitarana (1987)
Gnathamitermes perplexus	Adults	*H. bacteriophora*	Laboratory	Yu *et al.* (2006)
		S. carpocapsae	Laboratory	Yu *et al.* (2006)
		S. feltiae	Laboratory	Yu *et al.* (2006)
		S. riobrave	Laboratory	Yu *et al.* (2006)
Heterotermes aureus	Adults	*H. bacteriophora*	Laboratory	Yu *et al.* (2006)
		S. carpocapsae	Laboratory	Yu *et al.* (2006)
		S. feltiae	Laboratory	Yu *et al.* (2006)
		S. riobrave	Laboratory	Yu *et al.* (2006)
Macrotermitinae	Workers, soldiers, nymphs, alates	*Heterorhabditis* spp.	Laboratory	Rouland *et al.* (1996); Benmoussa-Haichour *et al.* (1998)
		Steinernema spp.	Laboratory	Rouland *et al.* (1996); Benmoussa-Haichour *et al.* (1998)
Mastotermes darwiniensis	Workers	*Heterorhabditis* sp.	Field	Bedding and Stanfield (1981); Gouge (2005)
Reticulitermes flavipes	Adults	*H. bacteriophora*	Laboratory/field	Mauldin and Beal (1989); Wang *et al.* (2002); Yu *et al.* (2006)
		H. indica	Laboratory	Wang *et al.* (2002)
		S. carpocapsae	Laboratory/field	Mauldin and Beal (1989); Yu *et al.* (2006)
		S. feltiae	Laboratory/field	Mauldin and Beal (1989); Yu *et al.* (2006)
		S. riobrave	Laboratory	Wang *et al.* (2002); Yu *et al.* (2006)
Reticulitermes hesperus	Adults	*H. bacteriophora*	Laboratory	Poinar and Georgis (1989)
		S. carpocapsae	Laboratory	Poinar and Georgis (1989)
Reticulitermes santonensis	Adults	*H. bacteriophora*	Laboratory	Samarasinghe (1996)
		S. carpocapsae	Laboratory	Samarasinghe (1996)
Reticulitermes tibialis	Adults	*S. carpocapsae*	Laboratory	Epsky and Capinera (1988)
Reticulitermes virginicus	Adults	*H. bacteriophora*	Laboratory	Yu *et al.* (2006)
		S. carpocapsae	Laboratory	Yu *et al.* (2006)
		S. feltiae	Laboratory	Yu *et al.* (2006)
		S. riobrave	Laboratory	Yu *et al.* (2006)
Zootermopsis angusticollis	Adults	*H. bacteriophora*	Laboratory	Georgis *et al.* (1982)
		S. carpocapsae	Laboratory	Georgis *et al.* (1982); Samarasinghe (1996); Wilson-Rich *et al.* (2007)
Blataria: cockroaches				
Blattella germanica	Adults	*H. bacteriophora*	Laboratory	Locatelli and Parleaz (1987); del Pino and Morton (2001)
		Heterorhabditis sp.	Laboratory	Locatelli and Parleaz (1987)
		S. arenarium	Laboratory	del Pino and Morton (2001)
	Adults, nymphs	*S. carpocapsae*	Laboratory	Koehler *et al.* (1992); Appel *et al.* (1993); del Pino and Morton (2001); Pye *et al.* (2001)

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
	Adults, nymphs	*S. feltiae*	Laboratory	Locatelli and Parleaz (1987); del Pino and Morton (2001)
	Adults	*S. scapterisci*	Laboratory	Grewal *et al.* (1993)
Blatta orientalis	Adults, nymphs	*H. bacteriophora*	Laboratory	Kotlarska-Mordzinska *et al.* (2000)
		S. carpocapsae	Laboratory	Koehler *et al.* (1992)
		S. feltiae	Laboratory	Koehler *et al.* (1992); Kotlarska-Mordzinska *et al.*, (2000)
Periplaneta americana	Adults, nymphs	*H. bacteriophora*	Laboratory	Zervos and Webster (1989)
		S. carpocapsae	Laboratory	Koehler *et al.* (1992)
		S. scapterisci	Laboratory	Grewal *et al.* (1993)
Periplaneta brunnea	Adults	*S. carpocapsae*	Laboratory	Corpus and Sikorowski (1992)
Periplaneta fuliginosa	Adults, nymphs	*S. carpocapsae*	Laboratory	Koehler *et al.* (1992)
Supella longipalpa	Adults, nymphs	*S. carpocapsae*	Laboratory	Koehler *et al.* (1992)
Siphonaptera: cat flea				
Ctenocephalides felis	Larvae, pupae	*S. carpocapsae*	Laboratory/field	Manweiler (1994)
Phthiraftera: lice				
Bovicola ovis	Adults	*H. bacteriophora*	Laboratory	James *et al.* (2010)
		S. carpocapsae	Laboratory	James *et al.* (2010)
		S. feltiae	Laboratory	James *et al.* (2010)
		S. riobrave	Laboratory	James *et al.* (2010)
Pediculus humanus capitis	Larvae, nymphs	*H. bacteriophora*	Laboratory	de Doucet *et al.* (1998)
Pediculus humanus humanus	Larvae, adults	*S. glaseri*	Laboratory	Weiss *et al.* (1993)
Arachnida: Ixodida ticks				
Amblyomma americanum	Adults	*H. bacteriophora*	Laboratory	Kocan *et al.* (1998a,b)
		S. carpocapsae	Laboratory	Kocan *et al.* (1989b)
		S. glaseri	Laboratory	Kocan *et al.* (1989b)
		S. riobrave	Laboratory	Kocan *et al.* (1989b)
Amblyomma cajennense	Adults	*H. bacteriophora*	Laboratory	Kocan *et al.* (1998b)
		S. carpocapsae	Laboratory	Kocan *et al.* (1989b)
		S. glaseri	Laboratory	Kocan *et al.* (1989b)
		S. riobrave	Laboratory	Kocan *et al.* (1989b)
Amblyomma gemma	Adults	*H. bacteriophora*	Laboratory	Kaaya *et al.* (2000)
		S. carpocapsae	Laboratory	Kaaya *et al.* (2000)
Amblyomma maculatum	Adults	*H. bacteriophora*	Laboratory	Kocan *et al.* (1998b)
		S. carpocapsae	Laboratory	Kocan *et al.* (1989b)
		S. glaseri	Laboratory	Kocan *et al.* (1989b)
		S. riobrave	Laboratory	Kocan *et al.* (1989b)
Amblyomma variegatum	Adults	*H. bacteriophora*	Laboratory	Kaaya *et al.* (2000)
		Heterorhabditis sp.	Laboratory	Mauleon *et al.* (1993)
		S. carpocapsae	Laboratory	Mauleon *et al.* (1993); Kaaya *et al.* (2000)
Amblyomma spp.	Adults	*S. riobrave*	Laboratory	Kocan *et al.* (1998a)
		S. carpocapsae	Laboratory	Samish and Glazer (1992); Mauleon *et al.* (1993); Samish *et al.* (2000b)
Anocentor nitens	Adults	*S. carpocapsae*	Laboratory	Freitas-Ribeiro *et al.* (2009)
Argas persicus	Adults	*H. bacteriophora*	Laboratory	Hassanain *et al.* (1997)

Continued

Table 8.1. Continued.

Target insect	Life stage	Nematode species	Site	Reference(s)
Boophilus annulatus	Adults	*Heterorhabditis* sp.	Laboratory	Samish and Glazer (1992); Mauleon *et al.* (1993); Samish *et al.* (2000a, c)
		S. carpocapsae		Mauleon *et al.* (1993)
		S. riobrave		Kaaya *et al.* (2000)
Boophilus decoloratus	Adults	*H. bacteriophora*	Laboratory	Kaaya *et al.* (2000)
		S. carpocapsae	Laboratory	Kaaya *et al.* (2000)
Dermacentor variabilis	Adults	*H. bacteriophora*	Laboratory	Kocan *et al.* (1998a, b)
		S. carpocapsae	Laboratory	Kocan *et al.* (1989b)
		S. glaseri	Laboratory	Kocan *et al.* (1989b)
		S. riobrave	Laboratory	Kocan *et al.* (1989b)
Hyalomma dromedarii		*Steinernema* sp.	Laboratory	El-Sadawy (1998)
Rhipicephalus appendiculatus	Adults	*H. bacteriophora*	Laboratory	Kaaya *et al.* (2000)
		S. carpocapsae	Laboratory	Kaaya *et al.* (2000)
Rhipicephalus evertsi	Adults	*H. bacteriophora*	Laboratory	Kaaya *et al.* (2000)
		S. carpocapsae	Laboratory	Kaaya *et al.* (2000)
Rhipicephalus (*Boophilus*) *microplus*	Adults	*Heterorhabditis amazonensis*	Laboratory	Monteiro *et al.* (2010b)
		H. bacteriophora	Laboratory	Monteiro *et al.* (2010a)
		Heterorhabditis sp.	Laboratory	Mauleon *et al.* (1993)
		S. carpocapsae		Mauleon *et al.* (1993)
		S. glaseri	Laboratory	Reis-Menini *et al.* (2008); de Carvalho *et al.* (2010)
		S. riobrave		Kaaya *et al.* (2000)
Rhipicephalus sanguineus	Adults	*H. bacteriophora*	Laboratory	Kocan *et al.* (1998a, b)
		S. carpocapsae	Laboratory	Kocan *et al.* (1989b)
		S. glaseri	Laboratory	Kocan *et al.* (1989b)
		S. riobrave	Laboratory	Kocan *et al.* (1989b)
Rhipicephalus spp.	Adults	*S. carpocapsae*	Laboratory	Kaaya *et al.* (2000)
		S. riobrave	Laboratory	Samish and Galzer (1992); Samish *et al.* (1999, 2000b, c)

multiplicity of pests and the rapidity with which pest and disease outbreaks can occur in these environments. As components of IPM, EPNs are used successfully for the control of root-feeding pests, particularly black vine weevil, fungus gnats, thrips and mushroom flies. More importantly, EPNs have shown considerable promise for the control of above-ground pests such as thrips, leaf miners, white flies, cutworms and diamondback moths under greenhouse conditions (see Table 8.1).

Black vine weevil (*O. sulcatus*) is a serious pest of many ornamental plants, both in the greenhouses and in outdoor plant-production nurseries. The adult weevils feed on leaves and the larvae feed on roots. Bedding and Miller (1981a) were the first to demonstrate the susceptibility of black vine weevil larvae to EPNs, which led to the development of the first commercial nematode product for the control of the weevil larvae in the potted plants industry in Australia. Currently, EPNs provide the most effective means of controlling black vine weevil larvae in potted plants in Australia, Europe and North America. In fact, the weevil larvae and pupae are extremely susceptible to EPNs and can be wiped out from the growing media within a few days of application. Tests on *Astilbe* in pots showed that heterorhabditids were more effective than steinernematids, with *H. bacteriophora* (HP88 or GPS11 strain), *Heterorhabditis marelatus*, *H. megidis* and *H. indica* providing 90–100% control, and *S. carpocapsae* and *S. feltiae* providing 79 and 67% control, respectively, of the

overwintered weevil larvae (P.S. Grewal, 2002, unpublished data).

Fungus gnats (*Bradysia* spp.) are also an important pest of ornamentals in greenhouses and interiorscapes. Although they do not cause much direct feeding damage on plants, the larval root feeding predisposes the plants to attack by pathogenic fungi. Adult fungus gnats spread phytopathogens and are a nuisance to workers. Lindquist and Piatkowski (1993) was the first to show the susceptibility of fungus gnats to EPNs, which resulted in the introduction of *S. feltiae* products in the UK, Europe, Canada and the USA. In most situations, *S. feltiae* provides the best control, but high temperatures in summer months can reduce its efficacy in greenhouses. Recent research has indicated that relatively warm-adapted EPN species such as *H. bacteriophora* GPS11 strain and *H. indica* can serve as alternatives to control fungus gnats under warmer conditions (Jagdale *et al.*, 2007).

The adults, immature stages and pupae of western flower thrips (*Frankliniella occidentalis*) have been found to be susceptible to EPNs. Soil applications have been particularly effective (Tomalak, 1994; Helyer *et al.*, 1995; Ebssa *et al.*, 2001a, b, 2004). EPNs have also shown acceptable control of thrips on leaves and flower buds in greenhouse situations and hence are used commercially for controlling thrips on above-ground plant parts (S.J. Piggott, personal communication). EPNs have also shown effective control of leaf miners (*Liriomyza* spp.) (Harris *et al.*, 1990; Olthof and Broadbent, 1992; Hara *et al.*, 1993; Williams and Walters, 1994, 2000; Williams and MacDonald, 1995; Bennison *et al.*, 1998) and white flies (Cuthbertson *et al.*, 2007, 2008; Qiu *et al.*, 2008) in greenhouses. Although the larvae of the shore fly (*Scatella* spp.) have shown high susceptibility to EPNs under laboratory conditions (Gouge, 1994), control in commercial greenhouses has been largely unacceptable with a few recent exceptions (see Tomalak *et al.*, 2005, for further discussion).

Richardson (1987) was the first to demonstrate the potential of EPNs for the control of mushroom sciarid flies (*Lycoriella* spp.). This research led to the development of the first commercial *S. feltiae* product for the control of sciarid flies in the UK in 1989. *S. feltiae*-based products were also subsequently introduced in Canada, the USA and Europe for the control of different species of sciarid flies in mushroom houses (Jess *et al.*, 2005; Grewal, 2007). Although, several EPN species have been tested against sciarid flies (Table 8.2), *S. feltiae* has been found to be the most effective species against these dipteran pests, perhaps due to its evolutionary history with dipteran flies and intermediate foraging strategy (Grewal, 2007). EPNs have also been tested against other pest species including phorid flies (*Megaselia* spp.) and cecid flies (Richardson, 1987), but acceptable commercial control of these pests has not yet been reported in commercial mushroom-production facilities.

8.9.2 Urban ecosystems

EPNs have proved quite effective for pest control in various components of the urban landscape, including turfgrass in home lawns, athletic fields, public and private parks and golf courses, woody ornamentals in home yards and streets, and herbaceous ornamentals, small fruits and vegetables in home gardens. Some of the major target pests for the use of EPNs in urban landscapes include white grubs, billbugs, chinchbugs, crane fly larvae, flea larvae, armyworms, cutworms, webworms and ants in turfgrass, borers and defoliators in trees, and common coleopteran and lepidopteran pests in home gardens.

White grub control with EPNs has received by far the most attention from researchers and the lawn and landscape industry, mainly because of the high value of this niche market, high population damage thresholds and the relatively sheltered micro-environment for EPNs provided by turfgrass cover. Glaser and Farrell (1935) were the first to demonstrate the potential of *S. glaseri* for control of grubs of the Japanese beetle, *P. japonica*.

Table 8.2. Commercially produced *Heterorhabditis* and *Steinernema* species with their bacterial symbionts, foraging behaviour, temperature activity ranges and most common target insects.

Nematode	Symbiotic bacterium	Foraging behaviour	Temperature activity range	Target insect groups
H. bacteriophora HP88 strain	*Photorhabdus luminescens* subsp. *laumondii*	Cruiser	Intermediate to warm	White grubs, weevils, root borers
H. bacteriophora GPS11 strain	*Photorhabdus temperata* subsp. *stackebrandtii*	Cruiser	Intermediate to warm	White grubs, weevils, root borers
H. indica	*P. luminescens* subsp. *alhurstii*	Cruiser	Warm	White grubs, weevils
H. marelatus	*P. temperata* subsp. *tasmaniensis*	Cruiser	Cold	Weevils
H. megidis	*P. temperata* subsp. *temperata*	Cruiser	Cold	Weevils
S. carpocapsae	*Xenorhabdus nematophilus*	Ambusher	Intermediate	Cutworms, armyworms, fleas
S. feltiae	*Xenorhabdus bovienii*	Intermediate	Cold	Sciarid flies, fungus gnats
S. riobrave	*Xenorhabdus cabanillasii*	Intermediate	Warm	Weevils
S. scapterisci	*Xenorhabdus innexi*	Ambusher	Warm	Mole crickets

Interest in EPNs surged again in the 1980s when large-scale mass-production systems for EPNs were established (Bedding, 1981, 1984a; Friedman, 1990). Initial tests against white grubs conducted using the then commercially available species *S. carpocapsae* were not very successful (Georgis and Gaugler, 1991). It was later found that this species was a poor match for the subterranean white grub larvae due to the nematode's ambush-type host-searching behaviour, which is more suitable for hunting mobile insects on the soil surface. Substantial effort has since been expended in the identification of more effective strains and species of EPNs with cruising-type foraging behaviour for the control of different grub species (Grewal *et al.*, 2005b). It has been found that different white grub species can differ substantially in their susceptibility to the same nematode species or strain, and nematode species and strains can differ in their pathogenicity to the same grub species. In addition, younger grub instars are more susceptible to EPNs than the fully grown last-instar grubs. These studies have led to the commercial availability of several EPN species for control of Japanese beetle and other white grub species, particularly in North America and Europe (Grewal *et al.*, 2005b). Through more recent laboratory and field trials, three nematode species, *Heterorhabditis zealandica* X1 strain, *H. bacteriophora* GPS11 strain, and *Steinernema scarabaei* have been found to possess exceptional potential particularly for control of Japanese beetle, northern masked chafer (*Cyclocephala borealis*), oriental beetle (*A. orientalis*) and European chafer (*R. majalis*) grubs. In a number of field tests, these nematode species provided grub control equal to or better than the most widely used curative control insecticides such as diazinon and trichlorfon (Grewal *et al.*, 2005c). Currently, only *H. bacteriophora* GPS11 and *H. zealandica* X1 strains are commercially available due to difficulties in the mass production of *S. scarabaei*.

Mole crickets (*Scapteriscus* spp.) are another major target pest for EPNs in turfgrass. The tawny mole cricket (*S. vicinus*) and the southern mole cricket (*Scapteriscus borellii*) are the two most destructive species and are distributed throughout the coastal plain region of the south-eastern USA. Adult and nymphal mole crickets cause damage by feeding on

grass roots and shoots, and by tunnelling through the ground. A single mole cricket can create a 3–6 m tunnel in just one night, drying out the soil and causing serious damage to plant roots. The annual costs of controlling mole crickets are estimated to exceed US$50 million in Florida alone. EPNs have been successful in reducing mole cricket damage to turfgrass. *S. scapterisci*, which was originally isolated from infected mole crickets in Uruguay, showed 75–100% infection of adult and nymphal stages of mole crickets under laboratory conditions (Hudson and Nguyen, 1989a,b). This species has also shown potential for mole cricket control in the field (Hudson *et al.*, 1988), but, even more importantly, inoculative releases have been successful (Parkman *et al.*, 1993, 1994, 1996). Becker Underwood Ltd has recently acquired a licence for this nematode from the University of Florida and a product has become available in the market. Another nematode species, *S. riobrave*, which was originally isolated from soil in the Rio Grand Valley in Texas, USA, has also shown potential for mole cricket control. In one test, a 66–86% reduction in turf injury was observed with a single application of *S. riobrave* per acre in South Carolina (Gorsuch, 1995). A commercial product, Vector MC, developed by Biosys, Inc., has been marketed by Lesco, Inc. for the control of mole crickets in turfgrass.

Effective control of other turfgrass pests including billbug (*Sphenophorus* spp.), the annual bluegrass weevil or *Hyperodes* weevil (*Listronotus maculicollis*), the black cutworm (*A. ipsilon*), armyworms (*Spodoptera* spp. and *Pseudaletia* spp.), webworms (*Chrysoteuchia* spp.) and crane flies (*Tipula* spp.) has been demonstrated. For all these pests, *S. carpocapsae* and *H. bacteriophora* have shown good results, both in the laboratory and under field conditions (see Table 8.1 for a complete listing of EPN studies on these pests). Nematodes infect both adult and larval billbugs, but treatments against larvae are generally more effective. Trials often show nematodes to be more effective than standard insecticides when larval stages are treated. In fact, EPNs were the most effective control method for the hunting billbug (*Sphenophorus venatus vestitus*) on golf courses in Japan until preventative-use chloronicotinyl insecticides were registered.

EPNs provide very impressive control of root-, trunk- and branch-boring caterpillars infesting urban trees. Gill and Raupp (1997) reported 100% control of *Iris* moth larvae by applying *S. carpocapsae* to the soil and corms of *Iris germanica*. Injection of EPNs directly into borer galleries has proven equally effective for the control of carpenterworms (*Holcocerus insularis*) (Qin *et al.*, 1988) and clearwing borers (*Synanthedon culciformis* and *Paranthrene robiniae*) in alder and birch, respectively (Kaya and Lindegren, 1983; Kaya and Brown, 1986). EPN applications directly to tree bark have produced more variable results due to their rapid inactivation on exposed surfaces. EPNs have also shown promise for the control of leaf-eating caterpillars including the bagworm (*Thyridopteryx ephemeraeformis*) (Gill and Raupp, 1994), fall webworm (*Hyphantria cunea*) (Yamanaka *et al.*, 1986), and winter moths (*Operophtera* spp.) (Tomalak, 2003) on urban trees.

EPNs have also shown significant potential for the control of leaf-feeding beetles (Kaya *et al.*, 1981; Tomalak, 2004) on urban trees, but they have been less effective against wood-boring beetles (see van Tol and Raupp, 2005, for a review). Tomalak (2004) showed that *H. megidis* can eliminate the pupating leaf beetles *Altica quercetorum* and *Agelastica alni* in soil under the canopy of urban trees. Adult Japanese beetles cause defoliation of a diverse variety of ornamental, fruit and shade trees and shrubs in urban landscapes. While EPNs can inflict significant mortality on grubs, there is no relationship between grub control and adult population because of migration from neighbouring areas. Adult beetles are also susceptible to EPN infection (Lacey *et al.*, 1993; Morris and Grewal, 2011) and the infested or infected beetles can disseminate EPNs externally or internally (Lacey *et al.*, 1995). Lacey *et al.* (1993) modified a common pheromone trap

to attract and infect the beetles with EPNs and allowed them to escape the traps to disseminate the nematodes to other beetles during mating and feeding. However, there are many environmental factors that limit nematode survival in the traps and on the beetles after they exit the trap.

Although many ant species are considered useful predators of pest insects and weed species, some ant species such as carpenter ants (*Camponotus* spp.) can cause serious damage to buildings, pharaoh ants (*Monomorium pharaonis*) can transmit food borne pathogens and stinging ant species can cause injury to humans, especially by eliciting hypersensitive reactions. Fire ants (*Solenopsis* spp.), especially the red imported fire ant (*Solenopsis invicta*) injure humans, pets and livestock. Studies on nearly a dozen species of ants showed that they are susceptible to EPN infection (see Table 8.1 and Gouge, 2005, for a review), and EPNs may be used as tools in the integrated management of ants in the field. More recently, Zhang *et al.* (2010) reported the susceptibility of the newly mated queens of red imported fire ant to *S. carpocapsae* and *S. scapterisci*. They found that both nematode species caused 23–31 to 77–92% mortality of the queens after 3 days' exposure and 62–69 to 100% after 6 days' exposure.

Many species of termite play a useful role in the decomposition of plant material. However, some termite species pose significant problems as pests in agriculture, forestry and urban ecosystems. Formosan termites (*Coptotermes formosanus*), subterranean termites (*Reticulitermes* spp., *Gnathamitermes perplexus* and *Heterotermes aureus*), *Mastotermes* spp., livewood termites (*Glyptotermes dilatatus* and *Postelectrotermes militaris*) and dampwood termites (*Zootermopsis angusticollis*) have been shown to be susceptible to EPNs both in the laboratory and under field conditions (Table 8.1). A comprehensive review of this subject has been provided by Gouge (2005). Yu *et al.* (2006) reported that the subterranean termites *H. aureus*, *G. perplexus* and *Reticulitermes flavipes* were susceptible to *H. bacteriophora*, *S. carpocapsae*, *S. feltiae* and *S. riobrave*; the latter species consistently generated the highest infection levels and mortality of *H. aureus* (80%) in sand assays. Wilson-Rich *et al.* (2007) showed that *S. carpocapsae* caused dose-dependent mortality of the dampwood termite (*Z. angustocollis*), and the termites increased their frequency and duration of allogrooming, vibratory displays, abdominal tip-raising and self-scratching in response to nematode infection.

8.9.3 Horticultural ecosystems

EPNs also show tremendous promise for the control of pests in horticultural ecosystems such as production nurseries, orchards and forestry. There are several common pests that occur in these habitats such as white grubs, stem and root borers, leaf-eating beetles and caterpillars, and weevils. Much of the research on white grubs, stem and root borers, leaf-eating beetles and caterpillars described in the urban ecosystems section above applies to these habitats as well (see also van Tol and Raupp, 2005; Cowles *et al.*, 2005). Similarly the research on black vine weevil larval control described in the section on controlled environments above is relevant to these habitats, except that there are additional challenges for EPN-based control of black vine weevil under field conditions. Despite the high susceptibility of the weevil larvae to EPNs in potted plants, low temperatures and unsuitable soil conditions can reduce nematode efficacy in the field (van Tol and Raupp, 2005; Cowles *et al.*, 2005). For example, *H. bacteriophora* GPS11 strain provided 100% control of black vine weevil larvae in pots but only a 57–71% reduction in adult emergence in field-grown *Taxus* plantations when the nematodes were applied in the spring, mainly due to cooler soil temperatures (P.S. Grewal, unpublished data).

In addition to black vine weevil, there are several other root-, foliage- and fruit-feeding weevils that pose serious problems in soft fruits and orchard crops and in forestry. Schroeder (1987) was the first to

show susceptibility of the citrus root weevil (*Diaprepes abbreviates*) to EPNs. This research quickly led to the introduction of the first commercial nematode product for control of the root weevil in citrus plants. Much research has occurred since then, which has revealed factors affecting nematode efficacy and has identified more effective nematode species and strains for control of the citrus root weevil (see Shapiro *et al.*, 2005, for a review). The EPN species *S. riobrave* and *H. indica* are currently used for the control of this weevil in citrus orchards in Florida. EPNs have also shown promise for the control of other weevils including the adult pecan weevil (*Curculio caryae*) (Shapiro-Ilan, 2001b, 2003), plum curculio (*Conotrachelus nenuphar*) (Shapiro-Ilan *et al.*, 2002b, 2004; Kim and Alston, 2008), guava weevil (*Conotrachelus psidii*) (del Valle *et al.*, 2008), banana weevil (*C. sordidus*) (Laumond *et al.*, 1979; Figueroa, 1990; Treverrow *et al.*, 1991), large pine weevil (*Hylobies abietis*) (Brixey, 2000; Collins, 2003, Everard *et al.*, 2009; Girling *et al.*, 2010), strawberry root weevil (*Otiorhynchus rugosostriatus*) (Booth *et al.*, 2002) and red palm weevil (*Rhynchophorus ferrugineus*) (Dembilio *et al.*, 2010).

Specialized root, stem and fruit borers in fruit crops are also important targets for the use of EPNs. Effective field control has been achieved for grape root borer larvae (*Vitacea polistiformis*) (Williams *et al.*, 2002, 2010), currant borer (*Synanthedon tipuliformis*) (Bedding and Miller, 1981b; Miller & Bedding, 1982), clearwing moth borers (*Synanthedon culiciformis* and *Synanthedon resplendens*) (Kaya and Brown, 1986), dogwood borer (*Synanthedon scitula*) (Davidson *et al.*, 1992), peach tree borer (*Synanthedon exitiosa*) (Gill *et al.*, 1992; Cottrell and Shapiro-Ilan, 2006), lesser peach tree borer (*S. pictipes*) (Shapiro-Ilan *et al.*, 2010b; Cottrell *et al.*, 2011), litchi stem borer (*Arbela dea*) (Xu and Yang, 1992), litchi longhorn beetle (*Aristobia testudo*) (Xu *et al.*, 1995; Han *et al.*, 1996), raspberry crown borer (*Pennisetia marginata*) (Miller and Bedding, 1982; Capinera *et al.*, 1986), western cherry fruit fly (*Rhagoletis indifferens*) (Patterson Stark and Lacey, 1999; Yee and Lacey, 2003), cranberry girdler (*C. topiaria*) (Dapsis, 1993; Berry and Liu, 1998; Simard *et al.*, 2002), navel orangeworm (*Amyelois transitella*) (Lindegren *et al.*, 1987; Siegel *et al.*, 2004) and codling moth (*C. pomonella*) (Kaya *et al.*, 1984; Lacey and Unruh, 1998; Lacey and Chauvin, 1999; Unruh and Lacey, 2001). Williams *et al.* (2002) found that *H. zealandica* X1 strain and *H. bacteriophora* GPS11 strain were the most effective nematodes, providing nearly 100% control of grape root borer larvae embedded in the roots in the laboratory. In commercial vineyards in Ohio, North Carolina and Georgia in the USA, *H. zealandica* and *H. bacteriophora* reduced adult emergence of the grape root borer by 60–75% with a single application in the spring. They also found that only the indigenous *H. bacteriophora* persisted for 2 years in vineyards (Williams *et al.*, 2010). Siegel *et al.* (2004) reported that *S. feltiae* and *S. carpocapsae* applied to almond and pistachio nut mummies on the ground can almost wipe out navel orangeworm larvae.

8.9.4 Agroecosystems

Many insect pests affecting field crops have been shown to be highly susceptible to EPNs under laboratory conditions, but there have been fewer successes in the field. Some of the factors limiting the success of EPNs in field crops are: (i) the high cost of treatment relative to chemical pesticides; (ii) the lack of availability of sufficient quantities of EPNs to treat high-acreage crops such as maize; (iii) a lack of irrigation for application; (iv) rapid inactivation of EPNs due to environmental extremes such as temperature, moisture and UV radiation, particularly on exposed soil and plant surfaces; (v) unpredictable seasonal demands; and (vi) limited storage stability. More details of the factors affecting the success of EPNs in agroecosystems can be found in reviews by Shapiro *et al.* (2002a), Grewal *et al.* (2005b) and Georgis *et al.* (2006). Nevertheless, field efficacy of EPNs has been demonstrated against a range of

pests and some successes have been obtained in certain situations. Below is a brief discussion on the efficacy of EPNs in controlling some major target pests in agroecosystems.

The adults, larvae and pupae of several weevil species have been targeted for use of EPNs in many field crops. In field trials, mortality of the sugarbeet weevil (*Temnorhinus mendicus*) varied from 40 to 93% (Boselli *et al*., 1991, 1994, 1997; Curto *et al*., 1992), sweet potato weevil (*Cylas formicarius*) from 25 to 85% (Jansson *et al*., 1990, 1993) and strawberry root weevil (*Otiorhynchus ovatus*) and black vine weevil (*O. sulcatus*) from 70 to 100% (Shanks and Agudelo-Silva, 1990). EPNs also caused up to 100% mortality of cotton boll weevil (*Anthonomus grandis*) (Cabanillas, 2003). While *H. bacteriophora* provided a 38–80% reduction in carrot weevil (*Listronotus oregonensis*) damage to carrots, *S. carpocapsae* provided only a 14% reduction (Miklasiewicz *et al*., 2002).

EPNs also show potential for the control of Colorado potato beetle (*L. decemlineata*), cucumber beetle (*Diabrotica undecimpunctata*), maize rootworm (*Diabrotica virgifera*), mint flea beetle (*Longitarsus ferrugineus* and *Longitarsus waterhousei*), striped flea beetle (*Phyllotreta striolata*) and various scarab species under laboratory and field conditions. However, no commercial use of nematodes has occurred against these beetle pests in field crops, perhaps due to the lack of irrigation available and the high cost of nematodes in these low-value, high-acreage field crops. Hiltpold *et al.* (2010) reported that genetic selection of *H. bacteriophora* that are attracted to volatiles produced from the roots can enhance nematode host finding and improve control of *D. virgifera virgifera*, while Toepfer *et al.* (2010) suggested that soil type can have a large influence on the effectiveness of EPNs against the same insect. Although the cabbage maggot (*Delia radicum*) appears to be a good target for EPNs, larval control and plant damage reduction in the field has been limited (Bélair *et al*., 2005).

Good control of lepidopterous pests of field crops has been obtained with EPNs. Although initial studies produced poor results, subsequent studies demonstrate excellent control of maize earworm (*Helicoverpa zea*) with *S. riobrave* when the nematodes were targeted against the prepupal and pupal stages (Raulston *et al*., 1992; Cabanillas and Raulston, 1994a,b; Cabanillas *et al*., 1994). Cabanillas and Raulston (1995) obtained 100 and 95% *H. zea* mortality by applying the nematodes when 50% of the larvae were late instars and still in the maize ears, and when 10% of the larvae had left the ears to pupate in the soil, respectively. Cabanillas and Raulston (1996a) demonstrated that irrigation method, timing and nematode concentration were important factors in the success of the nematodes: application of 200,000 IJs/m^2 resulted in 95% insect mortality when applied via in-furrow irrigation compared with 84 and 56% mortality when applied after or before surface irrigation, respectively. Another reason for these exceptional results is the higher heat tolerance of *S. riobrave* compared with *S. carpocapsae*; the latter caused no insect mortality in maize fields in Texas (Cabanillas and Raulston, 1996b). Feaster and Steinkraus (1996) obtained similar results on maize earworm control in Arkansas with *S. riobrave*.

Pink bollworm (*Pectinophora gossypiella*) is another excellent target for the use of EPNs in cotton. Although pink bollworm pupae are not susceptible to EPNs (Henneberry *et al*., 1995), the diapausing larvae in soil during the winter are susceptible (Gouge *et al*., 1999). Due to lower temperatures during winter months, *H. bacteriophora* has been found to be more effective than *S. riobrave* for the control of pink bollworm (Gouge *et al*., 1999). Similarly, tobacco budworm (*Heliothis virescens*) is a good target for the use of EPNs (Bell, 1995). Bari (1992) demonstrated that artichoke cuttings soaked in *S. carpocapsae* suspension protected the transplanted cuttings from artichoke plume moth (*P. carduidactyla*) infestation. This demonstration led to the commercial introduction of a nematode product in the artichoke market.

EPNs can provide excellent control of cutworms (*Agrotis* spp.) in many different field crops, which often exceeds the level of control provided by insecticides (see Table 8.1 for a complete listing of the studies). Field application of *S. carpocapsae* reduced black cutworm (*A. ipsilon*) damage by 50% on maize (Capinera *et al.*, 1988). Levine and Oloumi-Sadeghi (1992) found that a single application of *S. carpocapsae* reduced the number of cut maize plants by 76–83% during the 1–10 days after treatment. Yokomizo and Kashio (1996) reported that a single ground spray of *S. carpocapsae* at 1 billion IJs/ha or two applications of 0.5 billion IJs/ha with an 8-day interval caused 80 and 67% mortality of turnip moth (*Agrotis segetum*) larvae, respectively.

Diamondback moth (*Plutella xylostella*) is a major pest on many vegetable and ornamental plants. Although the larvae are susceptible to EPNs, acceptable field control has been more difficult to achieve (Baur *et al.*, 1998). Addition of adjuvants containing antidesiccant or UV protective action to the spray mixture can improve nematode survival on the exposed foliage and enhance control of the diamondback moth larvae, as described above (Vyas *et al.*, 2000; Schroer and Ehlers, 2005).

EPNs can provide effective control of the mint root borer (*Fumibotys fumalis*) in mint. Accurate application timing is critical to obtain effective control of the mint root borer due to the prolonged emergence of adults, the formation of resistant hibernacula and the limited persistence of the nematodes (Grewal and Georgis, 1999). When applied before the formation of hibernacula, *S. carpocapsae* can provide effective control of this pest (J. Takeyasu, 1992, unpublished data).

Insect pests affecting stored products have also been shown to be susceptible to EPNs. The larvae of the Mediterranean flour moth (*Ephestia kuehniella*), lesser grain borer (*Rhyzopertha dominica*), confused flour beetle (*Tribolium confusum*) and rice weevil (*Sitophilus oryzae*) have all been shown to be highly susceptible to *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* in stored wheat grains under laboratory conditions (Athanassiou *et al.*, 2008, 2010). No commercial use of EPNs has yet occurred in the stored-products industry.

8.9.5 Human and animal pests

Fleas pose problems for both humans and domesticated animals. In addition to their nasty bites, they can also transmit diseases. The cat flea (*Ctenocephalides felis*) is a cosmopolitan parasite of dogs and cats, and has also been reported feeding on humans. Adult fleas spend most of their time feeding on mammal hosts where mating and egg laying also occur. The eggs eventually drop off the animal and the emerging larvae feed on organic debris in pet bedding, lawns, carpeting and upholstered furniture. EPNs have been extremely effective in controlling flea larvae and pupae in home lawns. Silverman *et al.* (1982) were the first to report the susceptibility of cat flea larvae to EPNs. In tests performed in North Carolina in the USA, *S. carpocapsae* applied at 1 billion IJs/acre caused over 90% mortality of flea larvae within 24 h (Silverman *et al.*, 1982). *S. carpocapsae* also caused 91–97% mortality of flea pupae in cocoons in a test in Louisiana (Manweiler, 1994). EPNs are most effective against flea larvae in turf and soil when the outdoor temperatures are above 14°C and the soil is moist. Biosys, Inc. developed the first biocontrol product based on *S. carpocapsae* for the control of flea larvae in home lawns as a part of an integrated flea-control programme (Manweiler, 1994). *S. carpocapsae*-based products sold under the trade names Interrupt and Bio Flea Halt became extremely popular in 1994 and 1995 (Grewal and Georgis, 1999). There are currently many more products on the market.

EPNs have also been shown to possess potential for the control of ticks. Samish and Glazer (1991) were the first to report that EPNs were capable of killing engorged females of cattle ticks. Ticks belonging to the genera *Amblyomma*, *Argas*, *Boophilus*, *Dermacenteor*, *Hylomma* and *Rhipicephalus* have been found to be susceptible to nematode infection (Glazer *et al.*, 2005).

Reis-Menini *et al.* (2008) reported susceptibility of the cattle tick (*R. microplus*) to *S. glaseri* and found the nematodes to be compatible with low levels of a commonly used acaricide. Monteiro *et al.* (2010a) reported that *H. bacteriophora* HP88 strain affected the egg mass weight, oviposition period, survival period, hatching percentage, percentage egg production and nutritional index of the non-parasitic phase of the engorged *R. microplus*. The tick mortality was greater than 90% at all nematode concentrations, with 1200 IJs per tick providing 99% mortality. Similarly, Monteiro *et al.* (2010b) found that *Heterorhabditis amazonenesis* had a deleterious affect on many biological parameters of *R. microplus* including oviposition and survival period, with application of nematodes resulting in an overall tick mortality of 67.8%. de Carvalho *et al.* (2010) determined that a 2 h exposure of engorged female *R. microplus* ticks to *S. glaseri* was sufficient for infection but a minimum 24 h exposure to the nematodes was necessary for greater than 90% treatment efficacy.

Susceptibility of the body louse (*Pediculus humanus humanus*) to EPNs was first demonstrated by Weiss *et al.* (1993). They reported that *S. carpocapsae* and *S. glaseri* caused over 85% mortality of female lice within 24 h. The head louse (*Pediculus humanus capitis*) has also been found to be susceptible to EPNs, and *H. bacteriophora* was most effective among the EPN species tested (de Doucet *et al.*, 1998). James *et al.* (2010) found that the sheep lice (*Bovicola ovis*) were susceptible to *S. carpocapsae*, *S. feltiae*, *S. riobrave* and *H. bacteriophora* Petri dish bioassays at 30°C, with *S. riobrave* causing significantly higher mortality than *S. carpocapsae* at 37°C. They also reported that all species were able to locate and infect the lice in wool when formulated in water with 8% Tween 80. In the wool assays, *S. riobrave* caused higher mortality of lice than the *H. bacteriophora*. James *et al.* (2010) concluded that *S. riobrave* is likely to be most effective when applied to live sheep, because of its high temperature tolerance and cruising-type host-finding behaviour.

There are over 4000 species of cockroach, but only a few inhabit human dwellings around the world. Three species, the German cockroach (*B. germanica*), oriental cockroach (*Blatta orientalis*) and American cockroach (*Periplaneta americana*), are predominant in human dwellings including homes, restaurants, bakeries, food-processing plants and grocery stores. Cockroaches pose problems to humans mainly as carriers of human pathogens and through allergenic responses in sensitive individuals. Skierska *et al.* (1976) was the first to demonstrate the susceptibility of *B. germanica* to *S. carpocapsae* in Petri dishes. Since then, several other cockroach species have been found to be susceptible to EPNs (see Glazer *et al.*, 2005, for a review). Successful control of cockroaches in human dwellings has also been reported. Appel *et al.* (1993) applied *S. carpocapsae* in bait stations in apartment buildings and obtained a 67% reduction in the *B. germanica* population 8 weeks after application; this level of control was similar to the standard insecticide bait treatment. Similarly, Pye *et al.* (2001) obtained over 80% control of *B. germanica* by applying *S. carpocapsae* in a bait station containing polysaccharide gel with food and pheromone attractant.

Yellowjackets (*Vespula* spp.) pose serious problems for humans and animals as they are attracted to food and can also sting. The workers of *Vespula* spp. have been found to be susceptible to EPNs, and attempts to control them in the field have met with some success (see Gouge, 2005, for a review). EPNs have been applied either directly to the nest to eliminate the colonies or to the baits to which workers are attracted to infect them.

The house fly (*M. domestica*) is another serious pest of humans and animals throughout the world. The flies cause annoyance to humans and animals, but can also transmit over 100 different pathogens. Geden *et al.* (1986) were the first to demonstrate the susceptibility of the fly larvae and adults to *S. carpocapsae* and *H. bacteriophora* in Petri dishes. Subsequently, more species and strains of EPN were evaluated for virulence against the fly larvae

and adults under laboratory conditions, and field tests were conducted to control the pest (see Glazer *et al.*, 2005, for a review). In field trials, EPNs applied either directly to animal or chicken manure or to bait stations have demonstrated significant reductions in fly populations.

8.10 EPN Interactions with Plant-parasitic Nematodes

Commercial applications of EPNs can also result in improvements in plant growth that are not associated with control of the target insect pest. Early analyses of soil samples from EPN-treated areas of turfgrass and citrus revealed fewer plant-parasitic nematodes (PPNs) than the untreated plots. Systematic evaluations in the field have provided support for these observations. For example, *H. bacteriophora* reduced the populations of *Tylenchorynchus* spp. and *Pratylenchus pratensis* (Smitley *et al.*, 1992), *S. riobrave* reduced *Meloidogyne* sp., *Belonolaimus longicaudatus* and *Criconemoides* sp. (Grewal *et al.*, 1997), *S. carpocapsae* reduced *Globodera rostochiensis* (Perry *et al.*, 1998), *Trichodorus* sp. (Jagdale *et al.*, 2002) and *Aphelenchoides fragariae* (Jagdale and Grewal, 2008), and *S. feltiae/S. glaseri* reduced *Meloidogyne javanica* (Fallon *et al.*, 2002, 2006). There have also been reports showing no effect of EPNs on certain PPN species (see Lewis and Grewal, 2005, for a review).

Several mechanisms have been proposed to explain the unexpected interactions between EPNs and PPNs. These have included the following: (i) EPNs attracted to plant roots may be interfering with PPNs finding root or feeding sites (Bird and Bird, 1986); (ii) EPNs applied to soil for insect control may stimulate the build-up of general nematode antagonists and predators (Ishibashi and Kondo, 1986; Ishibashi and Choi, 1991); and (iii) EPN-infected cadavers filled with both symbiotic bacteria and developing nematodes can release high concentrations of ammonia (Grewal *et al.*, 1999b; Shapiro *et al.*, 2000; de Nardo *et al.*, 2006) and other allelochemicals, which can be toxic to PPNs (McInerney *et al.*, 1991; Grewal *et al.*, 1999b). The secondary metabolite 3,5-dihydroxy-4-isopropulstilbene, from EPN-infected cadavers, inhibited egg hatching of the PPN *Meloidogyne incognita* and caused significant mortality of the fungal-feeding nematodes *Aphelenchoides rhytium* and *Bursaphelenchus* spp. (Hu *et al.*, 1999). Indeed, both *Xenorhabdus* and *Photorhabdus* bacteria produce large amounts of ammonium in culture, which have been shown to be toxic to second-stage juveniles of *M. incognita* (Hu *et al.*, 1999; Grewal *et al.*, 1999b). *S. riobrave* and *S. feltiae/S. glaseri* applied in infected host cadavers significantly reduced the number of egg masses of *Meloidogyne partityla* (Shapiro-Ilan *et al.*, 2006) and *M. javanica* (Fallon *et al.*, 2006), respectively. Jagdale and Grewal (2008) demonstrated that soil application of insect cadavers infected with EPNs and their symbiotic bacteria suppressed populations of the foliar nematode *A. fragariae* in the rhizosphere. In addition, they found that the cadaver treatments significantly reduced the multiplication of *A. fragariae* in the leaves, even though there was no direct contact between *A. fragariae* and the EPNs and their symbiotic bacteria.

Jagdale *et al.* (2009) have suggested a new mechanism of EPN–PPN interactions. They investigated the effects of *S. carpocapsae* and its symbiotic bacterium, *X. nematophilus*, on the pyrogallol peroxidase (P-peroxidase), guaiacol peroxidase (G-peroxidase) and catalase activities in *Hosta* spp. and *Arabidopsis thaliana* plants as components of induced systemic resistance. P-peroxidase activity was significantly higher in the leaves from *Hosta* plants treated with *S. carpocapsae* IJs and *S. carpocapsae*-infected insect cadavers than in the leaves from the control plants 2 weeks after treatment. G-peroxidase activity was significantly higher in *S. carpocapsae*-infected cadaver and *X. nematophila* treatments 10 and 15 days after treatment (DAT) and in *S. carpocapsae* IJs at 5 and 15 DAT. Catalase activity in *Hosta* leaves was significantly higher in *S. carpocapsae*-infected cadaver and *X. nematophilus* treatments compared with

the control at 5 and 15 DAT and following *S. carpocapsae* IJ treatment at 5 and 10 DAT. Furthermore, the catalase activity in the *A. thaliana* leaves was significantly higher following *S. carpocapsae* IJ treatment than in the control at 7 DAT. They also determined the effects of *S. carpocapsae*-infected cadavers and *S. carpocapsae* IJs on expression of the *PR1* gene (which is expressed in response to a variety of pathogens) in transgenic *A. thaliana* leaves through a β-glucuronidase activity assay and found that the *PR1* gene was expressed in leaves from all treatments except the control. Thus, it was concluded that EPNs and their symbiotic bacteria can induce systemic resistance in plants, which may explain the elusive antagonistic interactions between EPNs and PPNs.

8.11 Conservation Strategies

Although EPNs are used primarily as inundative pest-control agents with relatively short-term effects, they do possess the potential to provide sustainable pest control. The role of nematodes in regulating insect pest populations is not well understood, but the reported naturally occurring insect mortality from EPNs ranges from 8 to 71% (Mrácek, 1986; Georgis and Hague, 1988; Klein, 1990; Akhurst *et al.*, 1992; Raulston *et al.*, 1992; Cabanillas and Raulston, 1994a; Campbell *et al.*, 1998). *S. glaseri* was reported to have been sustained in the field for 14 years in New Jersey with Japanese beetle (*P. japonica*) larval densities of less than 54 per m^2 (Gaugler *et al.*, 1992). In coastal California, endemic *H. marelatus* was dynamically linked with populations of a root-feeding insect and its host plant (Strong *et al.*, 1995, 1996, 1999). These EPNs indirectly protect the yellow bush lupin (*Lupinus arboreus*) by killing the root-feeding hepialid moth (*Hepialus californicus*) larvae. Lupins suffer heavy root damage and subsequent mortality from these larvae in the absence of *H. marelatus*. The nematodes cause high mortality of the larvae, and the spatial distribution of *H. marelatus* is correlated with long-term fluctuations in bush lupine cover (Strong *et al.*, 1995). Campbell *et al.* (1995, 1998) found a negative correlation between the abundance of *H. bacteriophora* and Japanese beetle larvae in turfgrass; they suggested that this pattern resulted from density-dependent suppression of the larval population. Campbell *et al.* (1995) found low percentages of samples to be positive for *H. bacteriophora* and *S. carpocapsae* in turfgrass plots, although there were differences of three orders of magnitude in the numbers of IJs in positive samples. The mean population density for each sample date showed no seasonal pattern of abundance. However, other studies (del Pino and Palomo, 1997; Efron *et al.*, 2001) have documented seasonal variation in nematode population density, and suggest a relationship with the seasonal abundance of hosts.

Studies provide evidence for in-undatively applied EPN reproduction in targeted pest insects and population increases over time. Grewal and Richardson (1993) demonstrated that *S. feltiae* reproduced in the sciarid fly (*Lycoriella auripila*) in mushroom houses and provided more effective control of second-generation than first-generation larvae. Klein and Georgis (1992) observed that *H. bacteriophora* applications made in the autumn provided 60–65% control of Japanese beetle grubs in turfgrass in Ohio, USA, and that this control increased to 95% in the following spring. Successful inoculative release of *S. scapterisci* against mole crickets in pastures in Florida has also been documented (Parkman and Smart, 1996). The persistence of EPNs beyond a season following their application against white grubs has also been reported in other studies (Sexton and Williams, 1981; Poinar *et al.*, 1987; Klein and Georgis, 1992; Choo *et al.*, 2002), suggesting the potential impact of EPNs over multiple generations of target pests. Susurluk and Ehlers (2008a) found evidence for sustainable control of black vine weevil with *H. bacteriophora* and Susurluk and Ehlers (2008b) showed that *H. bacteriophora* often persisted for over a year after application, particularly in beans followed

in rotation by wheat, with red clover as a cover crop. Compared with the 24.8 day half-life of *H. bacteriophora* in soil at 8°C, Susurluk and Ehlers (2008b) concluded that the nematodes recycled in host insects in the field to persist for 12 months. Koppenhöfer and Fuzy (2009) recovered inundatively applied *S. scarabaei* from turfgrass plots for 4 years, but significant control of the oriental beetle (*A. orientalis*) grub was observed only for about 18 months. These observations of EPN recycling and pest suppression suggest that conservation biological control by EPNs should be feasible, a conclusion supported by Akhurst *et al.* (1992), who reported that two *Heterorhabditis* spp. caused an epizootic extending over 5 ha in four species of white grubs feeding on sugarcane roots.

8.12 Genetic Improvements

Although the benefits of genetic selection have been demonstrated, there has been little commercial use of genetically improved strains. Gaugler *et al.* (1989) were the first to demonstrate the use of selective breeding for enhanced host finding in *S. carpocapsae*. The first reports on genetic selection for enhanced infectivity of *S. feltiae* to mushroom sciarid fly larvae was by Tomalak (1994) and for enhanced temperature tolerance of *H. bacteriophora*, *Steinernema anomaly* and *S. feltiae* were by Grewal *et al.* (1996a,b). Hiltpold *et al.* (2010) demonstrated that selection of *H. bacteriophora* for enhanced responsiveness to (*E*)-*β*-carophyllene, a volatile root signal produced by maize, can enhance control of western corm rootworm under field conditions. Zioni *et al.* (1992) were the first to isolate a morphological mutant, and O'Leary and Burnell (1997) were the first to isolate desiccation mutants in *H. bacteriophora*. Hashmi *et al.* (1995) were the first to report on the establishment of a transgenic strain of *H. bacteriophora* expressing a heat-shock protein gene from *Caenorhabditis elegans*. The first report on expressed sequence tags (ESTs) of *H. bacteriophora* GPS11 strain was by Sandhu *et al.* (2005), which was followed by a more comprehensive EST analysis of the TT01 strain of *H. bacteriophora* by Bai *et al.* (2009). The full genome of *H. bacteriophora* TT01 strain has now been sequenced and is anticipated to be in the public domain in the near future.

8.13 Conclusions and Future Directions

EPNs have emerged as excellent alternatives to chemical pesticides. They are now serving as effective tools in IPM in diverse ecosystems. EPNs have been evaluated against nearly 200 insect pest species of which almost all can be controlled effectively under field conditions. A broad host range and the ability to seek and kill insects in soil and in cryptic habitats such as plant roots and tree trunks where most chemical pesticides fail to reach make EPNs especially attractive. The ease of application via standard pesticide spray equipment and using irrigation systems has facilitated the adoption of EPNs in diverse ecosystems. Their compatibility with numerous agrochemicals including insecticides, miticides, fungicides, herbicides, wetting agents, plant growth regulators and spray adjuvants enhances the use of EPNs in IPM systems. In addition to additive effects, some pesticides can even enhance EPN pathogenicity/effectiveness through synergistic effects.

Although EPNs are most widely used against the soil-dwelling stages of insect pests, applications against above-ground pests have also been shown to be successful. Foliar applications have shown particular promise in certain situations, such as in greenhouses and in some outdoor situations, for the control of pests such as thrips, leaf miners, white flies and cutworms. EPN applications in insect galleries in tree trunks have provided excellent control of wood-boring insects. EPNs have been proven effective in prophylactic applications to protect plant-propagation material and growing plants against pest attacks. Slow-release formulations such as

alginate and polyacrylamide gels and EPN-infected cadavers hold promise for the prophylactic use of EPNs, particularly for their delivery via the growing media commonly used in greenhouses and the potted-plant industry. Successes have also been reported in the use of EPNs in traps designed to lure and kill significant pests such as cockroaches, houseflies, grasshoppers, mole crickets, cutworms and weevils. EPNs have also shown promise for the control of human and animal pests including fleas, yellowjackets, ants, termites, lice and ticks.

Further expansion of the use of EPNs is hampered by their sensitivity to environmental extremes including temperature, desiccation and UV radiation and the less than optimal longevity of the IJs, which limits the shelf life of these products. Grewal *et al*. (2005c) performed a critical analysis of the factors limiting expansion of the use of EPNs, presenting ideas for improvement and identifying research needs. They identified high cost, limited availability, suboptimal ease of use and suboptimal efficacy as the main factors limiting expansion, even in the markets in which they are currently used. Although progress has been made since 2005, these factors still remain a challenge for researchers and the industry to overcome. End-user education and marketing support for the adoption of EPN products is extremely limited compared with the competing chemical products. Expansion of use in new markets such as agroecosystems and outdoor horticultural production systems, in which EPNs have already been shown to be effective, needs further research, particularly in development of new application technologies and strategies. It is exciting to see recent expansion in research activity in the areas of EPN conservation biology, population ecology, genomics and molecular mechanisms of bacteria–nematode symbiosis and nematode and bacterial pathogenicity. It is expected that new fundamental research will provide the needed boost to further commercial development of EPNs.

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9 Microbial Control of Crop Pests using Entomopathogenic Fungi

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9.1 Introduction

Fungal diseases are widespread in nature, with various species found in diverse habitats. The association between entomopathogenic fungi and insects dates back to approximately 25 million years ago in the Oligocene–Miocene epochs based on fossil records (Poinar and Thomas, 1982, 1984; Poinar, 1993; Poinar and Poinar, 2005). Fungal diseases play an important role in the natural regulation of their hosts through epizootics, which decimate large portions of insect populations. Entomopathogenic fungi comprise genera or species that are proven to be pathogenic to insects, as well as to spiders and mites, which are closely related.

Approximately 750 species of entomopathogenic fungi are known to be associated with arthropods in plants, soil and aquatic environments (Fuxa, 1987; McCoy *et al.*, 1988). The majority are found in the orders Entomophthorales and Hypocreales. The host range of entomopathogenic fungi is rather diverse among different species. Many species are ubiquitous, including those in the genera *Beauveria* Vuillemin, *Hirsutella* Patouillard, *Isaria* Persoon: Fries (= *Paecilomyces* Bainier), *Lecanicillium* Gams and Zare (= *Verticillium* Nees per Link) and *Metarhizium* Sorokin, whereas others are somewhat limited to a few groups of insect and invertebrate hosts, such as those in the genera *Aschersonia* Montagne and *Hypocrella* Saccardo (scales and whiteflies), *Batkoa* Humber (homopterans and flies), *Conidiobolus* Brefeld (aphids), *Erynia* (Nowakowski ex Batko) Remaudière and Hennebert (culicids and chironomids), *Furia* (Batko) Humber (muscoids and noctuids), *Fusarium* Link (scales and other insects), *Neozygites* Witlaczil (aphids and thrips), *Nomuraea* Maublanc (noctuids and spiders), *Pandora* Humber (planthoppers and aphids), *Tolypocladium* W. Gams (small dipterans), *Torrubiella* Boudier (homopterans and spiders) and *Zoophthora* Batko (aphids and beetles). There are species with very narrow host ranges in the genera *Ascosphaera* Spiltoir and Olive (bee larvae), *Gibellula* Cavara (spiders), *Entomophaga* Batko (lepidopterans), *Entomophthora* Fresenius (flies and aphids) and *Massospora* Peck (cicadas). The ability to invade hosts directly through the cuticles makes fungi unique among entomopathogens, as oral ingestion is not required prior to invasion. This is extremely important for insects with piercing/sucking mouthparts such as aphids, as they are incapable of ingesting other pathogens (e.g. bacteria) that infect through gut walls.

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Microbial control utilizes fungi, bacteria and other disease organisms to suppress pest populations. About 25 fungal species have been evaluated as microbial control agents against insect and invertebrate pests, including: (i) *Aschersonia aleyrodis* Webber against whiteflies; (ii) *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin against various crop and soil insects, *Beauveria brongniartii* (Saccardo) Petch against European chafers and sugarcane borers; (iii) *Coelomomyces* spp., *Culicinomyces clavisporus* Couch, Romney and Rao, and *Tolypocladium cylindrosporum* W. Gams against mosquitoes; (iv) *Entomophaga maimaiga* Humber, Shimazu, Soper and Hajek against gypsy moth; (v) *Hirsutella thompsonii* Fisher against citrus rust mite; (vi) *Isaria farinosa* (Holmsk.) Fries against potato beetle and beet armyworm; (vii) *Isaria fumosorosea* Wize against whiteflies; (viii) *Lecanicillium* spp. against aphids, whiteflies and thrips; (ix) *Metarhizium anisopliae* (Metschnikoff) Sorokin against spittle bugs, locusts and grasshoppers; (x) *Metarhizium flavoviridae* W. Gams and Rozsypal against brown planthopper and black vine weevil; (xi) *Neozygites fresenii* (Nowakowski) Batko against aphids; (xii) *Nomuraea rileyi* (Farlow) Samson against lepidopterans; and (xiii) *Pandora neoaphidis* (Remaudière and Hennebert) Humber and *Zoophthora radicans* (Brefeld) Batko against aphids.

Previous reviews on the utilization of fungal pathogens as microbial control agents of insect pests include Steinhaus (1949, 1957), Ferron (1978), Fuxa (1987), McCoy *et al.* (1988), Tanada and Kaya (1993), Feng *et al.* (1994), Hajek and St Leger (1994), Hajek (1997), Boucias and Pendland (1998), Wraight and Carruthers (1999), Inglis *et al.* (2001), Shah and Pell (2003), Roberts and St Leger (2004), Castrillo *et al.* (2005) and Goettel *et al.* (2010). This chapter introduces the principles and practices of microbial control with entomopathogenic fungi in field crop systems, starting by reviewing the history of fungi as entomopathogens, followed by the most recent developments on taxonomic status of this group of organisms, and then focusing on their general biology and disease processes, as well as environmental factors that affect fungal infection and field epizootics. Strain characterization and development towards their commercialization and field application of selected species are also covered. Successful microbial control programmes with specific entomopathogenic fungi are reviewed as case studies. The future of fungal pathogens as microbial control agents in integrated pest management (IPM) is also discussed.

9.2 Fungi as Entomopathogens: the History

Chinese sericulturists were the first to record fungal diseases on silkworms in 700 BC (Tanada and Kaya, 1993). However, modern insect pathology did not take root until 1835 when the Italian entomologist Agostino Bassi (1773–1856) identified *Botrytis* (= *Beauveria*) *bassiana* as the causal agent of widespread silkworm disease in Italy and France. He also demonstrated that this fungus was contagious and could be transmitted not only to healthy silkworms but also to other species of insects. By recommending constant disinfection of the rearing facilities and timely removal of infected caterpillars, he was credited with the rescue of the economically important silk industry in Europe. He laid the foundation for the study of infectious diseases, which formed the basis for the germ theory that is still in use today. He preceded Louis Pasteur (1822–1895) and Robert Koch (1843–1910) with his contributions in plant pathology, microbiology and vertebrate pathology.

John LeConte (1825–1883), an American entomologist, introduced the concept of 'microbial control' in 1874 when he made recommendations to overhaul the study of economic entomology in the USA (LeConte, 1874). At the same time, Pasteur suggested the use of pebrine (microsporidia) for the control of grape phylloxera, *Daktulosphaira vitifoliae* Fitch (Homoptera: Phylloxeridae).

In 1878, Elie Metchnikoff (1845–1916), a pioneer Russian microbiologist, became

the first scientist to study entomopathogenic fungi systematically for microbial control of crop pests. During his study on grain beetle (*Anisoplia austriaca* Herbst; Coleoptera: Scarabaeidae), a devastating pest of cereal crops in Russia at that time, he observed that beetle population cycles corresponded with fungal outbreaks in the field. He identified the fungus as *Entomophthora anisopliae*. It was later renamed *Metarhizium anisopliae* by Sorokin to honour Metchnikoff's contribution to the field of insect pathology and microbial control. He suggested that fungus-killed cadavers from beetle-infested fields be collected and dispersed in other areas for management of this pest. He also recommended the establishment of production facilities to produce inocula for the initiation of field epizootics through artificial dissemination. He even envisioned laboratory manipulation of the fungus to improve its virulence. His work inspired generations of scientists in Europe and North America to study the theories and applications of entomopathogenic fungi in pest-management practices.

Field application of entomopathogenic fungi in the management of crop pests was first attempted in 1888. In Russia, Krassistschik established a production plant to mass produce *M. anisopliae* on beer mash for the control of the sugarbeet curculio (*Cleonus punctiventris* Germar; Coleoptera: Curculionidae) (Lord, 2005; Faria and Wraight, 2007). In the USA, Lugger initiated the first microbial control project against the chinch bug (*Blissus leucopterus* Say; Hemiptera: Lygaeidae), a major pest of cotton in the Midwest plains, by disseminating *B. bassiana*-infected bugs to initiate epizootics in Minnesota fields (Lugger, 1888). At the same time, Snow started a similar programme in Kansas and established an experimental station to mass produce *B. bassiana* conidia to be distributed free of charge to local farmers. A few decades later, the so-called 'friendly fungi' were found widely distributed among scales and whiteflies feeding on citrus in Florida. This fungal group consists of species in the genera *Aschersonia*, *Agerata*, *Verticillium*, *Sphaerostilbe*, *Podonectria*, *Myriangium* and *Hirsutella*. Although attempts to utilize these fungi in pest management resulted in mixed reviews for their efficacy (Fawcett, 1944; Fisher, 1947, 1950; Steinhaus, 1957), the benefit of some *Aschersonia* species such as *A. aleyrodis* and *Aschersonia placenta* Berkeley was realized later in Florida and in other countries as they were successfully utilized as biological control agents of citrus pests (McCoy, 1978; McCoy *et al.*, 1988). The establishment of the first Laboratory of Insect Pathology at the University of Berkeley in 1945 and the publication of *Principles of Insect Pathology* in 1949 by Edward Steinhaus (1915–1969) highlighted the further advance in the field of microbial control in the 1940s.

The need for control of major crop pests created opportunities for fungal-based microbial control agents across the globe. In the former USSR, Boverin, a *B. bassiana*-based mycoinsecticide, was developed for management of the Colorado potato beetle (*Leptinotarsa decemlineata* (Say); Coleoptera: Chrysomelidae) in 1965. In the USA, a mycoacaricide against citrus rust mite (*Phyllocoptruta oleivora* (Ashmead); Acari: Eriophyidae) based on *H. thompsonii*, Mycar, was granted full registration by the US Environmental Protection Agency (EPA) in 1981 (McCoy and Couch, 1982). Since then, hundreds of products have been developed for the management of insects, mites, ticks and nematodes. These include: (i) the *B. bassiana* strain GHA-based BotaniGard and Mycotrol for grasshoppers, whiteflies, thrips, aphids and other insects in the USA; (ii) the *M. anisopliae* var. *acridum* Driver and Milner (= *M. flavoviride*)-based Green Muscle against locusts and grasshoppers in Africa; and (iii) the *Lecanicillium muscarium*-based Mycotal for whiteflies and thrips, and the *Lecanicillium longisporum* (Petch) Zare and W. Gams-based Vertalec for aphids in Europe (Faria and Wraight, 2007).

Historically, entomopathogenic fungi have been explored for pest suppression just like chemical insecticides, with inundative application over large areas as the first priority. In Japan, *B. bassiana* has

been used against the pine moth (*Dendrolimus spectabilis* Butler; Lepidoptera: Lasiocampidae) since the 1930s (Kunimi, 2007). In China, annual production of *B. bassiana* conidia in the late 1980s reached 10,000 t, an amount large enough to treat 0.8–1.3 million ha (Feng *et al.*, 1994; Feng, 2001; Li *et al.*, 2010). *B. bassiana* was used primarily in large-scale aerial applications as oil or oil-emulsion formulations for pine caterpillars (*Dendrolimus* spp.), one of the major forest pests in the country. More than 30,000 ha were treated over a 5-year period, with mortality ranging from 43 to 93% (Lü and Zhao, 1988; Pan and Zheng, 1988). In Europe, *B. brongniartii* was developed for control of the subterranean pasture pest, the European cockchafer (*Melolontha melolontha* L.; Coleoptera: Scarbaeidae) in Switzerland and the sugarcane white grub (*Hoplochelus marginalis* Fairmaire; Coleoptera: Scarabaeidae) in France. *M. anisopliae* was used for the control of the black vine weevil (*Otiorhynchus sulcatus* (F.); Coleoptera: Curculionidae) and the western flower thrips (*Frankliniella occidentalis* (Pergande); Thysanoptera: Thripidae) in Germany (Butt *et al.*, 1999). In addition, a strain of *L. longisporum* has been commercialized for the management of aphids on chrysanthemum plants in greenhouses (Milner, 1997). In Brazil, an estimated 1 million ha was treated with *M. anisopliae* for control of spittlebugs in 2008 (Li *et al.*, 2010). In sub-Saharan West Africa, the use of microbial control agents had been limited to experimental trials with no control options or commercial products available. However, in 1997, the registration of Green Muscle, a mycoinsecticide based on *M. anisopliae* var. *acridum* (strain IMI 330189) for grasshopper and locust control, may have changed the dynamic (Lomer *et al.*, 1997). This oil-based formulation, the result of many years of an internationally funded research programme, LUBILOSA, can be applied at ultralow volume and is effective against target insects even under low relative humidity and higher temperatures.

The introduction of IPM in the late 1960s gradually changed the theories and practices of microbial control with entomopathogenic fungi. In South-east Asia, significant efforts have been made over the past two decades to promote IPM concepts because of concerns over conventional pesticides about their environmental safety and pest resistance. National IPM programmes exist in all South-east Asian countries, with microbial control as an integral part (Skovmand, 2007). In Malaysia, *Metarhizium* sp. was used against the coconut rhinoceros beetle (*Oryctes rhinoceros* (L.); Coleoptera: Scarabaeidae), whereas *B). bassiana* was used to control the oil palm bagworm (*Metisa plana* Walker; Lepidoptera: Psychidae) (Moslim *et al.*, 2004) and cocoa pod borer (*Conopomorpha cramerella* Snellen; Lepidoptera: Gracillariidae) (Vega and Posada, 2005). In Vietnam, efforts focused on the cost-effective production of *Beauveria* for control of the brown planthopper (*Nilaparvata lugens* (Stal); Hemiptera: Delphacidae) and rice earhead bug (*Leptocorisa acuta* Thunberg; Hemiptera: Alydidae) (Nguyen and Vo, 2005). *B. bassiana* was also used in Indonesia for management of a broad range of agriculture pests (Jakel, 2004). In response to the increasing resistance to many chemical pesticides, the Thai government invested in a national biological control programme. As a result, several microbial control products, including one based on *B. bassiana*, were imported and registered for management of a variety of crop pests (Grzywacz, 2004).

Alternative approaches such as classical introduction, inoculative release and species conservation are also considered. For classical biological control, *E. maimaiga* was introduced from Japan to the USA for management of the gypsy moth (*Lymantria dispar* (L.); Lepidoptera: Lymantriidae) (Hajek *et al.* 1995). In Australia, *Z. radicans* was imported from Israel to control the spotted alfalfa aphid (*Therioaphis trifolii* (Monell) f. *maculata*; Homoptera: Aphdidae) (Milner *et al.*, 1982). For inoculative release, *B. brongniartii*-inoculated barley kernels were applied to the soil in Europe to control European

cockchafer larvae in orchards and meadows (Keller, 1992; Keller *et al.*, 1997). Field epizootics of *N. rileyi* were either protected or artificially initiated for natural control of the velvetbean caterpillar (*Anticarsia gemmatalis* (Hübner); Lepidoptera: Noctuidae) in the USA and Brazil (Sprenkel and Brooks, 1975; Ignoffo *et al.*, 1976a; Moscardi and Sosa-Gomez, 1996), and the cotton bollworm (*Helicoverpa armigera* (Hübner); Lepidoptera: Noctuidae) and rice cutworm (*Spodoptera litura* (F.); Lepidoptera: Noctuidae) in India (Uma Devi *et al.*, 2003). Efforts were also directed to habitat manipulation and practice modification to enhance existing fungal populations. A strong diagnostic system was established to monitor the epizootics of *N. fresenii* against cotton aphid (*Aphis gossypii* Glover; Homoptera: Aphididae) in several southern states in the USA (Steinkraus *et al.*, 1991, 1995; Pell *et al.*, 2001; Shah and Pell, 2003). Harvesting time of lucerne was manipulated in Kentucky to encourage field epizootics of *Zoophthora* (= *Erynia*) *phytonomi* (Arthur) Humber, Ben-Ze'ev and Kenneth against the lucerne weevil (*Hypera postica* (Gyllenhal); Coleoptera: Curculionidae) (Brown and Nordin, 1986). In Switzerland, non-crop plants along field margins were preserved as refugia for alternative hosts as well as their associated fungal pathogens, which were responsible for reductions of pest aphid populations in adjacent annual crops later in the season (Keller and Suter, 1980).

9.3 Taxonomic Status

Fungi have been recognized as a separate kingdom along with Bacteria, Protista, Plants and Animals since the works of Whittaker (1957, 1959, 1969). Traditionally, fungi are divided into two divisions, Myxomycota for plasmodial forms and Eumycota for non-plasmodial forms that contain mycelia, with entomogenous fungi found in Eumycota within the subdivisions of Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina (Ainsworth *et al.*, 1973; Roberts and Humber, 1981; Tanada and Kaya, 1993). No known entomogenous forms are found in the division of Myxomycota (slime moulds), which is no longer considered part of the Fungi kingdom. Oomycetes (water moulds) in the subdivision of Mastigomycotina were later recognized as filamentous protists other than fungi due to cell-wall content (cellulose) and diploidy of filamental nuclei. True fungi contain haploid nuclei in the filaments with a cell wall composed of chitin. Until recently, there were only four established phyla within the Fungi kingdom, including Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota (Alexopoulos *et al.*, 1996; Margulis and Schwartz, 1998). This classification relies mainly on the sexual fruiting structures to assign fungal species into natural groups based on genetic relationships. However, uncertainty persists for a large group of fungi that have lost their ability to produce sexually through evolution. An artificial class, Hyphomycetes, was established within the subdivision of Deuteromycotina (Fungi Imperfecti) to cover these species, including some of the most commonly encountered entomopathogenic taxa such as *Beauveria*, *Metarhizium*, *Nomuraea*, *Isaria* and *Lecanicillium*. Members of this class are characterized by the mycelial forms that bear asexual spores (conidia) on specialized conidiogenous cells (conidiophores).

Recent advances in phylogentic studies based on molecular genetics have changed fungal classification dramatically. Broad relationships in every major group of fungi except Microsporidia have been addressed in the last decade using multiple genes to some extent. James *et al.* (2006a) completed a phylogenetic analysis of fungi using synthesized sequence data of six genes sampled in nearly 200 species from every major clade of fungi including Microsporidia. Based on these results, Hibbett *et al.* (2007) proposed a higher-level phylogenetic classification that divides the Fungi kingdom into one subkingdom, seven phyla and ten subphyla (Table 9.1).

Table 9.1. Genera of entomopathogenic fungi (Hibbett *et al.*, 2007).

Phylum Subphylum Class Order	Exemplar genera
'Basal Fungi'	
Chytridiomycota	
Chytridiomycetes	
Chytridiales	*Batrachochytrium,*
Spizellomycetales	*Powellomyces*, *Spizellomyces*
Manoblepharidomycetes	
Neocallimastigomycota	(rumen chytrids)
Neocallimastigomycetes	
Blastocladiomycota	
Blastocladiomycetes	
Blastocladiales	*Catenaria*, *Coelomomyces*, *Coelomycidium*
Microsporidia[a]	
Metchnikovellomycetes	
Microsporomycetes	
Nosemales	*Paranosema*
Glomeromycota	(mycorrhizal fungi)
Glomeromycetes	
Mucomycotina[b]	
Mucorales	*Mortierella*, *Mucor*, *Phycomyces*, *Sporodiniella*, *Syncephalastrum*
Kickxellomycotina[b]	
Kickxellales	*Ramicandelaber*, *Rhizophlyctis*
Harpellales	*Barbatospora*, *Harpellales*, *Smittium*
Zoopagomycotina[b]	
Zoopagales	
Entomophthoromycotina[b]	
Entomophthorales	*Basidiobolus*, *Batkoa*, *Conidiobolus*, *Entomophaga*, *Entomophthora*, *Erynia*, *Eryniopsis*, *Furia*, *Microbiotophthora*, *Massospora*, *Neozygites*, *Pandora*, *Schizangiella*, *Strongwellsea*, *Zoophthora*
Subkingdom Dikarya	
Ascomycota	
Taphrinomycotina	
Taphrinomycetes	
Neolectomycetes	
Pneumocystidomycetes	
Schizosaccharomycetes	
Saccharomycotina	
Saccharomycetes	
Saccharomycetales	*Candida*, *Geotrichum*
Pezizomycotina	
Arthiniomycetes	
Dothideomycetes	
Capnodiales	*Cladosporium*,
Dothideales	*Bipolaris, Podonectria*
Myriangiales	*Myriangium*
Pleosporales	*Phoma*
Eurotiomycetes	
Eurotiales	*Aspergillus*, *Penicillium*
Onygenales	*Myriodontium*

Table 9.1. Continued.

Phylum Subphylum Class Order	Exemplar genera
Laboulbeniomycetes	
Lecanoromycetes	(lichenized fungi)
Leotiomycetes	
Lichinomycetes	
Orbiliomycetes	
Orbiliales	*Arthrobotrys*, *Dactylaria*, *Duddingtonia*, *Elaphocordyceps*, *Gamsylella*, *Geniculifera*, *Monacrosporium*
Pezizomycetes	
Pezizales	*Ostracoderma*
Sordariomycetes	
Hypocreales	*Acremonium*, *Aschersonia*, *Ascopolyporus*, *Beauveria*, *Cordyceps*,*Culicinomyces*, *Fusarium*, *Gibellula*, *Hyrposporium*, *Hirsutella*, *Hymenostilbe*, *Hypocrella*, *Isaria*, *Lecanicillium*, *Mariannaea*, *Metacordyceps*, *Metarhizium*, *Nomuraea*, *Ophiocordyceps*, *Stilbella*, *Tolypocladium*, *Torrubiella*, *Trichoderma*
Ophiostomatales	*Sporothrix*
Sordariales	*Syspastospora*
Phyllachorales	*Colletotrichum*
Basidiomycota	
Pucciniomycotina	
Pucciniomycetes	(rust fungi)
Cystobasidiomycetes	
Agaricostilbomycetes	
Microbotryomycetes	
Atractiellomycetes	
Classiculomycetes	
Mixiomycetes	
Cryptomycocolacomycetes	
Ustilaginomycotina	(smut fungi)
Ustilaginomycetes	
Exobasidiomycetes	
Agricomycotina	(mushrooms, toadstools)
Tremellomycetes	
Dacrymycetes	
Agaricomycetes	
Wallemiomycetes	
Entorrhizomycetes	

[a] Based on Weiser (1985).
[b] Phylum not assigned.

In this new classification, the clade containing Ascomycota and Basidiomycota is classified as subkingdom Dikarya to reflect the putative synapomorphy of dikaryotic hyphae (Tehler, 1988). Dramatic changes that have been proposed in the classification concern the 'basal fungal lineages', which include the taxa that have traditionally been placed in Zygomycota and Chytridiomycota. The Chytridiomycota is retained in a highly restricted sense. The Blastocladiales, a member of Chytridiomycota traditionally, is treated as a phylum based on new information from a number of molecular studies (James *et al*., 2006b; Liu *et al*., 2006). The Neocallimastigales is also elevated to a phylum due to its distinctiveness from other chytrids in terms of morphology and molecular phylogeny.

The phylum Zygomycota is no longer accepted in the new classification because of the pending resolution of relationships among the clades within the group. The traditional Zygomycota is distributed among one phylum (Glomeromycota) and four *incertae sedis* subphyla that have not

been assigned to any phylum, comprising the Mucoromycotina, Kickxellomycotina, Zoopagomycotina and Entomophthoromycotina (White *et al*., 2006).

The Microsporidia, the intracellular parasites of animals and protists with highly reduced mitochondria that used to be considered as protozoans, are included in the new classification as a phylum of fungi based on studies by Hirt *et al*. (1999), Keeling *et al*. (2000), Gill and Fast (2006), James *et al*. (2006a) and Liu *et al*. (2006). No subdivision of the Microsporidia is proposed by Hibbett *et al*. (2007) because of the lack of well-sampled multilocus analyses of this group. The class-level classification included in Table 9.1 is the traditional morphological taxonomic system based on Weiser (1985). Vossbrink and Debrunner-Vossbrink (2005) proposed a three-class system that includes the Aquasporidia, Marinosporidia and Terresporidia to reflect the habitat of each group. However, consensus on below-phylum classification of Microsporidia is still lacking.

Most entomopathogenic fungi are found in the orders Entomophthorales and Hypocreales. There are six families within the Entomophthorales based on the revision of Humber (1989), including four previously accepted families. Two new families (Completoriaceae and Meristacraceae) were proposed to accommodate the development in classification. Most entomopathogenic genera are included in the family Entomophthoraceae. In addition, the four subgenera in the genus *Zoophthora* were raised to genera as *Zoophthora*, *Erynia*, *Furia* and *Pandora* (Humber, 1989) (Table 9.2).

The most dramatic changes to the entomopathogenic fungi in this new classification system involve the ascomycetes and their conidial (anamorphic) states classified among the pyrenomycetous fungi in the order of Hypocreales. These species are now placed in the class Sordariomycetes based on genetic features, whereas the historic class Pyrenomycetes uses morphological features and fruiting bodies for classification. Under the old system, the majority of these species belonged to the family Clavicipitaceae, mostly in the genera *Cordyceps*, *Torrubiella* and *Hypocrella* (Ainsworth *et al*., 1973; Roberts and Humber, 1981; Tanada and Kaya, 1993). Sung *et al*. (2007) reclassified the Clavicipitaceae based on phylogenetic relationships and split it into three separate families (Table 9.3).

Changes were also made at the genera level. Traditionally, the genus *Verticillium* recognizes two sections: (i) *Verticillium* section *Verticillium*; and (ii) *Verticillium* section *Prostrata* (Gams, 1971). All species in the type section *Verticillium* are anamorphic (conidial) states of perithecial ascomycetes in the order Phyllachorales, which includes phytopathogenic species with well-differentiated erect conidiophores. However, species in the section *Prostrata* are linked to several families within the order Hypocreales. To meet the monophyletic standards of current systematics, this section has been eliminated, with former species spread among several genera (Gams and Zare, 2001; Sung *et al*., 2001; Zare and Gams, 2001a, b; Zare *et al*., 2000, 2001). Most entomopathogenic species in this group are now placed in the genus *Lecanicillium*. The type species *Lecanicillium lecanii* (= *V. lecanii*) was a species complex that contains at least *L. lecanii*, *L. muscarium* and *L. longisporum*. The highly variable *V. lecanii* is no longer recognized.

The genus *Paecilomyces* was originally divided into two sections: (i) *Paecilomyces* section *Paecilomyces*; and (ii) *Paecilomyces* section *Isariodea* (Samson, 1974). The type species *Paecilomyces variotii* Bainier and all species in section *Paecilomyces* are anamorphic states of cleistothecial ascomycetes in the class Eurotiomycetes. All species in section *Isariodea* appear to be anamorphic states of pyrenomycetous ascomycetes in the order Hypocreales. Sung *et al*. (2007) separated these species into three different families (Table 9.3). The *Paecilomyces*-like species in the family of Clavicipitaceae include *Paecilomyces carneus* and *Paecilomyces marquandii*, whereas *Paecilomyces lilacinus* belongs to the family

Table 9.2. Summary of major species in Entomophthorales (Humber, 1989, 1997, 2011).

Family	Genus	Major species	Hosts
Entomophthoraceae	*Batkoa*	*apiculata* (Thaxter) Humber	Homopterans and flies
		major (Thaxter) Humber	Diverse insects
	Entomophaga	*grylli* (Fresebius) Batko	Orthopterans
		maimaiga Humber, Shimazu, Soper and Hajek	Gypsy moth
	Entomophthora	*muscae* (Cohn) Fresenius	Muscoid flies
		culiicis (Braun)	Mosquitoes and blackflies
	Erynia	*aquatica* (Ande. and Anag.) Humber	Culicids
	Eryniopsis	*caroliniana* (Thaxter) Humber	Dipterans
	Furia	*virescens* (Thaxter) Humber	Noctuids
	Massospora	*cicadina* Peck	17-year cicada
	Pandora	*neoaphidis* (Rem. and Henn.) Humber	Aphids
		delphacis (Hori) Humber	Planthoppers
	Schizangiella	*serpentis* Humber	Snakes
	Strongwellsea	*castrans* Batko and Weiser	Dipterans
	Zoophthora	*radicans* (Brefeld) Batko – species complex	Aphids, moths
		phytonomi (Arthur) Batko	Weevils
Completoriaceae			Fern prothallia
Meristacraceae			Soil invertebrates
Ancylistaceae	*Conidiobolus*	*obscurus* (Hall and Dunn) Rem. and Keller	Aphids
		thromboides Drechsler	Aphids
	Macrobiotophthora	*vermicola* (McCulloch) Tucker	Nematodes
Neozygitaceae	*Neozygites*	*fresenii* (Nowakowski) Batko	Aphids
Basidiobolaceae	*Basidiobolus*	*haptosporus* Drechsler	Vertebrates

Ophiocordycepitaceae. *Isaria*, a genus long considered a synonym of *Paecilomyces* (Luangsa-ard *et al.*, 2004; Gams *et al.*, 2005; Hodge *et al.*, 2005), is retained and now contains former *Paecilomyces* species such as *Isaria amoenerosea* (= *P. amoeneroseus*), *Isaria cateniannulata* (=*Paecilomyces cateniannulatus*), *Isaria cateniobliqua* (= *P. cateniobliquus*), *Isaria cicadae* (=*Paecilomyces cicadae*), *Isaria coleopterora* (=*Paecilomyces coleopterorus*), *I. farinosa* (=*Paecilomyces farinosus*), *I. fumosorosea* (=*Paecilomyces fumosoroseus*), *Isaria ghanensis* (=*Paecilomyces ghanensis*), *Isaria javanica* (=*Paecilomyces javanicus*) and *Isaria tenuipes* (=*Paecilomyces tenuipes*) (Luangsa-ard *et al.*, 2005; Sung *et al.*, 2007).

It is important to recognize that the taxonomic status of many fungal groups is still uncertain, in spite of monumental work over the past decade to overhaul the entire classification system. More phylogenetic analyses based on molecular studies will certainly shed light on the relationships among different groups, especially those with unknown teleomorphic states. In the meantime, default taxa should be used on a provisional basis for all communication purposes.

Table 9.3. Summary of major species in Hypocreales (Sung *et al.*, 2007; Humber, 2011).

	Genera			
Family	Teleomorphs	Anamorphs	Major species	Hosts
Clavicipitaceae	*Aciculosporium*	*Aschersonia*	*Aschersonia aleyrodis* Webber	Whiteflies
	Atkinsonella	*Ephelis*		
	Balansia	*Metarhizium*	*Metarhizium anisopliae* (Metschnikoff) Sorokin	Diverse insects
			Metarhizium flavoviride Gams and Rozsypal	Locust and grasshoppers
	Claviceps	*Neotyphodium*		
	Epichlö	*Nomuraea*	*Nomuraea rileyi* (Farlow) Samson	Noctuids
	Heteroepichlöe	*Paecilomyces*-like	*Paecilomyces marquandii* (Massee) Huges	Nematodes
	Hypocrella	*Pochonia*		
	Metacordyceps	*Sphacelia*		
	Myriogenospora	*Verticillium*-like		
	Neoclaviceps			
	Parepichlöe			
	Regiocrella			
	Shimizuomyces			
Ophiocordycipitaceae	*Elaphocordyceps*	*Haptocillium*		
	Ophiocordyceps	*Harposporium*		
		Hirsutella	*Hirsutella thompsonii* Fisher	Mites
			Hirsutella citriformis Speare	Leafhoppers and planthoppers
		Hymenostilbe	*Hymenostilbe dipterigena* Petch	Flies
		Paecilomyces-like	*Paecilomyces lilacinus* (Thom) Samson	Nematodes
		Paraisaria		
		Synglíocladium		
		Tolypocladium	*Tolypocladium cylindrosporum* W. Gams	Mosquitoes
		Verticillium-like		
Cordycipitaceae	*Ascopolyporus*	*Beauveria*	*Beauveria bassiana* (Balsamo) Vuillemin	Diverse insects
			Beauveria brongniartii (Saccardo) Petch	Scarabaeids
	Cordyceps	*Engyodontium*		
	Hyperdermium	*Isaria*	*Isaria farinosa* (Holmsk.) Fries	Diverse insects
			Isaria fumosorosea Wize	Diverse insects
	Torrubiella	*Lecanicillium*	*Lecanicillium lecanii* (Zimmerman) Zare and W. Gams	Diverse insects
			Lecanicillium muscarium (Petch) Zare and W. Gams	Diverse insects
			Lecanicillium longisporum (Petch) Zare and W. Gams	Diverse insects
			Lecanicillium fusisporum (W. Gams) Zare and W. Gams	Diverse insects
		Mariannaea-like		
		Microhilum		
		Simplicillium		

9.4 General Biology

Fungi are microscopic organisms consisting of single cells (yeasts and hyphal bodies) or, more commonly, branched filaments (hyphae) that form a mycelium. The hyphae are uninucleate or multinucleate segments, or coenocytic with numerous nuclei not separated by transverse walls. They are 2–10 μm in diameter and up to several centimetres in length. Fungi are unique among all organisms in containing both glycans and chitin in the cell wall, which separates them from plants (glycans only) and animals (chitin only). Fungi grow by branching or bifurcating (forking) at the tips of the hyphae. The combination of apical growth and parallel extension leads to the development of a mycelium.

As heterotrophs, fungi obtain their nutrition either from decomposed matter (saprophytic) or from living organisms (parasitic) with the help of various enzymes and proteins. Both obligate and facultative species are found in fungi associated with insects. Obligate species require living hosts to complete their life cycles, whereas facultative species can survive both *in vivo* and *in vitro*. Most species in the Entomophthorales are obligate parasites, whereas many species in Hypocreales are facultative.

Infection of insects is usually initiated by various propagules such as spores, conidia, zoospores, planonts, ascospores, sclerotia or sporodochia through the integuments or body openings. For Entomophthorales, the infective units are primary conidia from infected hosts or secondary conidia produced by primary conidia. For ascomycetes, conidia of the anamorphs and ascospores or secondary spores for the teleomorphs are infective. The only entomogenous basidiomycetes are found in the Septobasidiales, with both basidiospores and hyphae capable of infecting scale insects. Environmental resistant stages are not infective directly but can produce infective propagules after germination.

9.4.1 Host range and specificity

Almost all insects are susceptible to fungal infection, with the most common hosts found in the orders Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera and Hymenoptera. Fungal infection is found mostly in the immature (nymphal and larval) stages. However, adults of many insect species can also be affected. The pupal stage of most insects is infrequently attacked and infection of the egg is very rare (McCoy *et al.*, 1988; Tanada and Kaya, 1993).

Host range can vary significantly among different fungal species, or even different strains of the same species. Obligate species usually have a narrow host range. Examples in this group include *Massospora cicadina* Petch from the single genus of the gregarious cicadas (Soper, 1974) and *Strongwellsea castrans* Batko and Wiser from anthomyiid flies (Eilenberg and Michelsen, 1999). The host range for facultative species is much more diverse, with fungi such as *B. bassiana* and *M. anisopliae* infecting over 100 species in several insect orders (McCoy *et al.*, 1988).

Host range is determined by specificity. Specificity is the reciprocal adaptation between a pathogenic organism and the entirety of its host species (Fargues and Remaudiere, 1977). Factors affecting fungal host specificity are both ecological (temperature, humidity and solar radiation) and physiological (immune response and host defence). Together they regulate successful surface attachment, spore germination, cuticle penetration and proliferation in the haemocoel.

There are differences between the physiological (laboratory) and ecological (field) host ranges for fungal species/strains in pest-management practices. Many susceptible insect hosts under laboratory conditions are not found infected by the same pathogens in the field (Goettel *et al.*, 1990; Glare and Milner, 1991). Many fungal species are extremely host specific under naturally occurring epizootics, especially for members of the Entomophthorales (Nair and

McEwen, 1973; Wilding, 1981). Physiological host range is based on optimized inoculum doses and environmental conditions that may not occur in the field. Furthermore, differences in host behaviour and strain characteristics between the laboratory and the field, as well as spatial and temporal divergence between fungal pathogens and their hosts, may help to explain the discrepancy between the two types of host range.

9.4.2 Reproduction

Fungi can reproduce asexually or sexually. Asexual (anamorphic) reproduction is by various kinds of propagules. Zoospores, the motile asexual reproduction cells with a single posterior-directed whiplash flagellum, are present in the phyla Chytridiomycota, Neocallimastigomycoya and Balstocladiomycota. In Glomeromycota, Mucoromycotina, Kickxellomycotina, Zoopagomycotina and Entomophthoromycotina, asexual reproducation is by non-motile conidia that are passively carried away by wind, water or other agents. Asexual reproduction by means of conidia is very common in Ascomycotina with the formation of special reproduction structures such as phialide, conidiophore, sporodochium, pycnidium and acervulus. Asexual reproduction is less commonly found in the Basidiomycota.

The sexual (teleomorphic) reproduction of fungi involves the union of two different cells. Some species allow fusion only between individuals of opposite mating type (heterothallic), while others can mate and reproduce sexually with any other individuals or themselves (homothallic). In the Chytridiomycota, sexual reproduction occurs when the nuclei of motile gametes unite (planogamy) or motile male gametes fuse with larger stationary female gametes (oogamy). The resulting spores may germinate to produce diploid vegetative mycelia or undergo meiosis to produce haploid mycelia. The diploid mycelium can also produce resting sporangia to generate haploid zoospores. In the Glomeromycota, the haploid hyphae of two individuals fuse to form a gametangium. Zygospores are produced after the union of the gametes. A dikaryotic stage in which the nuclei inherited from two different mating types do not combine after cell fusion exists for ascomycetes and basidiomycetes. In the Ascomycota, the dikaryotic hyphae of the spore-bearing hymenium form a hook at the hyphal septum to ensure proper distribution of the newly divided nuclei. An ascus is then formed in which karygamy (nuclei fusion) occurs. Meiosis and mitosis follow immediately to produce ascospores within the ascus. After dispersal, the oospores may germinate to produce a new haploid mycelium. In the Basidiomycota, a similar structure (clamp connection) is formed at the hyphal septum at the beginning. A basidium is formed to produce basidiospores.

9.4.3 Transmission

Although vertical transmission is frequently reported in the Microsporodia, transmission for other groups of fungi is almost entirely horizontal by environmental contamination. Transovarial transmission has not been demonstrated with other fungal groups except in *Coelomycidium simulii* Debaisieux (Blastocladiomycota: Blastocladiales) in black flies (Tarrant and Soper, 1986). Spore dispersal in the environment can be active or passive depending on the structures and characteristics of the spores and sporophores. Some species rely on air currents or free water for spore dispersal, while others may attach to passing invertebrates or vertebrates for long-distance dispersal. Many entomopathogenic fungi are capable of forcibly discharging their spores from specialized structures such as asci in the Ascomycota and basidia in the Basidiomycota.

9.4.4 Survival

Survival during periods of unfavourable conditions (e.g. dry seasons, extremely high or low temperatures, lack of insect hosts) is critical for the persistence of entomopathogenic fungi. Obligate species can persist as dormant stages, while facultative species grow saprophytically until suitable hosts become available again. Most entomophthoralean species are obligate pathogens that produce thick-walled resting spores under such conditions, whereas hypocrealean species range from obligate to facultative and survive as conidia or hyphal bodies within mummified host cadavers. Species in the genus *Sorosporella* survive as thick-walled vegetative chlamydospores (Steinhaus, 1949). Soil provides a natural reservoir for entomopathogenic fungi to survival for long periods of time.

9.4.5 Life cycle

A typical life cycle for an entomopathogenic fungus encompasses both asexual and sexual stages, in which a haploid phase alternates with a diploid phase. A dikaryotic (double haploid nuclei) stage may also be present in between these two phases. In general, two sexually compatible mycelia (*N*) unite to form a zygote (2*N*) after passing through plasmogamy (fusion of cytoplasm), a heterokaryotic stage and karyogamy (fusion of nuclei). Meiosis follows to restore the haploid phase, which gives rise to a spore-producing structure. Spores are disseminated actively or passively before germinating on suitable hosts to form mycelia. The asexual stage contains only the haploid phase, where special reproduction structures are formed by haploid mycelia to produce haploid spores. Spores germinate under optimal conditions to give rise to haploid mycelium to complete the cycle.

Different patterns of life cycles exist for various fungal groups. It has long been believed that most Hypocreales have a simple asexual life cycle. However, recent advances in fungal genetics reconciled the anamorphs of many species with their teleomorphs under the auspice of three new families (Sung *et al.*, 2007). Species within the Chytridiomycota and Neocallimastigomycota undergo a haploid life cycle, whereas most species within the Blastocladiomycota have a haploid/diploid life cycle. A haploid/dikaryotic cycle is usually found in most species within the Ascomycota and Basidiomycota, and a dikaryotic life cycle is normal for smut fungi and some yeasts.

9.4.6 Endophytic growth

Entomopathogenic fungi can also grow as symbiotic endophytes in plant tissues, with most fungal endophytes found in the Ascomycota (Arnold and Lutzoni, 2007). Grass endophytes in the genus *Neotyphodium* (Clavicipitaceae) may help improve plant performance and its ability to resist both insect and mammalian herbivores (Saikkonen *et al.*, 2006).

Various genera of entomopathogenic fungi have been isolated as endophytes from different plant species, including: (i) *Acremonium alternatum* Likn: Fries from coffee; (ii) *B. bassiana* from maize, cotton, jimsonweed, ironwood, western white pine and coffee; (iii) *I. farinosa* and *L. lecanii* from ironwood; and (iv) *Paecilomyces* spp. from banana and rice (Vega, 2008). Although the relationship between the endophytes and their hosts is not yet well understood, studies in recent years are investigating the possibilities of introducing fungal pathogens such as *B. bassiana* and *Fusarium solani* (Mart.) Sacc. as plant endophytes for the biological control of specific insect pests such as cereal stem borers and plant-parasitic nematodes (Langewald and Cherry, 2000).

9.5 The Disease Process

In general, fungi infect insects through the surface of the integument. This mode of

infection makes it possible for fungi to attack insects independently from their feeding activities. However, other avenues have also been used to gain access into the host. Schabel (1976) reported *M. anisopliae* infection of the pale weevil (*Hylobius pales* (Herbst); Coleoptera: Curculionidae) through the mouthparts, whereas penetration of fungi through the alimentary tract has been observed in the black imported fire ant (*Solenopsis richteri* Forel; Hymenoptera: Formicidae) (Broome *et al.*, 1976) and gypsy moth (Wasti and Hartmann, 1975). Infections through spiracles and tracheae were also observed on elaterid larvae with *M. anisopliae* (McCauley *et al.*, 1968) and lucerne weevil with *B. bassiana* (Hedlund and Pass, 1968).

9.5.1 Spore germination

Upon landing on the host, fungal spores need to adhere to the cuticle to initiate pathogenesis. This process can be specific or non-specific depending on the fungal species. For some, the adhesion of spores on to the insect cuticle is a passive mechanism that involves mucilaginous materials and spore surface structures (McCoy *et al.*, 1988; Samson *et al.*, 1988). The surface of entomophthoralean conidia is covered with an amorphous mucus for adhesion, while the dry conidia of hypocrealan fungi contain well-organized fascicles or rodlets that can provide hydrophobicity (Boucias *et al.*, 1988). There are also cases where both mucus and rodlets are found on the conidial surface (Latgé *et al.*, 1986). For others, this process is rather specific, although the basis for host specificity is still largely unknown. Electrostatic forces and molecular interaction may be involved in adhesion (Samson *et al.*, 1988). Glycoproteins may serve as specific receptors for spores. The motile spores of aquatic *Coelomomyces* spp. can utilize sugars or complex carbohydrates as recognition cues for attachment (Travland, 1979; Kerwin and Washino, 1986). Hemagglutins, glucose and *N*-acetylglucosamine are some of the compounds found on spore surfaces (Latgé *et al.*, 1986).

Spores may or may not germinate after adhesion, depending on the surface conditions. Low humidity and unfavourable temperatures may prevent certain species from germinating. The inability to utilize available nutrients on the surface and the failure to recognize susceptible hosts contribute to early abortion of infection (Boucias and Pendland, 1984; St Leger *et al.*, 1987). Inhibitory compounds such as phenols, quinines and lipids on cuticle surfaces may present formidable barriers to fungal invasion (Smith and Grula, 1981; Kerwin, 1984). Upon germination, a fungal spore may penetrate the epicuticle directly with the germ tube, or form an appressorium on the surface and use the infection peg underneath to breach the cuticular barriers (Madelin *et al.*, 1967; Zacharuk, 1970a; Perkrul and Grula, 1979; Boucias and Pendland, 1991).

9.5.2 Penetration of the integument

The insect integument consists of three principle layers: the cellular epidermis, acellular basement membrane and acellular cuticle. The cuticle is further divided into a thin outer epicuticle with cement, wax and cuticulin, and a thick inner procuticle with polysaccharide and chitin embedded in a protein matrix. Fungi penetrate the insect cuticle using a combination of mechanical pressure and enzymic degradation (Zacharuk, 1970b; St Leger, 1991, 1993; Boucias and Pendland, 1998). The resistant epicuticle needs to be disrupted physically before enzymes can work their way through the chemically complex procuticle. Pressure at the tip of an invading hypha usually distorts the cuticle layers, causing structure fractures at the invasion point. Various enzymes such as proteases, aminopeptidases, lipases, esterases and *N*-acetylglucosamidases (chitinases) detected on germ tubes are responsible for the digestion of the integument. Host resistance to fungal invasion reflects the thickness and tensile strength of the cuticle, as well as the degree of cuticle sclerotization. Instead of targeting

the integument, penetration of insects with heavily sclerotized body segments usually occurs at other body openings such as the buccal cavity, spiracles and pores of sense organs (Hedlund and Pass, 1968; McCauley *et al.*, 1968; Charnley, 1989).

9.5.3 Replication in the haemocoel

After penetrating the insect integument and entering the haemocoel, many fungal species grow vegetatively by budding as yeast-like blastospores or hyphal bodies. Some entomophthoralean species produce wall-less protoplasts within the haemolymph. These types of growth forms do not normally exist outside the hosts. However, they are important for the survival and growth of fungi inside the hosts, as they provide not only increased surface area for nutrient acquisition but also the ability to multiply and disperse rapidly through circulation within the haemolymph, as well as the ability to evade or dissipate the host immune response. Complete colonization of the haemocoel may or may not occur before the death of the host, depending on the fungal species involved.

Penetration of host cuticle does not necessary guarantee successful fungal growth and reproduction, as the host may mount behaviour, humoral and cellular responses to counter the infection. Specific behaviours such as sun basking and behavioural fever are used by grasshoppers and house flies to help defend against potential lethal infections in the early stages (Carruthers *et al.*, 1992; Watson *et al.*, 1993; Ouedraogo *et al.*, 2004). Humoral responses from insect hosts include increased antifungal activity by a complex of inducible protease inhibitors (Boucias and Pendland, 1987; Wojda *et al.*, 2009). The sugar content in cell walls is thought to be crucial for host insects to recognize non-self fungal cells. Protoplasts lack cell walls and hence can easily escape host recognition, while recognizable walled hyphal bodies may become encapsulated by haemocytes in the haemolymph (Beauvais *et al.*, 1989). The encapsulation is rapidly melanized. Phagocytosis occurs when granulocytes are attracted to the invasive cells. Plasmatocytes are recruited to form a pseudo-tissue that later becomes a granuloma (nodule) in which the fungus may be lysed (Götz, 1986; Lee *et al.*, 2005). Only invasion from weak pathogens can be prevented with this type of cellular antifungal defence mechanism. In the case of virulent strains such as *B. bassiana*, hosts are either unable to form nodules or the encapsulation is overcome by fungal growth with the help of secondary metabolites produced by the fungus (Hung *et al.*, 1993). When innate defences are overcome, most insect hosts will be killed by fungal pathogens in a relatively short period of time (Pendland and Boucias, 1993).

Fungi kill their hosts within a few days to a few weeks by nutrient depletion and mycotoxic activities. Several toxins have been isolated from various fungal species, including: (i) destruxins A, B, C and D and desmethyldestruxin B from cultures of *M. anisopliae* (Roberts, 1966, 1969; Suzuki *et al.*, 1971; Samuels *et al.*, 1988, Vey *et al.* 2001); (ii) beauvericin from *B. bassiana* and *I. fumosorosea* (Hamill *et al.*, 1969; Gupta *et al.*, 1994); (iii) oosporein and bassianolide from *Beauveria* spp. (Eyal *et al.*, 1994); (iv) efrapeptins from *Tolypocladium* spp. (Weiser and Matha, 1988; Krasno *et al.*, 1991); (v) hirsutellin from *H. thompsonii* (Mazet and Vey, 1995); and (vi) toxic substances from entomophthoralean species (Yendol *et al.*, 1968; Prasertphon and Tanada, 1969). While the role of most toxins in pathogenesis is still not very clear, destruxins are correlated with fungal toxicity by paralysing host cells and causing dysfunction of the midgut, Malpighian tubules, haemocytes and muscle tissues (Samuels *et al.*, 1988).

The death of the host marks the end of parasitic development of the fungus. Saprophytic growth starts in the haemocoel to form a mycelial mass that turns into a sclerotium. Reproductive spores are produced within the sclerotium or on specialized sporophores. Outgrowth usually starts in the integumental regions of the host. Sporulation generally occurs on

cadavers but can also be observed on live insects such as cicada with *Massospora* spp. (Soper, 1974) and mirid bugs with *Entomophthora erupta* (Dustan) Hall (Ben-Ze'ev *et al.*, 1985).

9.5.4 Spore dispersal

The time to death for the insect host after initial infection varies among fungal species as well as among different strains of the same species. Strain virulence, infection level, environmental temperature, and size and stage of the host when it was infected also play a role in the process. For many entomopathogenic fungal species, production of aerially dispersing spores requires high (>95%) relative humidity. As mentioned above, spores are either actively discharged or passively picked up by wind, rain or other insects and invertebrates after sporulation. A few spores will land successfully on suitable hosts to start new infection cycles, whereas those which fail to do so need to find ways to survive in the environment. To compensate for the loss of the majority of spores during this process, a large number of spores is usually produced from each host cadaver. For example, fifth-instar gypsy moth larvae infected by *E. maimaiga* produce an average of 2.6×10^6 conidia per larva (Shimazu and Soper, 1986). Posada and Vega (2005) found that all 50 strains of *B. bassiana* isolated from the coffee berry borer (*Hypothenemus hampei* (Ferrari); Coleoptera: Curculionidae) had a spore production of at least 1×10^6 conida per beetle, with 14 of them producing over 2.6×10^6 conidia per beetle. In addition, many entomophthoralean species frequently produce two types of spores: (i) short-lived infective spores that are actively dispersed; and (ii) long-lived resting spores that are environmentally resistant.

Fungi can also increase their chances of dispersal by altering the behaviour of infected hosts. Aphids, ants, grasshoppers, planthoppers and flies infected with various fungi are all known to move to elevated locations before dying, with rhizoids developing underneath to attach cadavers in position (Samson *et al.*, 1988; Evans, 1989). Other hosts die in positions with wings or elytra spread open to expose sporulating areas. These kinds of behaviour clearly enhance the efficiency of spore dispersal, as nearby or passing through living hosts are more likely to be inoculated (Wilding, 1981). It has been hypothesized that selective pressure in the evolution of host–pathogen interactions may have forced this change of host behaviour to promote spore dispersal.

9.5.5 Pathogenicity

Pathogenicity is the quality or state of being pathogenic and the potential ability to produce disease (Steinhaus and Martignoni, 1970). Pathogenicity is different from virulence, although it is not uncommon to see them used interchangeably by many workers in the literature. Virulence is the disease-producing power of the pathogen and the degree of pathogenicity within a group or species. Pathogenicity is a prerequisite of virulence, as no virulence will be measured if an organism is not pathogenic, whereas a virulent organism must be pathogenic. On the other hand, a pathogenic organism could be non-virulent to a host if it fails to infect. A pathogen realizes its pathogenicity by the measure of virulence through successful infection. Highly virulent species kill their hosts within a few days, whereas less virulent species produce chronic and prolonged infection.

For a specific fungal species or strain, virulence against a targeted insect host is usually expressed as the concentration (LC_{50}) or dose (LD_{50}) required to kill 50% of exposed hosts. Fungal species have numerous strains that differ significantly in their virulence and pathogenicity. In general, a strain isolated from a specific host is more virulent to that host than those isolated from other hosts. Successful transmission among host populations may also enhance the virulence of that strain. In contrast, continuous *in vitro* passage usually results in diminished virulence.

Sublethal effects should also be considered when discussing fungal pathogenicity. There are cases where targeted hosts survive the fungal infection because of inadequate inocula received during the process. However, reproductive potential and offspring quality for exposed hosts are negatively affected. A reduction in adult feeding, female fecundity and egg fertility after sublethal infection have been observed for many insect hosts, including: (i) the Colorado potato beetle (Fargues *et al.*, 1991); (ii) desert locust (*Schistocerca gregaria* (Forskål); Orthoptera: Acrididae) (Blanford and Thomas, 2001); (iii) Mediterranean fruit fly (*Ceratitis capitata* Wiedemann (Diptera: Tephritidae) (Castillo *et al.*, 2000); (iv) western corn rootworm (*Diabrotica virgifera virgifera* LeConte; Coleoptera: Chrysomelidae) (Mulock and Chandler, 2001); (v) western tarnished plant bug (*Lygus hesperus* Knight; Hemiptera: Miridae) (Noma and Strickler, 2000); (vi) Asian long-horned beetle (*Anoplophora glabripennis* (Motschulsky); Coleoptera: Cerambycidae) (Hajek *et al.*, 2008); and (vii) emerald ash borer (*Agrilus planipennis* Fairmaire; Coleoptera: Buprestidae) (Liu and Bauer, 2008). Furthermore, insects surviving infection may have reduced resistance to cold and other environmental stresses, which could lead to additional mortality that is not normally accounted for by virulence. Host invasion by fungal mycelia and the action of toxic metabolites are thought to be responsible for various sublethal effects that are frequently recorded (Hajek and St Leger, 1994).

9.5.6 Signs and symptoms

No significant signs and symptoms are visible at the early stage of a fungal infection. By the late stages, however, infected insects become sluggish and show a lack of coordination. A reduction in food consumption is also very common. Epigeous species often move to high places and soil insects rise to the surface to expose themselves (Samson *et al.*, 1988; McCoy, 1990).

Some fungal species or strains produce pigments during the infection, which leads to a colour change of the host. For example, larvae of the pear thrips (*Taeniothrips inconsequens* (Uzel); Thysanoptera: Thripidae) infected with *L. lecanii* turn from white to pink after infection (Skinner *et al.*, 1991). Similar patterns were also observed in Japanese wax scale (*Ceroplastes japonicus* Green; Hemiptera: Coccoidae) (Liu *et al.*, 2009).

Cells and tissues of the infected insect may begin to disintegrate prior to or immediately after its death. Continuous growth of hyphae inside the host haemocoel eventually results in mummification of the host. This explains why most fungus-killed insects retain their form and shape following death. The most significant sign of fungal infection, of course, is the fungal outgrowth and sporulation on the cadaver, starting from areas between integuments and eventually completely covering the entire cadaver.

9.6 Ecological Considerations

9.6.1 Effects of environmental factors

A variety of abiotic or biotic factors may influence the success of fungal pathogens against insect hosts. Abiotic factors such as temperature, relative humidity, solar radiation and agrochemicals affect host susceptibility, as well as pathogen persistence and survival, transmission and dispersal, germination and growth, and development and reproduction. Biotic factors include host populations and other biological control agents that may alter the life cycle, dispersal, transmission and persistence of fungal pathogens.

Temperature

The development of a fungal pathogen and evolution of the disease process depend on temperature. The optimal temperature for most entomopathogenic fungi falls between 20 and 30°C, although various

degrees of infection and disease can occur at temperatures ranging from 5 to 35°C. Temperatures beyond these limits may cause inhibition of spore germination and vegetative growth in most species (Hywell-Jones and Gillespie, 1990). High temperatures are detrimental to the survival of fungi, whereas low temperatures may provide protection to the spores. For *N. rileyi* conidia, infectivity was lost within 1 day of exposure at 50°C while no loss of infectivity was observed after 448 days at –70°C (Ignoffo *et al.*, 1985). Low temperatures may also force fungi to produce environment-resistant stages such as resting spores or chlamydospores. The thermal death point, defined by Cochrane (1958) as 'the least temperature at which all spores or conidia are killed in 10 minutes' is about 55°C for *B. bassiana* (Studdert *et al.*, 1989; Varela and Morales, 1996), whereas for *M. anisopliae* it is between 49 and 60°C (Zimmermann, 1982; Liu *et al.*, 2003). Dry spores are twice as stable as wet spores at intermediate temperatures (25–40°C) (Ignoffo *et al.*, 1985).

It is well documented that an ambient temperature affects the rate of infection and the speed of kill for insects treated with entomopathogenic fungi. Vestergaard *et al.* (1995) showed that a decrease in temperature of 3–5°C from the optimum increased the time to death by a day for adult western flower thrips treated with *M. anisopliae*. Under field conditions, daily temperatures can fluctuate substantially between the low and high limits. Rapid spore germination and hyphal growth are extremely important for successful infection as optimal conditions may not always exist. The ability of conidia to survive short periods of exposure to high temperatures is also highly desirable as such conditions may occur in the field. Fungal species/isolates with rapid germination and hyphal growth rates may have an advantage as biological control agents because host infection can potentially occur much more quickly (Hajek and St Leger, 1994; Varela and Morales, 1996).

Humidity

Humidity is closely related to the survival and development of fungi. Desiccation is one of the frequent causes of spore mortality in the environment, whereas high moisture content may reduce conidia longevity in storage. For example, conidia of some *Entomophthora* spp. cannot survive humidities below 75% (Wilding, 1973; Newman and Carner, 1975). In contrast, conidia longevity for *M. flavoviride* increases with a decrease in relative humidity between 4.6 and 31.8% (Hong *et al.*, 1998). Similar results were also observed in *B. bassiana*, *I. farinosa* and *M. anisopliae* (Clerk and Madelin, 1965; Kawakami and Mikuni, 1965; Magalhaes and Boucias, 2004). Mid-range humidities are detrimental to conidia survival.

Humidity is a critical factor for spore germination. Although spores of some entomopathogenic fungi are capable of germinating at low ambient humidities (45–70%) (Steinkraus and Slaymaker, 1994; Milner *et al.*, 1997; James *et al.*, 1998), most species require high relative humidity (>90%) for at least a short period of time to germinate (Hajek, 1997; Fargues and Luz, 1998; Arthurs and Thomas, 2001).

Fungi start saprophytic development after the death of their host. Sporulation on host cadavers depends on relative humidity and is affected by time, temperature and light regimes (Ferron, 1978; Tanada and Kaya, 1993). For many species, sporulation occurs mostly in the early morning hours when humidity is high and temperature and light conditions are favourable. No spores are produced for *B. bassiana* when the relative humidity is less than 75% (Ramoska, 1984), whereas sporulation on the green peach aphid (*Myzus persicae* (Sulzer); Homoptera: Aphididae) is delayed for *L. lecanii* when the humidity drops below 100% (Milner and Lutton, 1986).

Solar radiation

All fungal propagules (conidia, spores, hyphae, hyphal bodies) are sensitive to solar

radiation, especially to medium-wave ultraviolet (UVB) within a wavelength range of 280–320 nm. Exposure to UVB damages the DNA, which may block its synthesis and create high levels of mutations. It can also lead to the production of highly reactive and deleterious radicals such as peroxide. As a result, the stability of the exposed fungus is rapidly reduced, while its insecticidal activity is severely limited (Moore *et al.*, 1993; Inglis *et al.*, 1995).

For most entomopathogenic fungal species, a few hours of direct exposure to radiation in the field are sufficient to fully inactivate the conidia. In addition, UV radiation has also been demonstrated to cause delays in the germination of surviving conidia (Zimmermann, 1982; Moore *et al.*, 1993). Fungi in developing stages (e.g. germination) are more susceptible to radiation (Braga *et al.*, 2001). The conditions of the microhabitats where the fungus is used in the field may influence its persistence. For example, survival of conidia deposited on the underside of leaves or in shaded areas is substantially prolonged compared with those on the surface, although indirect radiation will also eventually kill these conidia (Smits *et al.*, 1996).

The reduction in available inocula due to conidial inactivation and germination delay will negatively impact on the efficacy of these fungi as bioinsecticides for field crops. Considerable efforts have been directed to protect fungal pathogens by adding solar blocks or UVB-absorbing chemicals (e.g. sunscreens) in formulations (Moore *et al.*, 1993; Alves *et al.*, 1998). Tolerance to solar radiation is also a key criterion in the selection of fungal strains for further development as biocontrol agents (Morley-Davies *et al.*, 1995; Fargues *et al.*, 1996).

Agrochemicals

All classes of agrochemicals are potential inhibitors of entomopathogenic fungi, including insecticides, fungicides, herbicides and growth regulators that are commonly used in most commercial farming systems. Many have demonstrated inhibitory effects on fungal germination and growth *in vitro*, although in some cases the fungistatic properties of a chemical pesticide may be attributable to the associated carriers or adjuvants. However, many agrochemicals are compatible with entomopathogenic fungi in the field through sublethal effects (Quintela and McCoy, 1998; Ramakrishnan *et al.*, 1999; Furlong and Groden, 2001; Alizadeh *et al.* 2007). For some agrichemicals, the sublethal effects may predispose the insects to disease from physiological stresses and behaviour modifications.

In general, fungicides are the major concern for entomopathogenic fungi in pest management of field crops, although herbicides and other agrochemicals may also be detrimental to their survival and performance (Klingen and Haukeland, 2006). Fungicides such as tebuconazole, benomyl and maneb are highly inhibitory to Pandora (*Erynia*) *neoaphidis* (Rem. and Henn.) Humber, *B. bassiana* and *L. lecanii* under laboratory conditions (Olmert and Kenneth, 1974; Latteur and Jansen, 2002). Negative impacts of fungicides on entomopathogenic fungi have also been observed in the field. For example, application of carboxin reduced *N. fresenii* prevalence in cotton aphid populations during the early seasons (Smith and Hardee, 1996), whereas treatment of chlorothalonil caused a week-long delay in fungal epizootics (Wells *et al.*, 2000). However, extrapolation of field inhibition from *in vitro* results may prove to be inaccurate, as many pesticides do not exhibit the same activity under field conditions compared with in the laboratory due to concentration and compartmentalization (Clark *et al.*, 1982; Loria *et al.*, 1983; Mietiewski *et al.* 1997; Jaros-Su *et al.*, 1999).

Host conditions

The morphological and physiological conditions of individual insect hosts can influence their susceptibility to fungal infection. Not all stages in the life cycle are equally vulnerable to fungal disease. Host age within the same stage may also play a

role in the initiation and development of infection (Boucias *et al.*, 1984). It is well documented that stressed insects are more susceptible to fungal infection than those not under stress (Steinhaus, 1958; Vago, 1963). Host insects can suffer from stresses caused by crowding, malnutrition, starvation, adverse chemical or environmental conditions, and physiological mechanisms (Donegan and Lighthart, 1989). Insects living under crowded conditions tend to be more stressed as space, air, food, water and other essential life supplies may be limited. Inadequate nutrition or starvation generally leads to smaller and weaker individuals that are more susceptible to diseases (Hare and Andreadis, 1983; Ramoska and Todd, 1985; Furlong and Groden, 2003). Chemical and environmental stressors usually reduce the fitness of insects, and a depressed immune response will certainly weaken defence mechanisms of the host against fungal infection.

The conditions of the host population will affect the efficacy of the fungus. Density dependence is common among various entomopathogenic fungal species (Fuxa and Tanada, 1987). Increased host density means a higher probability of disease transmission within the population, with more host materials and a better chance of interaction between sick and healthy individuals. It is also hypothesized that the establishment, persistence and epizootics of fungal pathogens are determined by certain thresholds of their host populations (Anderson and May, 1980).

Other biological control agents

Other biological control agents such as predators, parasitoids and entomopathogens may interact with entomopathogenic fungi synergistically or antagonistically. Interguild predation by certain predators on infested hosts reduces the impact of entomopathogenic fungi (Roy and Pell, 2000). Several species of coccinellids feed on aphids at the late stage of *P. neoaphidis* infection under laboratory conditions (Pell *et al.*, 1997; Roy *et al.*, 1998). However, predators and parasitoids may help enhance the transmission and dispersal of entomopathogenic fungi through their foraging activities (Roy *et al.*, 2001).

9.6.2 Epizootiology

Epizootic is a term used to define 'an unusually large number of disease cases' in a host population at a given period of time (Fuxa and Tanada, 1987). Epizootiology deals with the study of the characteristics, ecology and causes of the epizootics. Key factors in epizootiological studies include pathogen populations, host populations, transmission mechanisms, environmental factors, and spatial and temporal patterns of the outbreaks.

Information on previous disease levels in the population is needed to determine whether an epizootic is under way. A stable and low prevalence of certain diseases among the population does not constitute an epizootic. However, a sudden increase in the number of infected individuals within the population triggered by biotic or abiotic factors may lead to an epizootic over time.

9.7 Strain Characterization and Development

The development of a fungal microbial control agent requires several important steps. First, candidate isolate with the potential to suppress the target pest population has to be identified through a series of laboratory bioassays, greenhouse trials and field experiments. Secondly, the characteristics of this isolate such as growth rate, spore production potential, storage stability and environmental persistence need to be evaluated carefully. Thirdly, mass-production techniques must be developed to minimize production costs and maximize yield and quality. Fourthly, suitable formulations need to be explored to ensure their safe delivery during application and their field efficacy against target pests. Finally, official registration must be pursued to allow the general marketing of the new microbial pesticide.

9.7.1 Isolation and strain selection

Entompathogenic fungi can be isolated directly from diseased host insects, or indirectly from the soil and other substrates from the environment. To ensure successful isolation, designed sampling schemes should be followed correctly using a series of field and laboratory techniques before pure cultures can be obtained. Knowledge of the general biology and ecology of both the fungus and its hosts is helpful in this process. Detailed isolation methods and techniques have been described by Goettel and Inglis (1997) for Hypocreales (Hyphomycetes) and by Papierok and Hajek (1997) for Entomophthorales.

Fungal strains within the same species vary greatly in terms of virulence against host insects. Only a few of them may have the potential for further development as microbial control agents. It is therefore critical for the right strains to be selected for this purpose. Strain selection usually starts with the screening of hundreds or even thousands of isolates through efficacy bioassays in the laboratory. Recent advances in molecular technology may help improve selection efficacy by using phenotypic or genotypic markers in the process. Pilot trials are then performed on a few highly virulent strains under greenhouse and field conditions for their suitability and applicability as microbial control agents (Vidal *et al.*, 1997; Liu *et al.*, 2002; Montesinos, 2003).

9.7.2 Strain characterization

Following selection, candidate strains need to be characterized to ensure their taxonomic status, as individual isolates may display considerable variation in host range and virulence. It is becoming increasingly apparent that identification at the species level using morphological characteristics is no longer adequate. Molecular techniques (e.g. RAPD-PCR) based on DNA polymorphisms are now commonly used for subspecies classification (Tigano-Milani *et al.*, 1995; Hegedus and Khachatourians, 1996; Mor *et al.*, 1996).

Other biological and physiological characteristics, such as enzyme and mycotoxin production, conidia viability, germination speed, hyphal growth rate, infectivity, spore production and response to environmental factors, may also influence efficacy of the microbial control agents. In general, strains with rapid spore germination and fast hyphal growth stand a better chance in infecting and colonizing their hosts. Enzymes and mycotoxins produced by such strains will help the process. The ability to produce large quantities of spores and to persist in the environment constitutes the basis for utilization in pest management. Examples of how fungal strains have been characterized can be found in the development of microbial control agents for whiteflies (Faria and Wraight, 2001) and plant bugs (Leland *et al.*, 2005).

9.7.3 Mass production, formulation and delivery

For commercial development, selected fungal strains must be produced on an industrial scale with the highest yield possible at the lowest cost. Methods used for industrial scale-up include liquid or solid-phase fermentation (Fravel *et al.*, 1999). Liquid-phase (submerged) fermentation may be appropriate for some species that readily produce thin-walled blastospores, whereas solid fermentation utilizes inexpensive medium for the production of aerial spores on the wet surface. Biphasic fermentation, which uses vegetative mycelia produced by liquid batch culture to produce conidia on a solid nutrient carrier, successfully provides high yields of stable conidia for *B. bassiana* (Rombach *et al.*, 1988) and *M. anisopliae* (Jenkins *et al.*, 1998).

The final product should be formulated to improve its storage stability and field performance. Additives used in the formulation include wetting and dispersal agents, humectants, UV or osmotic protectants, and nutrients to stimulate

germination and growth. Formulated fungal products have the potential to provide better and more consistent results in pest-management practices (Burges, 1998; Wraight *et al.*, 2001).

Delivery of a lethal dose of fungal products to the targets in the field is always a challenge, as direct contact is required to start the infection process. Spraying equipment, nozzle size and angle, spray pressure and volume, and the crop system all play a role in determining the final results of microbial control with entomopathogenic fungi (Wraight and Carruthers, 1999). Different crop systems may require different spray technologies and configuration to achieve good coverage. Cost and efficiency need to be balanced between traditional hydraulic equipment and portable sprayers. Nozzle size, angle, spray pressure and volume have direct impacts on the direction and deposition of the products used (Bateman, 1992; Hislop *et al.*, 1993; Giles, 1997).

9.7.4 Biosafety

Biosafety is a critical aspect of product development, as fungi are living organisms with the ability to persist in the environment. Concerns over the potential impacts on non-target organisms, vertebrates and mammals and on human health need to be addressed before full registration can be pursued. The data required for registration vary from country to country (Kabaluk *et al.*, 2010). In the USA, the EPA sets uniform guidelines for all biopesticides including fungal-based agents (EPA, 2010).

The safety features of a fungal product are determined by its host range, the production of metabolites and its fate in the environment. A wide host range is desirable for commercial products, but this may raise concerns for non-target organisms. Mycotoxins help fungi overcome host defences, yet they could become the source of environmental toxicity. Persistence in the environment is critical for the fungus to continue its life cycle after application; however, unintended consequences such as species displacement and environmental contamination may also be an inevitable part of the process. It is important to recognize the needs at both ends of the spectrum.

Historical evaluations have shown that entomopathogenic fungi are generally weak pathogens towards vertebrates (fish, amphibian, reptiles and birds) and mammals, with pathogenicity, toxicity and allergic reactions among the top concerns. Detailed reviews on the safety aspects of entomopathogenic fungi have been provided by Burges (1981), Goettel and Jaronski (1997), Goettel *et al.* (2001), Vestergaard *et al.* (2003) in general, and by Zimmermann (2007a,b) for *B. bassiana*, *B. brongniartii* and *M. anisoplae*.

9.7.5 Patenting, registration and commercialization

Before the commercial potential of a selected fungal strain can be fully exploited, the developer may wish to seek legal protection of its biotechnological invention by means of patenting. A patent grants a set of exclusive rights to a developer for a limited period of time in exchange for public disclosure. The filing of patents for biopesticides is governed by a series of national and international treaties. Not all treaties are recognized by all countries, and different countries may have different requirements for such filings.

Despite a relatively large number of patents for fungal-based biopesticides worldwide, only a few have been registered for use in agriculture. According to Faria and Wraight (2007), since the 1960s, a total of 171 products have been developed as mycoinsecticides and mycoacaricides based on 12 species or subspecies (varieties) of entomopathogenic fungi. Among these, *B. bassiana*, *M. anisopliae*, *I. fumosorosea* and *B. brongniartii* are the most commonly used active ingredients. Approximately 75% of the listed products are currently registered, undergoing registration or commercially available, whereas 15% are no longer available.

9.8 Use of Fungi in IPM Systems

Entomopathogenic fungi have been utilized in various pest-management programmes as biological control agents under four broadly defined categories: classical, inundation, inoculation and conservation.

9.8.1 Classical biological control

Classical biological control is the intentional introduction and permanent establishment of an exotic biological agent for long-term pest control (FAO, 1996). The major steps of a typical classical biological programme include target selection, literature reviews, surveys for natural enemies in both native and newly expanded ranges, species selection, importation, quarantine and exclusion, non-target and environmental impact evaluation, field release and colonization, and efficacy evaluation. If successful, this method provides sustainable and economical control of the target pest.

E. maimaiga *against gypsy moths in the USA*

Gypsy moth is a major pest of hardwood trees in the eastern USA. It was first introduced accidentally from Europe to Massachusetts in 1869 and spread dramatically over the next few decades. The first outbreak occurred in 1889. By 1987, it had become established throughout the entire north-eastern region. Infestations were also found in Indiana, Michigan, North Carolina, Ohio, Virginia, West Virginia and Wisconsin. *E. maimaiga* was introduced from Japan to the USA in the early 1900s in an attempt to control this pest. Infected cadavers were released on to tree trunks with resident gypsy moth populations. By the end of 1911, no transmission had been documented and the programme was discontinued because a viral epizootic had decimated the local host populations (Hajek *et al.*, 1990).

Another attempt to reintroduce this fungus from Japan was made in 1984 following the exceptionally damaging gypsy moth outbreak in the USA in 1981. As a result, *E. maimaiga* was released in New York in 1985 and Virginia in 1986. However, these releases were considered to have failed as transmission to native host populations was extremely low or non-existent. Furthermore, no recovery of *E. maimaiga* was made at the release sites in the following years (Hajek *et al.*, 1995).

During early June 1989, *E. maimaiga* was found to be causing high levels of infection in gypsy moth populations in south-eastern Connecticut (Andreadis and Weseloh, 1990). Since then, natural infection has been widely documented throughout the north-eastern USA (Elkinton *et al.*, 1991). Natural spread by airborne conidia and artificial spread by human manipulation are believed to be responsible for the range expansion of this fungus. The origin of this virulent and fast-spreading strain remains unclear, although Weseloh (1998) concluded that this fungus must have become established and started spreading around 1971. To help *E. maimaiga* establish in new environments, soil containing resting spores or infected cadavers from current epizootics were collected and redistributed. Currently, *E. maimaiga* is distributed over much of the area infested by gypsy moth in the USA. Studies on mass production, storage, germination and infectivity of resting spores for better utilization of this fungus are also under way.

Z. radicans *against spotted alfalfa aphids in Australia*

The spotted alfalfa aphid is a pest of legume plants indigenous to the western Palaearctic region. It was first recorded in Australia in 1977 and quickly spread from Queensland southward and westward across the mainland, causing widespread devastation to lucerne (*Medicago sativa*) in pastures (Passlow, 1977; Hughes *et al.*, 1987).

Z. radicans was known to cause dramatic epizootics in spotted alfalfa aphid populations in Israel (Kenneth and Olmert, 1975). A strain of *Z. radicans* was imported from Israel to Armidale in New

South Wales for the biological control of spotted alfalfa aphid in 1979. The fungus was released at four selected sites on to lucerne plants by placing infected but still alive aphids, cadavers or sporulating cultures in Petri dishes. Infection by *Z. radicans* on the spotted alfalfa aphid was recorded within 5 weeks of the initial release (Milner *et al.*, 1982). Field releases have also been made in Tasmania in southern Australia, western Australia and other parts of New South Wales. Extensive epizootics were recorded in host populations in southern Queensland and northern New South Wales in the autumn of 1981 (Milner and Lutton, 1983).

Within 6 years of the first devastating appearance of the potted lucerne aphid in Australia, the pest ceased to be an economic problem. *Z. radicans*, together with *Trioxys complanatus* Quilis (Hymenoptera: Aphidiidae), a parasitic wasp that was used simultaneously in the biological control programmes, is credited for the success of the management of this pest (Pell *et al.*, 2001).

9.8.2 Inundation biological control

Inundation biological control involves applying large quantities of fungal propagules over a short period of time for rapid pest suppression. For this approach, fungi are used as if they were chemical insecticides and hence are described as 'mycopesticides' or 'mycoinsecticides'. Inundative augmentation does not necessarily seek permanent establishment of the fungus or secondary infection from the initial application but rather pest population reduction to prevent crop loss in the current season. Hypocrealean species have great potential as inundation biological control agents as they are relatively easy to mass produce *in vitro* and can be formulated for use with conventional spray equipment. In contrast, constraints in *in vitro* mass production of entomophthoralean fungi have limited their utilization in inundation biological control.

B. bassiana *against Colorado potato beetles in the former USSR*

Colorado potato beetle is the most important insect defoliator of potatoes. Both adults and larvae feed on potato leaves. Heavy infestation can lead to complete destruction of potato crops in a single season. It is native to Mexico and is currently found in many countries in North America, Europe and Asia. Its complex life history and remarkable ability to develop insecticide resistance make it a challenging pest to manage in any cropping systems.

B. bassiana is a ubiquitous entomopathogenic fungus known to reduce Colorado potato beetle densities. Commercial-scale production and utilization of *B. bassiana* for the management of this pest first occurred in the former USSR. A *B. bassiana*-based biopesticide, Boverin, was developed in the early 1960s, with large quantities produced in the following decades (Roberts *et al.*, 1981). For 10 years, low-dose *B. bassiana* (1.2×10^{13} conidia/ha), combined with one-fifth of the regular rate of chemical insecticide (dichlorodiphenyltrichloroethane (DDT) or polychlorfinene) provided good control of the beetle. A higher dose ($3–4.5 \times 10^{13}$ conida/ha) of the fungus was recommended later to eliminate chemical insecticides (Ferron, 1978).

B. bassiana *against pine caterpillars in China*

Pine caterpillars, *Dendrolimus* spp. (Lepidoptera: Lasiocampidae), are serious forest defoliators in China, with more than 20 species found throughout the country. The Masson pine caterpillar (*Dendrolimus punctatus* Walker) is the most common species in southern China where outbreaks are recorded every 3–5 years. Larval feeding on the needles completely defoliates the affected pine trees. Repeat attacks eventually lead to tree mortality within a few years. Setae from late-stage larvae may also cause severe allergic skin reaction to humans.

Starting from the late 1960s, *B. bassiana* was tested against Masson's pine caterpillar in Fujian Province. In one trial, nearly 500 kg of *B. bassiana* dry culture was applied as a mist over 100 ha of infested pine stands, which resulted in an average mortality of 82% for the pest at 8 days after treatment (Li *et al.*, 1981). Similar results were also obtained in the neighbouring Guangdong Province when over 2000 ha of *D. punctatus*-infested pine stands were treated. Encouraged by the results, the Chinese central government and 13 provincial governments supported a project to mass produce *B. bassiana* for the large-scale suppression of pine caterpillars across the country. As a result, hundreds of small laboratories were established within a few years throughout the country to produce this fungus inexpensively to satisfy suppression needs. *B. bassiana* was cultured on solid media such as rice, wheat bran, or maize meal in containers with large surface areas. By the end of the 1970s, hundreds of metric tonnes of *B. bassiana* had been mass produced and hundreds of thousands of hectares of pine stands treated. Target pests were also expanded to include the Chinese pine caterpillar (*Dendrolimus tabulaeformis* Tsai and Liu) in the north, the Simao pine caterpillar (*Dendrolimus kikuchii* Matsumura) in the south and the Siberian silk moth (*Dendrolimus superans sibiricus* Tschetverikov) in the north-eastern part of the country (Li, 2007).

L. longisporum *against aphids in Europe*

Aphids are one of the most widespread groups of pests in agricultural systems that cause crop losses through direct feeding and disease transmission. One or more aphid species are potential pests of most crops grown in protected cultivation. The green peach aphid is the most common species, attacking a wide range of host plants including chrysanthemum.

L. longisporum is a common pathogen of aphids and scale insects in tropical and subtropical regions. Recent classification of the former genus *Verticillium* using ribosomal RNA gene sequencing placed all former *Verticillium* insect pathogens into the new genus *Lecanicillium*, which includes at least *L. lecanii*, *L. longisporum* and *L. muscarium* (Zare *et al.*, 2000; Gams and Zare, 2001; Zare and Gams, 2001a,b). In previous studies, *L. longisporum*, along with other species in the new genus, was referred as *V. lecanii*.

A strain of *L. longisporum* has been commercialized in Europe as Vertalec against aphids on chrysanthemum plants in greenhouses. Blastospores are produced by liquid fermentation and formulated with a nutrient source to allow colony growth on leaf surfaces (Milner, 1997). A good efficacy against a number of different aphid species has been demonstrated (Hall, 1981; Milner, 1997; Burges, 2000). One application of Vertalec provides control of green peach aphid as well as melon aphid (*A. gossypii* Glover; Homoptera: Aphididae) for 3 months. This product is currently available in Denmark, Finland, the Netherlands, Norway and the UK, with registration pending for France, Spain and Turkey.

M. anisopliae *var.* acridum *against locusts and grasshoppers in Africa*

Locusts and grasshoppers (Orthoptera: Acrididae) are devastating pests of crops and pasture grasses in Africa. They were traditionally controlled by the use of dieldrin through treatment of barrier strips and aerial applications over swarms. The outbreaks of desert (*S. gregaria* (Forskål)), migratory (*Locusta migratoria capito* Sauss) and red (*Nomadacris septemfasciata* Serville) locusts in the 1980s led to widespread applications of chemical insecticides for their control. However, the substitute organophosphate pesticides for dieldrin such as fenitrothion and malathion had shorter environmental persistence. As a result, repeated applications over large areas were needed. Concerns over environmental and economical costs moved locust and grasshopper control towards IPM.

In 1990, a collaborative research programme was initiated between research institutes in the UK, the Netherlands and

the Republics of Benin and Niger to investigate the possible use of entomopathogenic fungi as biological control agents (Prior and Greathead, 1989). Initial surveys of natural enemies revealed that *M. anisopliae* var. *acridum* was an important pathogen of locusts and grasshoppers (Shah *et al.*, 1997; Driver *et al.*, 2000). Isolate 330189 was then selected for commercialization after a series of bioassays and characterization, along with the development of mass-production technologies (Lomer *et al.*, 2001). Fungal conidia extracted from rice medium were dried to a low moisture content (<5%) for long-term storage. A formulation mixture of kerosene, groundnut oil and dried conidia was used in field applications. A total of 70–90% of treated locusts and grasshoppers were found to be infected or dead within 14–20 days after application (Lomer *et al.* 2001). As a result, a patented product, Green Muscle, was developed commercially and has been recommended by the Food and Agriculture Organization of the United Nations for locust and grasshopper control (Lomer *et al.*, 2001; Langewald and Kooyman, 2007).

Paranosema *(=* Nosema*)* locustae *against grasshoppers around the world*

P. locustae is a spore-forming pathogen that was first isolated from African migratory locusts (*Locusta migratoria migratorioides* Reiche and Fairmaire; Orthoptera: Acrididae) (Canning, 1953), but also occurs naturally in grasshopper populations in the Great Plains and Prairies of North America. It primarily infects the fat bodies of its hosts. Although infection may occasionally result in high levels of mortality among some acridid species (Streett and Henry, 1984), most infections usually lead to reduced feeding, delayed development and lower fecundity as host energy reserves are depleted over time (Johnson, 1997).

P. locustae is produced commercially on live grasshoppers such as *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae) (Henry, 1985). Spores are typically formulated as bait in wheat bran and applied through cyclone seeders and mechanical spreaders. It has been tested extensively in the field in the USA and Canada (Ewen and Mukerji, 1980; Henry and Onsager, 1982; Lockwood and Debrey, 1990). A standard application rate of 2.5×10^9 spores/ha can lead to a 50–60% population reduction for the current generation, with 35–50% of the surviving population remaining infected (Henry *et al.*, 1973).

P. locustae has been used in Argentina, Cape Verde, China and Mali to control grasshoppers. In Argentina, a considerable locust population reduction was observed in treated areas (Lange and De Wysiecki, 1996). In China, annual treatment of over 100,000 ha produced satisfactory results (Lomer *et al.*, 2001). However, its full potential as a microbial control agent may be limited by its low short-term efficacy and lack of host specificity, as well as challenges associated with formulation, production, storage, cost and application.

9.8.3 Inoculation biological control

Inoculation biological control involves the application of a small amount of fungal propagules early in the season for successful establishment and transmission of the fungus within pest populations. Under the right conditions, epizootics caused by the fungus over time will eventually keep pest populations below the economic threshold. The potential for many species of entomophthoralean fungi to cause epizootics in the field after introduction has attracted much attention in recent years for their possible utilization in inoculation biological control.

B. brongniartii *against cockchafers in Europe*

The European cockchafer is a sporadic pest of grassland, forests and orchards in central Europe. The adults feed on the blossoms and young leaves of fruit trees and other deciduous or ornamental species, while the larvae cause extensive damage to the root systems of various crops, meadow grasses and tree seedlings.

A biological control programme with a focus on *B. brongniartii* was established in the early 1970s to manage the European cockchafer. *B. brongniartii* forms blastospores in liquid culture, which can be inoculated on to barley seeds to produce mycelium and aerial conidia. The fungus was used against adults in forests through aerial application of blastospores in aqueous suspensions, as well as against larvae in orchards and meadows by pressing inoculated barley kernels 3–5 cm into the soil with a tractor-mounted seed drill. In several trial sites, substantial population reductions were observed for a period of 6–9 years (Keller, 1992; Keller *et al.*, 1997).

Z. radicans *against diamondback moths around the world*

The diamondback moth (*Plutella xylostella* (L.); Lepidoptera: Plutellidae) is one of the most destructive cosmopolitan insect pests of brassicaceous crops. It was the first crop pest reported to show resistance to DDT and has now developed significant resistance to almost all synthetic insecticides used in the field. Failures in chemical control of this pest in many regions have led to increase efforts for the development and implementation of IPM programmes worldwide (Sarfraz *et al.*, 2005).

The entomophthoralean fungus *Z. radicans* is a widespread pathogen of the diamondback moth that attacks larvae and occasionally pupae. It has been reported in many countries to cause periodical epizootics that lead to significant population reductions. Infected larval or pupal cadavers usually serve as a source of fungal inocula for the next infection cycle. However, naturally occurring field epizootics cannot always be relied on for management of the pest, as epizootics depend on favourable environmental conditions, which may not exist when needed.

An inoculative augmentation programme to induce field epizootics of *Z. radicans* through auto-dissemination using lure traps for the diamondback moth was studied in the UK. To start epizootics earlier than would have occurred naturally, adult pheromone traps were dusted with *Z. radicans* conidia before deployment. Male diamondback moths attracted to the traps were contaminated with fungal conidia. Conidia were then dispersed in the field by the males as they left the traps. Fundamental studies on virulence, persistence, horizontal transmission and field evaluation were carried out in Australia, Cuba, Mexico, Kenya and Malaysia (Pell *et al.*, 1993; Furlong and Pell, 1997, 2001; Furlong *et al.*, 1995; Vickers *et al.*, 2004).

H. thompsonii *against citrus rust mites in the USA*

Citrus rust mite is a major citrus pest in Florida. It attacks fruit, leaves and young twigs in early spring, with populations reaching high levels in a few weeks. Heavy infestation can reduce fruit growth, damage fruit quality and cause fruit to drop prematurely. Chemical control with multiple applications is usually required, particularly on citrus trees bearing fruits designated for fresh-fruit markets.

H. thompsonii was first reported by Spears and Yothers (1924) in association with the citrus mite. *H. thompsonii* can cause high mortality in wild populations of citrus mites and act as a key factor in the natural control of this pest (Fisher, 1950; Muma, 1955). However, the high incidence of fungal epizootics is normally not attained before mite damage to the fruit. It was assumed that fungal inocula in orchards are not high enough at the start of the season to initiate epizootics.

A programme was set up to introduce the fungus early in the season to keep the mite population under the economic threshold. In field trials, applications of *H. thompsonii* mycelia to citrus groves controlled citrus rust mites for up to 12 weeks compared with the 6 weeks of control obtained by chlorobenilate, with the mite population remaining low for 10–14 weeks (Selhime and McCoy, 1971). About 40% of the mites were infected by the fungus at 6 days after the treatment (Villalon and Dean, 1974).

N. rileyi *against velvetbean caterpillars in the USA and Brazil*

The velvetbean caterpillar is the most damaging foliage-feeding soybean pest in the southern USA and Brazil. It is native to tropical and subtropical areas in the western hemisphere. Larval feeding on foliage early in the season causes significant damage to soybean crops. Most insecticide applications in soybean fields are directed against this pest.

N. rileyi is a dimorphic insect fungal pathogen specific to larval lepidopterans, with a preference for species in the family Noctuidae (Ignoffo, 1981). More than 30 species have been identified as hosts, including the velvetbean caterpillar (Ignoffo *et al.*, 1976a). Field epizootics have been reported in noctuid populations in Argentina, Australia, Brazil, Ecuador, India, Mexico and the USA (Suwannakut *et al.*, 2005). In Brazil, *N. rileyi* is the key factor that prevents velvetbean caterpillar populations from reaching damage thresholds in soybean fields. Prevailing epizootics often preclude the need for insecticide applications against this pest (Fuxa, 1974; Kish and Allen, 1978).

Seasonal colonization of *N. rileyi* in soybean crops was considered. In the USA, artificial epizootics were induced when *N. rileyi* conidia and infected alternate hosts were applied (Sprenkel and Brooks, 1975; Ignoffo *et al.*, 1976b). In Brazil, farmers refrained from insecticide application when natural incidences of *N. rileyi* on caterpillars were observed. Furthermore, late planting was practised to avoid high populations of velvetbean caterpillars on soybeans, as *N. rileyi* would usually cause high pest mortality (Moscardi and Sosa-Gomez, 1996).

9.8.4 Conservation biological control

Conservation biological control focuses on the preservation and enhancement of existing natural enemies for pest management through habitat manipulation and farming or management practice modification. It seeks to identify effective indigenous pathogens, predators or parasitoids before cultural and management practices are adopted to conserve and promote their activities in the field. Manipulation and modification that favour fungal pathogens may include increasing relative humidity by irrigation, reducing pesticide and fungicide application, and establishing refugia for alternative overwintering hosts.

N. fresenii *against cotton aphids in the USA*

The cotton aphid is an important pest of cotton in the southern USA. High aphid populations can negatively impact on cotton yield, which leads to economic losses. Outbreaks of cotton aphid have often been associated with low activities of natural enemies and aphid resistance to chemical insecticides.

The entomopathogenic fungus *N. fresenii* is a common pathogen of the cotton aphid throughout the cotton-growing region. Field epizootics have been documented since 1989 in southern USA (Steinkraus *et al.*, 1991, 1995). This fungus is extremely specific with a host range limited to a few aphid species. Infection is usually initiated by capilliconidia from germinating primary conidia. *In vitro* cultivation of *N. fresenii* is very difficult. However, it can survive long periods of time as resting spores, mature hyphal bodies or early-stage conidiphores in dried and frozen aphid hosts (Steinkraus *et al.*, 1993; Vingaard *et al.*, 2003).

To take advantage of the situation for aphid management in cotton fields, a diagnosis service was set up in Arkansas in 1993 to track the prevalence of *N. fresenii*. Farmers were asked to collect cotton aphids during the season and send them in 70% ethanol to the diagnosis laboratory to assess the presence of the fungus. Information on fungal infection rates based on those samples could then be used by the farmers to determine whether pesticide application was necessary for the season. A 50% infection rate would indicate a strong likelihood of fungal epizootic within a few

days and hence no chemical treatment was warranted, whereas a 15% infection rate could lead to an aphid population decline within a week and therefore framers were increasingly advised against pesticide applications. This service was later expanded to Alabama, Florida, Georgia, Louisiana, Mississippi and South Carolina with significant economic and environmental benefits to local farmers (Pell *et al.*, 2001; Shah and Pell, 2003; Steinkraus, 2007).

Z. phytonomi *against lucerne weevil in the USA*

Lucerne weevil is a destructive pest of lucerne introduced to North America on multiple occasions between 1900 and 1960. Both larvae and adults feed on terminals, foliage and new crown shoots. Heavy infestation results in a reduced stand density and low yield in subsequent harvests. It is now found in all 48 contiguous states of the USA, parts of northern Mexico, and from Quebec to British Columbia in Canada.

Z. phytonomi is a pathogen of lucerne weevil first discovered in Ontario, Canada, in 1973 (Harcourt *et al.*, 1974) and is now found throughout the eastern USA (Hajek *et al.*, 1996). It overwinters as thick-walled resting spores in the soil. Resting spores germinate during the spring to produce capilliconidia, which infect lucerne weevil larvae. Infected larvae are usually killed within 4–5 days (Nordin *et al.*, 1983). Field epizootics are dependent on accumulated moisture of >91% and the continuing presence of a host weevil density >1.7 larvae per stem (Millstein *et al.*, 1982; Nordin *et al.*, 1983).

In Kentucky, the harvest timing of lucerne was manipulated to encourage field epizootics of *Z. phytonomi* (Brown and Nordin, 1986). The lucerne was cut a few days earlier than normal and left in windrows to provide warm and humid aggregating sites for the weevil. The sites were ideal for the onset of fungal infection among host larvae. As a result, earlier and more intense fungal epizootics were observed in the field (Brown and Nordin, 1986). Consequently, this practice was recommended as a standard procedure for the management of lucerne weevil across the country.

Refugia to preserve entomopathogenic fungi

Non-crop plants found in field margins can serve as refugia for alternate hosts as well as their associated fungal pathogens (Shah and Pell, 2003). For example, certain entomophthoralean species are more common at the edges of fields (Powell *et al.*, 1986) or in hedges and forest borders (Keller, 1998). These fungi are the source of inocula that initiate epizootics among pest populations in adjacent major crop fields.

In Switzerland, non-pest aphids in lucerne meadows harboured *P. neoaphidis* and *Conidiobolus obscurus* (Hall and Dunn) Rem. and Keller (Entomophthoromycotina: Entomophthorales) during the off-season, which were responsible for population reductions of pest aphids in adjacent annual crops (Keller and Suter, 1980). In Slovakia, Barta and Cagán (2003) discovered five species of entomophthoralean fungi infecting the common nettle aphid (*Microlophium carnosum* Buckton; Homoptera: Aphididae) in agricultural landscapes and hypothesized that the nettle patches served as reservoirs for fungal pathogens. In South Africa, a significantly higher occurrence of hypocrealean fungi including *B. bassiana* and *M. anisopliae* was found in soil samples taken from refugia compared with organically and conventionally cultivated citrus orchards (Goble *et al.*, 2010). Creating new and preserving existing refugia will help conserve natural populations of fungal pathogens.

9.9 Conclusions and Perspectives

The predominate use of synthetic chemical insecticides such as DDT and other organophosphates across the globe since the 1950s has generated many problems for pest management in all crop systems, including: (i) pesticide resistance from target insects; (ii) a negative impact on non-target insects

and native natural enemies; (iii) health risks to human and wildlife; and (iv) environmental contamination. The serious consequences of pesticide abuse of certain products were famously described by Rachel Carson in her 1962 book *Silent Spring*. Social and political concerns over the environmental safety of chemical insecticides forced researchers, manufacturers, growers and governments to address these problems while maintaining the sustainability of pest-management programmes. As a result, IPM was introduced as a sustainable approach to manage pests by combining biological, cultural, physical and chemical tools in a way that minimizes economic, health and environmental risks.

Entomopathogenic fungi are important components of biological control within the IPM system. They differ from other insect pathogens such as bacteria and viruses by invading hosts through the cuticle without being digested. Fungal spores landing on host bodies passively or actively have the ability to germinate within several hours under favourable conditions. Their germ tubes penetrate the host integument by a combination of mechanical pressure and enzyme degradation to reach the haemocoel. Massive replication of hyphal bodies within the haemocoel eventually depletes the host of nutrients and leads to death of the host with the help of various mycotoxins. Sporulation on host cadavers follows, producing inocula for the next life cycle.

Entomopathogenic fungi have great potential in the management of insect pests under different crop systems. With hundreds of species coevolving and coexisting with their hosts and literally thousands of specific strains in each species yet to be discovered in various habitats, their impact on the population dynamics of many economically important species of insects, spiders and mites should not be overlooked. Sustainable agriculture in the new millennium relies on environmentally friendly alternatives to reduce the use of synthetic chemical insecticides. Biopesticides based on entomopathogenic fungi are good substitutes for the phased-out chemicals with less pest resistance, low non-target impacts and few concerns over environmental safety. The organic farming movement since the late 1990s has provided excellent opportunities for the utilization of fungal pathogens as biological control agents.

With opportunities come challenges. The challenges for fungal-based microbial control agents arise from their biological features and public misconceptions. As living organisms, entomopathogenic fungi cannot be mass produced as easily and efficiently as conventional chemical insecticides. Storage stability is a major concern for many fungal products. Strict requirements on optimal environmental and host conditions for maximum performance are usually hard to meet in the field. These inherent attributes make fungal-based microbial insecticides less cost-effective compared with chemical insecticides. However, public perception still focuses exclusively on the efficacy against target insects without full appreciation of other aspects such as environmental safety and host selectivity.

The world pesticide market is estimated at US$25 billion. The synthetic pesticide market has been declining for the past couple of decades due to the growth of biopesticide development and the adoption of genetically modified crops. However, microbial insecticides account for only 1–2% of the pesticide market, with most from sales of biopesticides based on the bacterium *Bacillus thuringiensis*. The market share of fungal-based pesticides is even smaller (Thakore, 2006; Kiewnick, 2007). Despite optimistic predictions for the future of microbial control agents, fungal pathogens may face even more competition from new series of chemical insecticides as well as transgenic plants. Advances in virulence improvement, production and formulation are needed for the further development and implementation of fungal pathogens in the pest management of crop systems.

The future of entomopathogenic fungi in IPM of crop systems rests on a set of new paradigms in the development, production,

utilization and regulation of fungal-based microbial control agents to be shared by researchers, manufacturers, growers and governments. It may not be possible for any microbial control agent to provide sustainable control of insect pests individually under any agriculture or forestry systems (Lacey and Goettel, 1995; van Frankenhuyzen *et al.*, 2000); however, significant selective pest control can be achieved by using fungal pathogens as important components of integrated systems. Furthermore, fungal pathogens are most useful in environmentally sensitive areas where chemical insecticides are not appropriate, or in semi-artificial environments such as greenhouses, where a high efficacy of pest control can be expected. In general, fungal-based microbial control agents should not be considered as competitors of conventional chemical insecticides, but rather partners with specific market and application niches in integrated crop management systems. To accomplish this: (i) during strain selection and characterization, researchers need to treat fungal pathogens as natural enemies capable of providing long-term suppression; (ii) manufacturers need to develop new technologies and procedures to reduce product cost and increase field applicability; (iii) growers need to accept lower instant efficacy for sustainable pest management; and (iv) governments need to modify regulations governing fungal-based microbial control agents to encourage their development, registration and use. Only then can we envision the development and utilization of fungal pathogens that are not driven purely by the pesticide market but are promoted by the entire community under the general principles of integrated crop management. A quick review of successful cases of fungal pathogens in the past highlights the importance of co-operation among all stake-holders. Strong government support was credited for the management of the Colorado potato beetle in Russia and pine caterpillars in China. Grower involvement played a critical role in suppression of the spittlebug in sugarcane plantations in Brazil and the cotton aphid in the USA. Finally, international collaboration between researchers and government agencies was the key to the management of locusts and grasshoppers in Africa. Along the way, the shift from inundative application to inoculative release and species conservation is likely to continue.

The future of entomopathogenic fungi as microbial control agents may also depend on the development of new technologies. Recent advances in molecular technology makes strain isolation and selection more accurate and efficient, new formulations will make fungal products more stable in storage and in the field, genetic modification and strain hybridization have the potential to improve fungal virulence, and fungal endophytes may become part of crop systems in the near future.

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10 Microbial Control of Crop Pests using Insect Viruses

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10.1 Introduction

One of the main principles of integrated pest management (IPM) is the use of specific pesticides for specific control of those pests surpassing the economic damage threshold in a given crop. The use of chemicals in plant protection is becoming more and more restricted because many are harmful to non-target organisms and due to rigid tolerance levels for chemical residues not only in water but particularly in our food. For a number of insect pests, virus-based control agents are ideal tools for IPM strategies: (i) many have a narrow host range and are highly virulent against their insect host; (ii) they are environmentally safe; and (iii) they do not interfere with non-target organisms. Viral insecticides do not harm the naturally occurring antagonists, such as predatory or parasitoid insect species, in an agro-ecosystem and allow them to be integrated into a pest-control strategy.

More than 1100 insect viruses are known today. They are found in more than 20 virus families, including DNA as well as RNA viruses (Fauquet and Mayo, 2005). Some of these viruses can also occur in vertebrates or plants, but there are several viruses that exclusively infect arthropods. A typical feature of many arthropod-specific viruses is that their infectious particles are embedded in a proteinaceous matrix, a so-called occlusion body (OB). Protected by the OB against environmental factors, such as heat, pH or UV light, these viruses can persist for quite some time outside their insect hosts, until they are taken up by another individual. Occluded viruses are observed in three virus families: (i) *Baculoviridae* (baculoviruses); (ii) *Reoviridae* (cypovirus); and (iii) *Poxviridae* (entomopoxviruses) (Fauquet and Mayo, 2005). In addition to these occluded viruses, rhinoceros beetle (*Oryctes rhinoceros*) nudivirus and iridoviruses have also been used as pest-control agents. A brief introduction to these different groups of viruses is given in this chapter. Because of their high host specificity, high efficacy, safety to non-targets and ease of use, research and development of insect viruses as microbial control agents has been predominantly driven forward for baculoviruses. Therefore, the application of baculoviruses as pest-control agents will be the main topic of this chapter.

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10.2 Insect Viruses with Potential for Biological Control

10.2.1 Cypoviruses

Cypoviruses (CPVs) have a double-stranded RNA genome and comprise arthropod-specific members of the virus family *Reoviridae*. CPVs are found in about 250 species of insects, predominantly from the Lepidoptera, but also from the Diptera and a few Hymenoptera (Mertens *et al.*, 2005). Many CPVs have a broad host range, which may comprise several lepidopteran families. Viral replication takes place in the cytoplasm of midgut epithelial cells. CPVs often cause chronic infections. Infected individuals can pupate and develop to adults, which constrains the potential of CPVs as biocontrol agents. Larvae can also recover from viral infection by sloughing off infected cells. As an example, CPVs have been investigated for control of the processionary pine caterpillar (*Thaumetopoea pityocampa*) in Japan since the 1960s. The CPV-based product Matsukemin was the first microbial insecticide registered in France and Japan, but it lost its registration in 1995 (Yasuhisa, 2007).

10.2.2 *O. rhinocerus* nudivirus

Oryctes rhinoceros nudivirus (OrNV) is a double-stranded DNA virus that is infectious to several coleopteran insects in the family Scarabaeidae (Vlak *et al.*, 2008; Jehle, 2010). OrNV was first described as *Rhabdionvirus oryctes* (Huger, 1966) and later as OrNV. It was previously assigned to the family *Baculoviridae* but was recently orphaned. A new virus genus, the so-called nudiviruses, was proposed (Burand, 1998; Wang *et al.*, 2007). Control of the rhinoceros beetle using OrNV is a highly successful example of inoculative biological control using a virus. The rhinoceros beetle was accidentally introduced to South Pacific regions and is a major pest on coconut palms. The search for putative control pathogens was successfully carried out in the region of origin of the pest by collecting diseased larvae (Huger, 1966; reviewed by Jackson, 2009). Larval stages and adult beetles become infected with OrNV. In adults, the viral infection is initially restricted to the midgut epithelial cells. These cells become filled with newly synthesized viruses and are sloughed off into the gut lumen (Huger, 2005). Due to the continuous regeneration of the infected epithelial cells, large amounts of virus are produced and spread by the flying beetle in its habitat. This results in an extremely efficient horizontal spread of OrNV in the beetle populations and virus contamination of the nesting sites (Huger, 2005).

10.2.3 Baculoviruses

More than 600 viruses belong to the family *Baculoviridae*. To date, baculoviruses have been isolated only from three insect orders: Lepidoptera, Hymenoptera and Diptera. There are many historic accounts of hosts in other arthropod orders, but these have never been proven by molecular means (Adams and Bonami, 1991). Baculoviruses are DNA viruses with a double-stranded, circular genome of 80–180 kb (Theilmann *et al.*, 2005). The DNA is packed into rod-shaped particles called virions. The virions are embedded in a proteinaceous OB (Granados, 1980). The OB not only protects the virions from physical damage by environmental factors but also delivers the virus to the alkaline midgut of the host larvae (Hu *et al.*, 2003).

Baculovirus OBs can have a polyhedral or granular shape. Therefore, two major groups of baculoviruses have been described: (i) the nucleopolyhdroviruses (NPVs); and (ii) the granuloviruses (GVs) (Fig. 10.1). However, it should be noted that this grouping does not follow the natural phylogeny and the present classification of baculoviruses (Jehle *et al.*, 2006a). For NPVs, the OBs, which are 0.15–2 μm in size, are called polyhedra and contain several virions (Fig. 10.1a). The granule-like OBs of GVs are significantly smaller (120–300 nm) and contain typically one (rarely two or more) virions (Theilmann *et al.*,

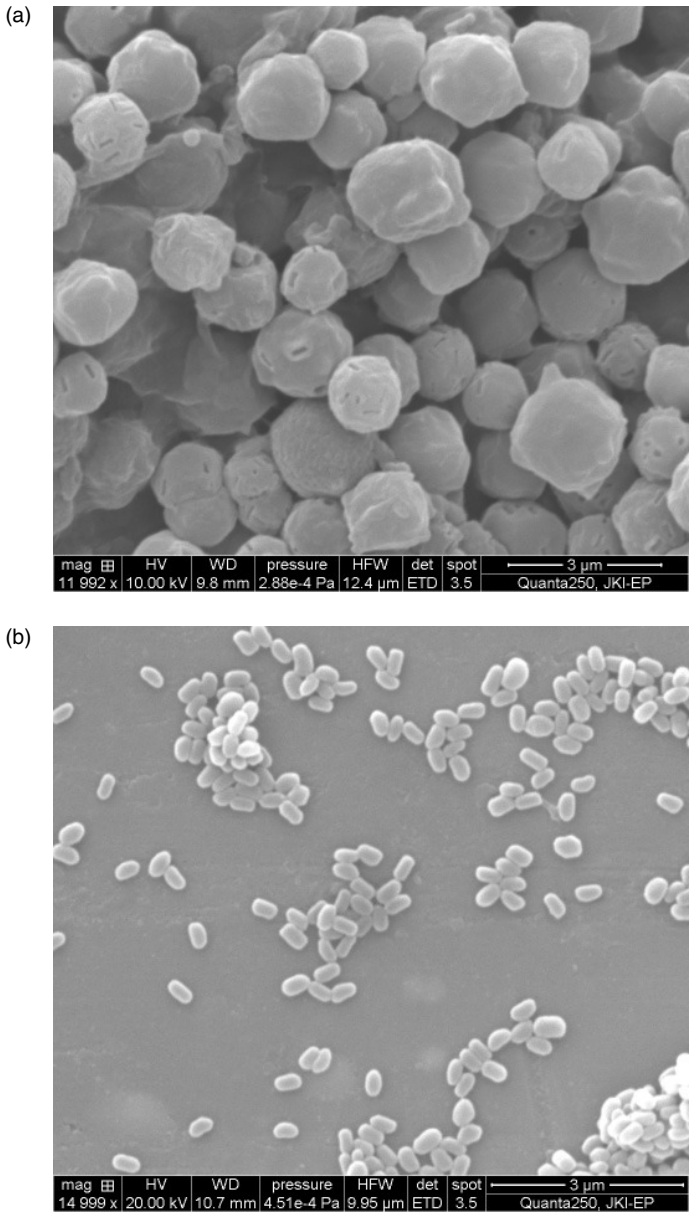

Fig. 10.1. Scanning electron micrograph of occlusion bodies of *Agrotis ipsilon* nucleopolyhedrovirus (AgipNPV) (a) and *Cydia pomonella* granulovirus (CpGV) (~0.2–0.4 µm long) (b). (Photo courtesy of Joerg Wennmann and Katja Richert-Poeggeler, Julius Kühn Institute, Germany.)

2005) (Fig. 10.1b). Among NPVs, virions with single nucleocapsids (SNPVs) or multiple nucleocapsids (MNPV) can be distinguished (Granados, 1980). As well as these occlusion-derived virions (ODVs) embedded into the OB, a second virion phenotype, the so-called budded virus (BV), is produced during the replication cycle (Fig. 10.2). This is genetically identical to the ODVs but has a distinct role. Whereas

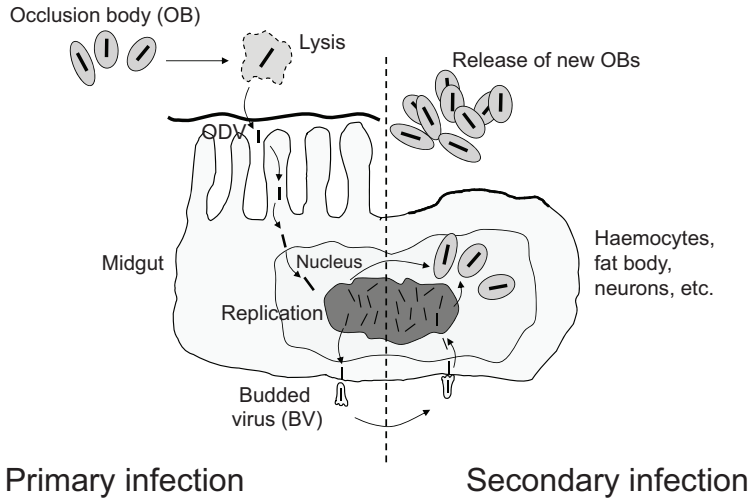

Fig. 10.2. Schematic infection cycle of *Cydia pomonella* granulovirus (CpGV). After the uptake of occlusion bodies (OBs) *per os*, the OBs are solubilized in the alkaline milieu of the insect midgut (lysis), releasing the occlusion-derived viruses (ODVs). Primary infection starts with fusion of ODVs with the midgut epithelial cells. The ODVs enter the nucleus, where virus replication starts. The newly synthesized viruses bud through the membrane of the epithelial cells. Secondary infection starts with the release of budded virus (BV) and the infection of other tissue cells. After replication, the viruses are embedded in the proteinaceous OBs late in infection. Following liquefaction of the host cell, the OBs are released into environment to start the next infection cycle.

OBs mediate oral infection from insect to insect, BVs enable the spread of infection from cell to cell within an infected larva (Federici, 1997) (Fig. 10.2).

Baculoviruses infect only larval instars. The natural route of infection is oral infection by ingestion of OBs, for example when an insect feeds on a plant. After virus uptake, the OBs are dissolved in the alkaline conditions (pH >10) of the insect's midgut, the ODVs are released and a primary infection of the midgut epithelium is initiated (Fig. 10.2). Transcription of viral genes and genome replication is induced to produce numerous copies of the virus. Virus replication leads to the development of a virogenic stroma and hypertrophy of the nucleus (Walker *et al.*, 1982). Enlargement of the nucleus is typical of the GV initial phase of replication, and is accompanied by the disintegration of its membrane (Federici, 1997). The newly synthesized genomes are packaged with viral proteins to form nucleocapsids. These nucleocapsids bud through the basal plasmalemma and in doing so acquire an envelope derived from the host-cell membrane, generating a BV (Granados and Lawler, 1981). The BVs spread via the trachea and/or haemolymph in the insect, causing a secondary infection (Fig. 10.2). During the secondary infection, the nucleus enlarges and ruptures, and cytoplasm and nucleoplasm become mixed. Nucleocapsids are assembled and become enveloped by membranes outside the stroma. The OB protein polyhedrin or granulin is highly expressed in the very late phase of infection, and the newly produced virions gain a protein matrix. The cell becomes filled with OBs (Federici, 1997). In the final stage of infection, the cells and tissues of the insects disintegrate. The insect tissues rupture and the OBs are released into the environment, ready to start a new infection cycle when they are ingested by another host larva.

10.3 Baculoviruses as Insecticides: Prospects and Limitations

Baculoviruses have several properties making them favourable for application in IPM. The most important feature in this regard is their extremely high host specificity. Baculoviruses are found in several families in the phylum Arthropoda, but in most cases, a given baculovirus infects only a few insect species, usually belonging to the same family or even to the same genus (Lacey, 1995). In many cases, just one single susceptible host is known. By applying baculoviruses in plant protection against a given pest species, only the target pest is affected, whereas beneficial and even neutral arthropod species in the same ecosystem are left unharmed. Thus, the whole potential of the natural antagonists of the pest can be exploited. Secondary pests are kept below economic damage levels and the necessity for additional plant-protection measures is greatly reduced. As a consequence, a treadmill situation, as is often the case when broad-spectrum pesticides are used, can be avoided. Another important point is that, in contrast to most microbes, baculoviruses do not produce toxins. If they kill their host, it is by the massive proliferation of virus in cells and organs. Thus, high host selectivity implies that baculoviruses are harmless for man, they are environmentally and ecologically safe, and they do not result in residue problems. An extensive record of biosafety studies has been established over the last four decades (Anon., 2002), and all studies showed that baculoviruses are safe to vertebrates, humans and the environment.

One of the most important features for the application of baculoviruses as biocontrol agents is the occurrence of the ODV form during their life cycle. The formation of the OB contributes tremendously to the virus stability. It allows easy handling of the viruses during the production and application process and enables product stability and a long shelf life. Due to the size of OBs, the virus concentration in a suspension can easily be estimated by counting OBs under a light microscope. In addition, their stability means that OBs can be applied in the field with conventional sprayers.

A further advantage compared with chemical insecticides is the fact that viruses are able to multiply in their host. After dissemination for pest control, the virus may persist in the population of the target pest and spread. Real epizootics can be initiated, maintaining the pest at a low level for several years and making further control measures unnecessary.

From the more than 1100 insect viruses known today, about 60% belong to the baculoviruses. For almost every forest or agricultural lepidopteran pest insect, a baculovirus has been isolated and described in the literature (van Beek and Sun, 2007). There are estimates that baculoviruses can be used against almost 30% of all the major pests of food and fibre crops. In Central America, by replacing chemical insecticides with insect viruses, pesticide consumption could be reduced by nearly 80% (Huber, 1990). This vast potential for pest control has barely been exploited so far. Whereas in the past most registrations were held by government agencies in the USA and Canada, recently several virus preparations have been produced and sold by private companies, particularly in Europe and North America (see Table 10.1). For less developed countries in particular, where costs for imported insecticides are high, the local production of baculovirus products provides a cost-saving alternative as well as a source of employment.

Nevertheless, the application of insect viruses in pest control has also been confronted with some reluctance. Insect viruses have some disadvantages, particularly with regard to their economic use in plant protection. The narrow host range of baculoviruses is the key feature that makes them advantageous from an ecological viewpoint. Their potential market, however, will always be restricted to single pest species and cropping systems. Thus, their host specificity and selectivity can entail an inherent economic disadvantage hampering their commercialization.

Table 10.1. Examples of baculoviruses developed as biocontrol agents for control of lepidopteran pests worldwide (Sun and Peng, 2007; Wang and Zengzhi, 2010; X. Sun, 2010, personal communication).

Host	Virus type	Country (products)	Crop
Adoxophyes orana	GV	Europe (pending in Annex I); Switzerland (Capex)	Apple
Anagrapha falcifera	NPV	USA (CLV-LC)	Vegetable
Anticarsia gemmatalis	NPV	Argentina (registration in progress); Brazil (Baculo-Soja, Baculovirus Nitral, Coopervirus PM, Protege)	Soybean
Autographa californica	NPV	China (three producers)	Vegetable
Buzura suppressaria	NPV	China (one producer)	Tea
Cydia pomonella	GV	Argentina (Cazporirusine, Madex); Canada (Virosoft Cp4); USA (Cyd-X, Virosoft Cp4); Europe (Granupom, Carpovirusine, Madex); New Zealand (Carpovirusine, Virex, Madex, Cyd-X)	Apple, pears, walnut
Ectropis obliqua hypulina	NPV	China (two producers)	Tea
Epinotia aporema	GV	Argentina (registration in progress)	Soybean
Gynaephora ruoergensis	NPV	China (one producer)	Grass
Helicoverpa armigera	NPV	Australia (Heliocide, Vivus Gold, Vivus Max); China (11 producers); Europe (provisionally listed on Annex I); India (Helicide, Virin-H, Helocide, Biovirus-H, Helicop, Heligard); Thailand; Vietnam	Cotton, tomato, pepper, tobacco
Helicoverpa zea	NPV	Australia (Gemstar, Vivus); USA (GemStar)	Cotton
Leucania separata	NPV	China (one producer)	Wheat, maize
Lymantria dispar	NPV	USA (Gypcheck)	Forest
Mythimna separata	NPV; GV	China (currently not marketed)	Rice
Neodiprion abietis	NPV	Canada (Abietiv)	Forest
Neodiprion lecontei	NPV	Canada (Lecontvirus)	Ornamentals, forest, woodlands
Orgyia pseudotsugata	NPV	Canada (Virtuss, TM Biocontrol-1)	Forest
Pieris rapae	GV	China (one producer)	Vegetables
Plodia interpunctella	GV	USA (FruitGuard)	Stored almonds and raisins
Plutella xylostella	GV	China (two producers)	Vegetables
Spodoptera exigua	NPV	Europe (Spod-X GH); Thailand; USA (Spod-X); China	Vegetables, ornamentals
Spodoptera littoralis	NPV	Europe (provisionally listed on Annex I)	Cotton
Spodoptera litura	NPV	China; India (Spodocide, Spodi-cide, Biovirus-S); Thailand; Vietnam	Cotton, vegetables, rice

The host specificity implies that each virus has to be propagated in its own host when producing baculoviruses on a large scale. Production of viruses at an industrial scale in living insects is labour-intensive. To guarantee a stable quality of virus, a healthy laboratory colony of the host insect needs to be established and reared over a whole year. Propagation of virus in living insects may lead to product variation in terms of composition and purity. Virus content and activity may be subjected to fluctuations; other microorganisms present in the insects may become contaminants in

the final product. Therefore, stringent quality controls including bioassays to test the biological activity of the virus are inevitable (Huber and Miltenburger, 1986). *In vitro* production in cell culture is possible but is also associated with high costs for cell-culture media and fermentation technology. In addition, the establishment of a specific insect cell line susceptible for the virus is complex and not always successful. Furthermore, baculovirus propagation in cell culture harbours the risk of losing genetic features such as *per os* infectivity, which is important in the living insect but not needed in tissue culture and might therefore quickly be discarded from the viral genome. There is also no selective pressure for producing OBs, so the baculovirus might degenerate into a virus stock defective in OB production.

A second disadvantage of the narrow host range occurs in the field when there is more than one pest species present in a crop. In this situation, in contrast to broad-spectrum chemical insecticides, additional control measures are needed, accompanied by additional costs and effort for the farmer.

In most countries, insect viruses have to be officially registered for use as insecticides and are subjected to the same regulations as chemical pesticides. Therefore, the cost of their commercialization is of the same order of magnitude as that for conventional insecticides. However, due to their high host selectivity, the market size and their sales potential are very limited – an aspect that is not attractive for a potential producer.

Furthermore, the use of selective control agents in the framework of IPM programmes requires good knowledge of the biology of the agent and its interactions with the target pest. Therefore, good education of the farmer in the use of biological control agents is an additional requirement. As insect viruses do not act on contact and are less persistent than most chemical pesticides, correct timing and application of the sprays needs more attention. Baculovirus control agents have a short residual activity on leaf surfaces and often need repeated applications. Furthermore, baculoviruses only infect the larval stages. This means that strict timing of applications is necessary to gain the maximum effect. In addition, inactivation by factors such as UV light and temperature extremes has to be considered in the field.

10.4 Genetic Engineering of Baculoviruses

The main drawbacks of baculovirus application in the field are their relatively slow speed of kill, their low virulence for older larval instars and their high specificity, often to a single host species. Although the narrow host range is advantageous from an ecological point of view as described above, it makes baculoviruses less attractive economically, as several baculoviruses are required when control of different insects is needed. In contrast to chemicals, which can kill pest insects within hours, the feeding damage caused by pest larvae continues for several days after the application of baculovirus sprays because the virus particle has to be taken up and virus infection needs to be initiated. This is often not accepted by the farmers. Genetic engineering has been applied since the early 1980s to overcome some of the drawbacks of baculovirus application compared with use of chemical insecticides. Different strategies have been applied, mostly based on the insertion of foreign genes into the virus genome expressing protein products that are deleterious for the insect: (i) physiological factors such as hormones and enzymes have been expressed in recombinant baculoviruses to interrupt the insect's metabolism; and (ii) insect-specific toxins or mutated versions of proteins have been introduced into the viral genome (Wood and Granados, 1991; Inceoglu *et al.*, 2006). One of the main target genes for genetic manipulation is the polyhedrin (*polh*) gene. Its protein product is the major protein found in the OB and it is produced in high quantities at the end of the viral infection process. Its promoter is very strong, allowing the production of high levels of additional insect control proteins

(Inceoglu *et al.*, 2006). Although economically less important, most research on genetic engineering of baculoviruses has been done using *Autographa californica* MNPV (AcMNPV), which serves as a molecular model of baculoviruses and has provided excellent experimental tools developed earlier as an expression vector for insect proteins in cell culture (Smith *et al.*, 1983; Summers and Smith, 1987). Various recombinant baculoviruses have been constructed to achieve two main purposes: (i) to improve the speed of kill of the viruses; and (ii) to extend the baculovirus host range.

10.4.1 Reducing time to kill

One of the first attempts to modify the insecticidal properties of baculoviruses by incorporation of a foreign gene was expression of the gene *BeIT*, an insect-specific paralytic neurotoxin of the scorpion *Buthus eupeus*, in AcMNPV to stop insect feeding more rapidly. Expression of *BeIT* under the *polh* promoter could be induced, but no paralytic activity was detected in assays with third- and fourth-instar larvae (Carbonell *et al.*, 1988). Since then, further insect-selective toxins have been expressed successfully. The toxin TXP-I from female *Pyemotes tritici* mites was expressed in AcMNPV and induced a strong paralytic response 2 days after infection of fifth-instar cabbage looper (*Trichoplusia ni*) larvae (Tomalski and Miller, 1991). Recombinant viruses were also constructed to produce *Bacillus thuringiensis* (*Bt*) toxin (Martens *et al.*, 1990) but showed no improvement in virulence (Bonning and Hammock, 1996; Inceoglu *et al.*, 2006). Recently, the cathepsin-L gene of the flesh fly (*Sarcophaga peregrine*) and the keratinase gene of the fungus *Aspergillus fumigatus* have been cloned into AcMNPV and tested against neonate and third-instar fall armyworm (*Spodoptera frugiperda*) larvae. Oral ingestion of virus by neonate larvae resulted in a 26% reduction in time to kill (Gramkow *et al.*, 2010). An alternative approach to insect toxins is the expression and overproduction of insect hormones to disrupt the host's homeostasis. Following this strategy, a synthetic diuretic hormone of the moth *Manduca sexta* was expressed by *Bombyx mori* NPV (BmNPV), disrupting the physiology of *B. mori* larvae. When injected into fifth-instar larvae of *B. mori*, the larvae died about 1 day earlier than controls infected with the wild-type BmNPV (Maeda, 1989). Eclosion hormone, involved in shedding of the cuticle during moulting, and juvenile hormone esterase, involved in controlling the larval moult, were also successfully expressed in recombinant baculoviruses (Hammock *et al.*, 1990; Eldridge *et al.*, 1991).

10.4.2 Host barriers to baculovirus infection

Several barriers in the virus–host interaction process affect the baculovirus host range and virulence. The determining factors of baculovirus host specificity are the ability to enter the host cells and tissues, to replicate there and to produce new virus particles (Thiem, 1997). The OB is the first limitation for host entry. Organisms lacking alkaline conditions in their midgut should not be susceptible to baculovirus infection, because the protein matrix of the OB cannot be dissolved. The peritrophic membrane of the insect's midgut may also present a physical barrier to baculovirus infection of midgut cells (Volkman, 1997). Fourth-instar larvae of *T. ni* can resist infection with AcMNPV by sloughing off infected midgut cells (Engelhard *et al.*, 1994). Further limitations to viral infection can occur at the level of virus entry into cells or by a block of replication in the selected cells (reviewed by Lu and Miller, 1997). Insect hosts have evolved several methods to block virus infection. Unlike vertebrates, insects lack an adaptive immune system, but they possess several innate defence mechanisms to resist infections (Tzou *et al.*, 2002). Phagocytosis, melanization and encapsulation are mediated by specialized cells called haemocytes. At the molecular level, the host range is limited by the ability

of the viral protein products to interact with host-cell components: molecular pathways of the host need to be compatible with the virus requirements concerning virus attachment, production of viral progeny and exit from the cells.

10.4.3 Extension of host range

Numerous studies have been performed to determine the genes involved in host-range limitation of baculoviruses. Over the last two decades, several genes involved in host-range determination have been identified. Several baculoviruses have been selected for a modified host range by simultaneous infection of insect cells with different virus strains. However, the detailed mechanisms behind these host-range variations have not been determined so far. At a molecular level, host range is limited by the ability of the viral protein products to interact with host-cell components. Several studies have been carried out using insect cell cultures transfected with BmNPV and AcMNPV. These two baculoviruses differ significantly in their infectivity for host cells, despite the fact that they show a high sequence identity at the predicted protein level. BmNPV replicates in *B. mori* (*Bm*N) cells, but not in *Spodoptera frugiperda* (*Sf*) cells. AcMNPV replicates in *Sf* and many other cell lines, but not in *Bm*N cells (for a review, see Rohrmann, 2008). After co-infection of *Sf*21 cells with both BmNPV and AcMNPV, a recombinant virus, eh-AcMNPV, infectious for both BmNPV and *Sf*21 cells could be isolated (Maeda *et al.*, 1993). eh-AcMNPV contained a small DNA stretch derived from the BmNPV helicase gene, indicating that the DNA helicase plays a role in baculovirus host-range definition (Maeda *et al.*, 1993). Another factor, host range factor-1 (*hrf-1*), was identified as a *Lymantria dispar* MNPV (LdMNPV) gene, which enabled AcMNPV to infect *L. dispar* cells after co-transfection with the two viruses (Thiem *et al.*, 1996). Further putative determinants of the baculovirus host range have been described (Thiem, 1997). Construction of recombinants with a modified host range is technically possible (Thiem, 1997; Chen *et al.*, 1997). However, to modify a baculovirus host range directed to target a specified second pest will need a detailed understanding of the molecular mechanisms of virus–host interaction. Host-range expansion may be possible in a very few cases, restricted to closely related species considered as hosts. The more distantly related the host species, the less possible such an approach will be. As very closely related pest species rarely occur in the same crop, this would not be of immediate benefit for the farmer. Hence, research into this direction seems to be more an academic exercise than a sustainable strategy for baculovirus application in the field.

10.4.4 Field application of genetically engineered viruses

Only a very few genetically engineered baculoviruses with modified insecticidal properties have left the laboratory and were tested in the field. One of the first field trials was performed with a recombinant AcMNPV expressing the insect-selective toxin AaHIT gene from the scorpion *Androctonus australis* against third-instar *T. ni* larvae (Cory *et al.*, 1994). As observed before in laboratory bioassays, a reduction in feeding damage of 23–29% was obtained using the recombinant baculovirus. The field stability of two genetically engineered variants of *Helicoverpa armigera* NPV (HearNPV) has been tested and compared with wild-type HearSNPV. Inactivation of the recombinants did not differ from the wild-type virus (Sun *et al.*, 2004). In general, the field data obtained so far with recombinant baculoviruses have not been encouraging, as the damage reduction in most cases, although statistically significant, was economically not satisfying. Hence, so far, no recombinant virus product has been commercialized for pest control (Summers, 2006). In the USA, the industry has ceased their efforts to register genetically engineered viruses (Inceoglu *et al.*, 2006).

For organic farmers, genetically engineered viruses are not an alternative to natural isolates, because the application of genetically engineered products is not tolerated by producers or by consumers of organic products, who already refuse chemical insecticides. In addition, there has been controversial discussion of the field release of genetically engineered organisms including viruses. Once released into the environment, their fate cannot be predicted and their existence cannot be withdrawn. There are mathematical models to evaluate the competition between wild-type and genetically engineered viruses in the field, but it is necessary to take into consideration additional information about natural factors such as overwintering of larvae in order to predict competitive interactions between strains (Dushoff and Dwyer, 2001). Several studies were performed on different non-target predators and parasitoids (*Solenopsis invicta*, *Geocoris punctipes*, *Hippodamia convergens* and *Microplitis croceipes*) using different virus constructs to test for shifts in the predator's life history and for the persistence of recombinants in non-target species (Li *et al.*, 1999; Smith *et al.*, 2000). No effect was found on predator feeding and fecundity (Li *et al.*, 1999). In addition, it was found that the probability of virus dispersal by parasitoids was low (Smith *et al.*, 2000). In contrast, it was found that predators could contribute to the survival and spread of recombinant baculoviruses in the ecosystem (Li *et al.*, 1999).

Most studies of genetically engineered baculoviruses demonstrating a reduced time to kill accompanied by a reduction in feeding damage are biased towards experiments using third- and fourth-instar larvae as targets. These larvae feed more than first- and second-instar larvae and are more susceptible than fifth-instar larvae. This experimental design cannot be transferred directly to the natural situation in the field. In the field, virus agents need to be targeted against early instars or against mixtures of young and older larvae. Early-instar larvae have dramatically lower feeding rates than older ones and will die before significant damage occurs. Studies on food consumption of different instars of the cabbage white butterfly (*Pieris rapae*) revealed that only 6.9% of total food consumption was due to first- to third-instar larvae, whereas 70.9% were due to fifth-instar larvae (Tatchel, 1981). For virus-infected cabbage looper (*T. ni*) larvae, it was found that first- and second-instar larvae consumed a maximum of 2% of their potential consumption, whereas older instars consumed up to 10% (Harper, 1973). Consistent with these results are studies investigating on the feeding damage by different instars of corn earworm (*Helicoverpa zea*) larvae treated with *H. zea* SNPV. When targeting the early instars, damage was reduced to more than 90% (Farrar and Shapiro, 2003). Therefore, an increased speed of kill of a recombinant baculovirus will result only in a minor reduction in damage when applied to early instars, as is normal good practice in crop protection. It also underlines the fact that the timing of application is much more crucial for successful control and prevention of damage than the speed of kill or cessation of feeding.

10.5 Examples of Successful Implementation

10.5.1 Control of *Anticarsia gemmatalis* using AgMNPV

The velvetbean caterpillar, *A. gemmatalis*, is the major defoliator on over 11 million ha of soybean in Brazil (Moscardi, 1989). Since 1979, research was performed towards the development of a biocontrol agent using *A. gemmatalis* MNPV (AgMNPV) to reduce the large amounts of pesticide applied every year to control the velvetbean caterpillar. Initial laboratory and field trials demonstrated a high virulence of AgMNPV for its host, inducing a high mortality even at low doses in the field. The use of AgMNPV has been described as the largest and the most successful programme for insect control using baculoviruses (Moscardi, 1999; Moscardi *et al.*, 2002;

Rangel and Faria, 2010). Its success has been based not only on the low-cost technologies carried to the farmers and the education programmes regarding AgMNPV but also on the cooperation of research, extension services and farmers. Implementation of the programme started in 1982 when small numbers of larvae were reared on an artificial diet. The virus-killed larvae were then distributed for treatment of field demonstration plots and further virus production in the field (Moscardi *et al.*, 2002). In 1986, a formulated AgMNPV product became available. This process was kept at a basic technical level: AgMNPV was applied as a suspension of infected larval raw material with kaolin in water (De Oliveira, 1998). Due to high costs, however, laboratory production of the virus was discontinued in the following years and AgMNPV field production was carried out instead. Infected larvae were collected in contracted fields treated with AgMNPV. Up to 600 kg of larvae killed by AgMNPV were collected per day, providing enough for virus treatment of about 30,000 ha (Moscardi *et al.*, 2002). Since this production was not sufficient to meet the increasing demand, further efforts were made for large-scale industrial production. From an estimated area of 200 ha of soybean treated with AgMNPV in 1982–1983 (De Oliveira, 1998), the treated area in Brazil was over 1,550,000 ha in 2001–2002 (Moscardi *et al.*, 2002). In 2004–2005, 2 million ha were treated with AgMNPV (Rangel and Faria, 2010). Recently, *in vivo* laboratory production of AgMNPV was performed by Coodetec (Coopervirus PM; Table 10.1), inoculating 800,000 larvae/day (Moscardi and Sosa-Gómez, 2007). For the growers, the application of AgMNPV has several advantages: (i) compared with chemical insecticides the costs are reduced by up to 30%, mostly because just one application of virus is enough to achieve insect control for the whole season; (ii) the collection of virus-killed larvae provides an additional income to many families; and (iii) a considerable reduction in chemicals can be achieved, supporting human and environmental health.

10.5.2 Control of *H. armigera* in China using HearNPV

Species of *Heliothis* are worldwide pests on food and fibre crops. With a rapidly growing population in the 1960s to 1970s, China faced the problem of protecting its resources against pest insects. Broad-spectrum chemicals were used intensively. As a result, species of *Heliothis* became resistant to all major chemical insecticides. In addition, many chemicals often affected non-target organisms and persisted in the environment (Ignoffo and Couch, 1981; van Beek and Sun, 2007). The cotton bollworm (*H. armigera*) is the major pest of cotton in China. As China is one of the largest cotton producers in the world – 24 out of 29 of China's provinces are cotton-growing regions – this pest is of great economic importance (Wu and Gou, 2005). Since the 1990s, IPM and microbial insecticides have been introduced to counteract the resistance problem and to reduce the application of chemical insecticides. The baculovirus HearNPV was registered in 1993 and then commercialized (Wang and Zengzhi, 2010). With an estimated 1600 t of formulated product in 2005, HearNPV is the most important viral biocontrol agent in China (Sun and Peng, 2007). By 2010, HearNPV formulations were being produced by 11 companies, and of a total of 35 viral biocontrol agents registered in China, 14 are based on HearNPV (Wang and Zengzhi, 2010; X. Sun, 2010, personal communication) (Table 10.1). Because of the labour-intensive production process, baculovirus production is mostly performed in factories in rural areas where manufacturing costs are comparatively low (Sun and Peng, 2007; van Beek and Sun, 2007). The virus is produced in insects fed on an artificial diet and is further processed into wettable powders or emulsions. The recommended field application is treatment of two to three times during one pest generation (Entwistle, 1998). The amount of formulated HearNPV was estimated to be approximately 500 t in 2009, applied on about 100,000 ha of cotton. The much lower level of use in 2009 compared with 2005 is

the result of the increase of transgenic *Bt* cotton during these years (X. Sun, 2010, personal communication).

10.5.3 Control of *Cydia pomonella* using CpGV

In nearly all pome-fruit-growing areas, the codling moth (*C. pomonella*) is the most devastating insect pest of apple and pear (Barnes, 1991). If codling moth control fails in an orchard, further pest control (e.g. mites, aphids, leaf rollers) is useless, because the production of high-quality fruits is not possible (Croft and Riedl, 1991). Multi-resistance to chemicals, the mediocre efficacy of chemical insecticides and residue load on apples have made biological control agents increasingly attractive in apple production.

The discovery of *C. pomonella* granulovirus (CpGV) prepared the way to solve several problems of conventional codling moth control using chemical insecticides. CpGV was identified from infected codling moth larvae collected in Mexico in 1963 (Tanada, 1964). It is highly specific to codling moths and therefore does not interfere with non-target organisms in the orchard. CpGV is extremely virulent; its 50% lethal dose (LD_{50}) for neonate codling moth larvae was determined as 1.2 granules per larva (Huber, 1986). In 1987, CpGV was the first viral pesticide registered for use on a food crop. Several commercial preparations are currently registered in North America (e.g. Cyd-X, VirosoftCp4) and in many European countries (e.g. Carpovirusine, Cyd-X, Granupom, Madex, Virgo), as well as in many other apple growing countries in South America, South Africa, Australia, New Zealand and others (Table 10.1). Most of these products are based on the original isolate from Mexico, CpGV-M.

So far, no cross resistance to chemical insecticides has been observed. However, there are some concerns of growers regarding CpGV application: (i) its inactivation by UV radiation requires repeated treatments during the season; (ii) after virus application, the larvae continue to feed for a few days, rendering superficial damage to the apples, which is not tolerated by many conventional growers; and (iii) production costs and consequently the shelf costs of CpGV products are higher than those of broad-spectrum insecticides. On the other hand, there is very good evidence that CpGV application contributes much more effectively to the reduction of codling moth populations and long-term pest pressure than many chemical insecticides, making CpGV attractive not only in organic but also in conventional production methods applying IPM (Huber & Dickler, 1976; Kienzle *et al.*, 2003). Conventional growers often combine CpGV applications with other control measures to benefit from this specific feature of CpGV. According to information from CpGV producers, CpGV is applied today on more than 100,000 ha in Europe, and 80% of the selling of CpGV products goes to IPM. In organic apple production, CpGV application is recommended in combination with mating disruption using the pheromone confusion method (Cross *et al.*, 1999; Lacey *et al.*, 2008).

10.6 Field Resistance to a Baculovirus Product: a New Challenge

For a long time, it was assumed that the development of resistance against viral pesticides was unlikely because of the complex replication cycle of baculoviruses (Cory and Myers, 2003; Rohrmann, 2008). It was possible to induce resistance in laboratory experiments, where insects were reared under virus selection pressure (Briese, 1986). However, these populations returned to their initial level of susceptibility within a few generations when kept without virus.

Codling moth populations with a reduced field susceptibility to CpGV products were first observed in two orchards in 2003 in southern Germany. The offspring of these field populations were tested in the laboratory and showed a 500–1000-fold

reduced susceptibility to CpGV than the sensitive laboratory strain (Fritsch *et al.* 2005). In addition, bioassay tests on a codling moth field population in France revealed a 13,000-fold reduced susceptibility compared with the internal laboratory population (Sauphanor *et al.*, 2006). Until 2006, codling moth populations of 13 German organic apple orchards were tested systematically in bioassays for their susceptibility to CpGV-M. Resistance ratios of 1000–10,000-fold were reported (Asser-Kaiser *et al.*, 2007). To date, CpGV resistance has been demonstrated in about 40 orchards in Germany, France, Italy, Switzerland, Austria and the Netherlands (Jehle *et al.*, 2010).

By single-pair crosses of a homogenous resistant codling moth strain and a susceptible laboratory strain, clear evidence for sex linkage to the Z chromosome and a concentration-dependent dominance has been provided (Asser-Kaiser *et al.*, 2007). These results are consistent with the observation of the rapid emergence of CpGV resistance. Selection for both homozygous and heterozygous resistant insects has been fostered by the fact that all commercially available products in Europe are based on only one isolate, CpGV-M, which was applied several times during a season for several years in the orchards. The resistance to CpGV-M observed in field populations of *C. pomonella* will require new strategies of virus application in biological control programmes.

To counteract the resistance problem, a search for further CpGV isolates was initiated. In 2006, a novel isolate, CpGV-I12, derived from Iran, was found to show improved efficacy against resistant codling moth populations in bioassays (Jehle *et al.*, 2006b; Eberle *et al.*, 2008). Its activity against resistant larvae was comparable to the activity of CpGV-M against susceptible larvae. Overcoming resistance was possible in all larval instars of the codling moth. CpGV-I12 and other resistance-overcoming CpGV isolates were tested successfully in the field (Zingg, 2008; Berling *et al.*, 2009a). Concurrently, a CpGV (Madex Plus) was selected by passaging CpGV-M through resistant codling moth larvae by Andermatt Biocontrol (Switzerland). In 2007, Madex Plus received registration in Switzerland (Zingg, 2008). By genome characterization and genome mapping of several CpGV isolates deriving from different geographic origins, different genome types of CpGV were identified (Berling *et al.*, 2009b; Eberle *et al.*, 2009). This genetic diversity is the basis for the development of new CpGV products, which are currently in the process of registration.

10.7 Future Prospects

Baculoviruses are highly promising agents for use in the biological control of many insect pest species, especially from the orders Lepidoptera and Hymenoptera. Intensive research carried out over the last 50 years has proven their efficacy as well as their safety to humans, non-target organisms and the environment. Despite their great potential and several economically successful examples, many promising viruses have never been registered or commercialized. The main reasons for this reluctance to introduce baculoviruses into insect pest-control practice are the difficulties and high costs of registration, which is discouraging for many small and medium-sized enterprises producing baculovirus products. This situation has hampered the registration of baculovirus products and other microbial pest-control agents in many countries. In Europe, the registration of a new baculovirus species as a biocontrol agent costs approximately US$1.5–3 million and may take 5–10 years, during which a company will not make any revenue (Ravensberg, 2011). Some progress in facilitating the registration of new baculovirus products was achieved recently in the European Union, where baculoviruses can be registered at a species level, facilitating the further registration of new isolates and strains of the same species (SANCO, 2008). However, this is only a first step, and more have to follow. If the registration process is not further facilitated and significantly accelerated, baculoviruses

will continue to be 'promising' but they will not be able to penetrate the pesticide market.

The emergence of codling moth populations resistant to CpGV, as observed in several in European countries, has revealed that resistance management strategies also need to be implemented with baculoviruses, especially if they are very intensely and frequently used as the only control measure, as occurs in many organic orchards. The finding and/or selection of isolates overcoming CpGV resistance also underlines the fact that the genetic diversity of baculoviruses is far away from being well understood and sufficiently exploited in plant-protection practice. Therefore, the availability of baculovirus isolates that can be developed further to become biocontrol agents will be pivotal.

There are several fields of research that are important to foster the future application of baculoviruses as biocontrol agents. These include: (i) the identification and biological and molecular characterization of baculoviruses; (ii) exploitation of the genetic diversity of baculoviruses; (iii) a better understanding of the population dynamics and virus–host interactions on a population level; and (iv) improving the implementation of baculovirus application into IPM.

As exemplified above, the economic success of baculoviruses has never been greater than in current times. Hence, it is regrettable that public funding and therefore scientific interest in baculovirus research has declined over the last decade. Today, the opportunities for baculoviruses being used as biological control agents are better than ever: (i) there is a rapidly growing demand for organic food as well as for crops with low or no chemical residues; (ii) there are more and more chemical pesticides banned for health and environmental reasons; (iii) there are a number of companies with experience of virus registration as well as the economic need for a baculovirus products; and (iv) there is a large number of known baculoviruses that are highly specific for the major pest insects of many crops. Further efforts are needed to bring academics, registration authorities, producers and extension services together in order to develop new virus products for use in biological control.

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11 Biological Control of Weeds with Plant Pathogens: Four Decades On

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11.1 Introduction

'Two of the major competitors for man's food are weeds and plant pathogens. It is a challenge to pit these two forces against one another and tip the biological balance in man's favour!' These were the concluding remarks from the first review on the subject of weed pathology, published some 40 years ago (Wilson, 1969). In fact, as the statement implies, the review was compiled before the science of weed pathology had been properly field tested as a management strategy and, in terms of classical biological control (CBC), before a 'silver bullet' had been fired in anger. As Wilson (1969) pointed out, there has been a long-standing interest in exploiting plant pathogens for the control of weeds, most notably by Cunningham (1927) in New Zealand who summarized the attempts of nearly a decade of research on 'the possible value of fungi as controllants of weeds and insects'. However, his conclusions on this form of weed management, or 'natural control' as he termed it, were not encouraging: 'The evidence produced indicates that it is but a waste of time to proceed with experiments along these lines' (Cunningham, 1927). In contrast, success had already been achieved with phytophagous insects, and the principles and concepts of using natural enemies for weed control had been laid down even earlier by entomologists who had demonstrated the efficacy of the strategy, not only for the management of seemingly intractable and highly problematic invasive alien weeds (McFadyen, 1998) but also for exotic insect pests (DeBach and Rosen, 1991).

However, CBC is still largely unknown to or misinterpreted by both scientists and the general public, despite the simplicity of the strategy and its long and successful history. Indeed, this approach to pest management – involving the deliberate release of host-specific exotic natural enemies into a new environment to control invasive species – most often provokes antipathy, bordering on hostility. This is because the information that filters through to the public is confused and often distorted or sensationalized by the media, based on horror stories of introductions of so-called biological control agents that have gone wrong. None of these much-publicized examples, such as the disastrous introduction of the cane toad as a control agent of sugarcane pests in Australia, had been scientifically vetted nor were they based on the principles of CBC: all have involved the release of generalist predators (arthropods,

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amphibians, molluscs, reptiles and mammals), rather than coevolved natural enemies – the hallmark stamp of CBC. The media have continued to pursue these inevitable disasters with vigour and persistence, while successful and scientifically underpinned biological control projects have tended to slip beneath their radar. After all, most media stories are founded on bad rather than on good news. An unfortunate consequence has been the subsequent use of these infamous examples of 'biological control gone wrong' by bureaucrats, particularly in developing countries, where there are no established protocols for biological weed control, as a reason to obstruct and delay projects (Wilson and McFadyen, 2000). Additionally, there has been an exaggerated concern within the biological control community about host specificity and potential non-target impacts by introduced CBC agents. These are totally irrelevant if compared with the truly catastrophic environmental problems and widespread ecosystem disruption caused by invasive alien weeds and insect pests (for which CBC is frequently the only sustainable remedy). Even in countries such as New Zealand, with a well-established tradition in CBC, fears of dilution of endemic biodiversity and homogenization of the flora and fauna have been raised as a reason for not introducing biological control agents (Emberson, 2000). This is indeed an entirely ill-founded allegation, as one of the principal aims of CBC is to prevent loss of biodiversity by controlling the exotic invaders. Even more worrying is that some of the most successful weed biocontrol projects in New Zealand would not have passed present-day legislation in that country (Groenteman *et al.*, 2011).

This chapter aims to allay the fears and the controversy – in particular the 'pathophobia' that inevitably surrounds the use of plant pathogens (Warner, 2011) – and to provide the many and varied stakeholders, as well as decision makers, involved directly or indirectly in weed management with up-to-date information on the 'state of the art' of biological control of invasive alien weeds involving the classical approach. However, because many plant pathogens can be cultured or manipulated *in vitro*, another management strategy not available to entomologists can also be employed against weeds – that of inundative biological control. This approach incorporates radically different concepts based on a much higher level of technology and, typically, has been targeted at indigenous or naturalized weeds in high-input agriculture. Moreover, in addition to the potential technological constraints, there are also economic, legislative and marketing hurdles to overcome whenever commercial interests are involved that sets this strategy apart from CBC. The current status of inundative biological control using bioherbicides is discussed, with an emphasis on the progress achieved over the past four decades and, in particular, on how the concepts have changed over time.

11.2 Classical Biological Control

11.2.1 Introduction to CBC

Perhaps never before in its long history, spanning over 120 years, has the classical approach been more relevant than it is today, as the negative impacts of increasing globalization come home to roost, with dramatically increasing movement of alien species between previously geographically separated continents, countries and biomes (Elton, 1958; Baskin, 2002; Denslow, 2003; Lee and Chown, 2009; Ascunce *et al.*, 2011). Invasion of natural ecosystems by alien species represents one of the biggest threats to biodiversity, as now acknowledged by scientists and governments throughout the world (IUCN, 2000; Mack *et al.*, 2000; Mooney and Hobbs, 2000; Perrings *et al.*, 2010). The impacts and knock-on effects of invasive alien species are immense and often irreversible. The geographical barriers of oceans, mountains and deserts have provided the isolation necessary for unique ecosystems and species to evolve over millions of years. However, these natural barriers have been rendered ineffective in

just a few hundred years, as the pace and volume of international trade has burgeoned since the industrial revolution and the world heads towards the 'McDonaldization' of its flora and fauna (Nee, 2004; Evans and Waller, 2009).

Customs and quarantine practices, developed in earlier times to guard against both human and agricultural diseases and pests, are often inadequate safeguards against species that threaten native biodiversity. Thus, the inadvertent ending of millions of years of biological isolation has created major ongoing problems that affect both developed and developing countries (IUCN, 2000). There are now numerous well-documented biological invasions involving organisms in all the major taxonomic groups, including animals, fungi, plants, algae and viruses (Mooney and Hobbs, 2000). Here, only invasions involving plants will be discussed. Such invasions can lead to: (i) replacement of diverse systems with single-species stands or monocultures of alien plants, threatening not only the indigenous flora but also the dependent fauna; (ii) disruption of food chains; (iii) interference with breeding sites; (iv) alteration of microclimates; (v) changes in soil chemistry and geomorphological processes; and (vi) modification of fire regimes, hydrology and biogeochemical cycles (Cronk and Fuller, 1995; Mack *et al.*, 2000). The impact of plant invasions is global and enormous, in both ecological and economic terms. As a combined consequence of all these factors on the ecological balance, an introduced plant species can provoke species extinctions and therefore can impact directly on biodiversity: the ecological cost is the irretrievable loss of native species and ecosystems (IUCN, 2000). In addition, the direct economic costs of invasive plant species run into many billions of dollars annually. Oerke *et al.* (1994) calculated that losses due to terrestrial weeds (based on eight major crops) average almost 13% of the world's agricultural output alone. It is highly significant that, for well-studied weed floras such as those of North America, the majority of the worst weeds are introduced species (Zwölfer, 1968; Andres and Goeden, 1971). For example, in the USA, it is estimated that about 65% of the weed flora comprises introduced species and that the total impact on the US economy is at least US$13 billion/year (Westbrooks and Eplee, 2004). In addition, exotic aquatic weeds have caused major ecological imbalances throughout the world and have impacted directly or indirectly on human activities through: (i) interference with transport, water supply systems, tourism, fishing, electricity generation and irrigation; (ii) increasing water loss through transpiration; (iii) promoting erosion and flooding; and (iv) escalating human diseases by favouring the breeding of mosquitoes and other disease vectors (Barret, 1989; Harley, 1990; Singh and Gill, 1996; Chikwenhere and Keswani, 1997; Kissmann, 1997, 2000; Moreira *et al.*, 1999; Lorenzi, 2000).

Ideally, all introductions of plants into new areas of the world should be subject to strict rules enforced by well-trained inspectors. Unfortunately, however, even in developed countries, as well as in areas known to be under extreme risk of invasion – such as the oceanic islands – undesirable species introductions are known to be very difficult to prevent. Even widely recognized agricultural pests have repeatedly reached supposedly inaccessible regions. Additionally, although prevention should be regarded as a priority among the strategies aimed at avoiding problems with invasive species, it is often very difficult to foresee them. Seemingly harmless plants that have been introduced as beneficial trees, such as tropical pines (*Pinus* spp.), wattle (*Acacia* spp.) and paper bark (*Melaleuca quinquenervia*), or as attractive ornamentals, such as *Miconia calvescens* or wild ginger (*Hedychium* spp.), later have become aggressive ecosystem invaders in regions where they have been introduced (Gagné *et al.*, 1992; Meyer and Florence, 1996; Henderson, 2001; Williams *et al.*, 2003; Loope *et al.*, 2004; Soares and Barreto, 2008a). There is no way such an outcome could have been anticipated for these examples, and for many others.

Potentially, evidence on the invasive capability of a plant species – as

demonstrated by invasions by the same species that took place in other regions of the world – could be used as an early-warning system. Unfortunately, even for major plant invaders, this information often remains neglected where plant introduction and distribution are concerned. For example, there are many publications encouraging the use and even the sale of propagating material of some of the worst weeds in the world for various purposes such as aquarium plants, medicinal plants, ornamentals and green manure, among others. This is exemplified by a Brazilian website (Embrapa Agrobiologia: http://www.cnpab.embrapa.br/) that promotes plant species as appropriate for fighting soil erosion but which includes some well-known, highly invasive exotic tree species, notably: *Acacia mangium*, *Acacia nilotica*, *Acacia saligna*, *Albizzia lebbek*, *Albizzia procera*, *Casuarina equisitifolia* and *Leucaena leucocephala* (Machado *et al.*, 2006). Although listed among the ten worst world weeds by Holm and Herberger (1969), *Imperata cylindrica* or cogon grass, a native of the Old World tropics, is still being sold in the USA as an ornamental under the name Japanese blood grass. *Chromolaena odorata*, the mis-named Siam weed, is actually native to the Neotropics and is responsible for disastrous and widespread ecosystem invasions in Africa and Asia, and has been the target of biological control programmes in several countries. However, unexpectedly, during a meeting of the Third International *Chromolaena* Workshop in Abidjan in 1993, where funding from the Food and Agriculture Organization of the United Nations (FAO) for an initiative aimed at its biocontrol was to be discussed, there was a strong lobby from several African-based international organizations claiming that it should not be controlled because it is a good cover crop in slash-and-burn agriculture, as well as having additional medicinal 'benefits', such as preserving bodies and inducing abortions. The fact that agricultural scientists should still be promoting slash-and-burn technology is a sad reflection of their ecological awareness. Conversely, in the Neotropics, this plant serves no useful purpose, even though indigenous peoples have been acquainted with it for millennia, while it has been present in Africa for less than 50 years. In the face of these fabricated conflicts of interest, the FAO had no choice but to withdraw its funding for the proposed programme (Barreto and Evans, 1996; Moore, 2001).

The responses to such weed invasions have been many and varied, beginning with attempted eradication through to long-term integrated pest management (Wittenberg and Cock, 2001; Veitch and Clout, 2002). In natural ecosystems, however, most of these measures have failed due to economic and/or environmental concerns and constraints, especially where vast tracts of marginal or 'valueless' land are involved. It is only in recent, more environmentally enlightened times, when the importance of biodiversity to the planet's future and the immense ecological damage caused by these invasions have been fully appreciated, that more sustainable solutions are now being considered. The emphasis here is on the word 'considered', as, despite theme-specific, global conferences and international agreements, pragmatic solutions have been limited with no coordinated funding directed towards the implementation of control strategies. In essence, there has been too much talk and too little action.

CBC is one approach for the management of invasive alien weeds that stands out because of its elegance, successful track record and cost-effectiveness. For CBC, the concepts are simple and follow a logical approach, initially developed and refined by entomologists over the last century. The premise is based on the enemy-release hypothesis or ERH (Keane and Crawley, 2002; Mitchell and Power, 2003) which posits that plants, once freed of their natural-enemy complex, become ecologically fitter and therefore more competitive than those subject to natural control. If climatic factors are favourable, then there are few barriers to regulate growth and fecundity, and this may result in population explosions with the subsequent development of weed invasions.

Thus, alien plant species are invariably introduced, either deliberately or accidentally, into a new geographical area without their coevolved natural enemies, or at best with only a partial complement. Recently, another theory, the endophyte-enemy-release hypothesis (E-ERH), has been proposed, which offers an additional explanation as to why some plants become invasive and how CBC can be evaluated more effectively as a management tool (Evans, 2008). CBC aims to redress this imbalance by searching in the centre of origin of the weed target, identifying the plant pathogens or arthropods that are attacking it, selecting and screening those that appear to impact most on that target and, finally, introducing and releasing approved agents in the exotic area (FAO, 1996). The single, most important criterion is that the selected agents must be highly specific to the target weed. The advantages and disadvantages that are recognized for CBC as a strategy for weed control are outlined in Table 11.1.

CBC fits well into an integrated, biologically based approach to pest management in agroecosystems, and is the only viable, long-term or sustainable option for the control of many invasive alien weeds in rangeland and natural environments (McFadyen, 1998; Charudattan, 2001). Entomologists had an early lead in CBC of weeds when the cochineal mealybug (*Dactylopus ceylonicus*) was taken from northern to southern India in 1836 to control the weedy cactus *Opuntia vulgaris*. Subsequently, in 1865, it was successfully transferred from India to Sri Lanka for the same purpose (Julien and Griffiths, 1998), while similar pioneering introductions of insect natural enemies have been made over more than a century in Hawaii targeted against lantana weed (*Lantana camara*) (Perkins and Swezey, 1924). During its history, CBC of weeds with insects has resulted in some spectacular success stories, including the pioneering project in the 1920–1930s by the Commonwealth Prickly Pear Board to control South American species of *Opuntia* cacti in Australia using a moth (*Cactoblastis cactorum*) introduced from Argentina (Dodd, 1936; Julien and Griffiths, 1998). More recently, excellent control of the neotropical aquatic plant *Salvinia molesta* (water fern) in Asia, Africa and Australasia has been achieved following the release of a shoot-feeding weevil, *Cyrtobagous salviniae*, originally collected in Brazil (Thomas and Room, 1986).

Although Wilson (1969) – surveying the subject of use of plant pathogens in weed control – stated that 'the idea of using them to control weeds is almost as old as the science of plant pathology itself', it was not until the 1970s that the involvement of plant pathologists in CBC was officially put into practice. Even earlier than this seminal publication, Oehrens (1963) conjectured on the potential role of rust fungi in weed biocontrol. Later, in contrast to the earlier obscure and discouraging experience of Cunningham (1927) in New Zealand, he undertook ground-breaking introductions of two rust pathogens against invasive European weeds in Latin America (Oehrens

Table 11.1. Advantages and disadvantages of CBC.

Advantages	Disadvantages
Inherently safe	Can have long lag phase
Cost effective	Long research phase
Target specific	Need for opinion of wide range of stakeholders
Practical	Potential conflicts of interest
Environmentally benign	Not always effective
Efficacious	Non-commercial
Sustainable	No eradication
Genetically stable	Dependent on public funding
Compatible with organic agriculture	Restricted to the control of exotics
Proven track record	No 'recall' of agent after release

and Gonzales, 1975; Oehrens, 1977). This led to the successful control of European blackberry (*Rubus constrictus*) by the rust *Phragmidium violaceum* imported from Germany into Chile. It would appear, therefore, that this is the first documented example of CBC of weeds using a plant pathogen and, patently, where quarantine procedures and risk assessments were not even considered. A similarly cryptic account of the importation of pathogens into Australia from the New World during the 1920s – to screen against the aforementioned prickly pear (*Opuntia* spp.) – appeared in the official reports of this highly successful programme (Dodd, 1936; Mann, 1970). No further details of their screening were reported, but several of these neotropical pathogens were subsequently recorded in Australia (Evans, 2002). Could these have contributed to the success of the now legendary Argentinian moth, which saved farming systems in southern Queensland?

Quimby (1982) discussed infamous examples of devastating plant diseases, such as potato late blight, coffee leaf rust, chestnut blight and eucalypt dieback, to highlight two issues. First, to demonstrate the potential of plant diseases to impose dramatic damage on plant populations, including noxious weeds, and secondly, the need to draw attention to the great care that should be taken in terms of safety to non-target plants. The first release of an exotic pathogen, however, had already been undertaken in Australia. This followed the pest risk protocol established by Wapshere (1974) and was based on the introduction of arthropods rather than of pathogens as CBC agents. The target was skeleton weed (*Chondrilla juncea*) and the CBC agent was the rust *Puccinia chondrillina*, from the plant's centre of origin in the Mediterranean region of Eurasia (Cullen *et al.*, 1973; Mortensen, 1986; Cullen and Hasan, 1988). The total estimated saving due to increased crop yields and reduced herbicide usage varies greatly in the biocontrol literature. However, the cost:benefit ratio has been put at 1:112 (Marsden *et al.*, 1980). Mortensen (1986) credited this pathogen with an annual saving of over US$12 million, and Marsden *et al.* (1980) estimated an overall saving of AU$260 million, projected up to the year 2000. Successful control of blackberry in Chile and of skeleton weed in Australia generated an upsurge of interest in the potential of this novel approach to weed management.

A number of comprehensive reviews of CBC of weeds using pathogens have followed from Wilson's original, spanning the last four decades (Hasan, 1974; Huffaker, 1976; Charudattan and Walker, 1982; Templeton, 1982, 1984; Wapshere, 1982; Adams, 1988; Cullen and Hasan, 1988; Ayres and Paul, 1990; Evans and Ellison, 1990; Hasan and Ayres, 1990; Watson, 1991; TeBeest *et al.*, 1992; TeBeest, 1993; Barreto and Evans, 1996; Julien and White, 1997; Charudattan, 2001; Evans *et al.*, 2001a,b; Evans, 2002; Barton, 2004; Yandoc-Ables *et al.* 2006a,b). Additionally, several reviews have been published that have addressed national or regional CBC programmes (Gardner *et al.*, 1995, 1996; Olckers and Hill, 1999; Ellison and Barreto, 2004; Julien *et al.*, 2007; Barreto, 2008), and even specific crops (Ellison, 2004).

11.2.2 Principles of plant pathology and CBC

Historically, plant pathologists have been dedicated to fighting crop pathogens and thus the discipline has always been oriented towards the control of diseases and mitigation of the losses caused to agriculture and forestry. Naturally, plant pathologists tend to regard such pathogens as adversaries. It is ironic, therefore, that the knowledge accumulated by a century and a half of research in this field now serves as the basis for CBC of weeds with pathogens where the aim is to promote plant diseases as beneficial. All topics representing the framework of the science of phytopathology, and dealt with in standard textbooks (e.g. Agrios, 2005), yield information that is of great relevance for pathosystems of interest to CBC of weeds, including:

- Disease diagnosis.
- Taxonomy of plant pathogens.
- Elucidation of disease cycles and life history of pathogens.
- Determination of how pathogens survive, disseminate, infect and colonize their host.
- Determination of the environmental conditions favourable for the pathogen and for disease outbreaks in the field.
- Host and pathogen genetic variability (particularly in terms of virulence and resistance to disease).
- Pathogen biology and plant disease epidemiology.

Many plant pathogens that are found attacking important invasive alien weeds in their centres of origin are poorly known or entirely new to science. This is often because the plants themselves have been little studied due to their rarity and/or non-economic interest in their native ranges. For these, the same basic steps expected to be fulfilled in the study of novel diseases must be followed. The pathogenic organisms must be described and named, pathogenicity must be demonstrated through Koch's postulates and *in vitro* and *in vivo* studies must be performed in order to generate the basic information needed for further evaluation. Such projects offer unique training opportunities for plant pathologists, as all of the fundamental areas of the discipline must be addressed for a particular organism if a viable evaluation of the CBC potential of a candidate agent is to be undertaken.

The disease triangle (Gäumann, 1950) illustrates one of the most fundamental paradigms in plant pathology, that is, the existence of a disease caused by a biotic agent requires the following interacting factors: (i) a susceptible host; (ii) a virulent pathogen; and (iii) environmental conditions favourable for disease development. There are variations of the disease triangle, such as that in Agrios (2005) where an extra factor is added (time) in a tetrahedron. A fifth factor is mentioned but not included, humans – meaning human impact on diseases through management actions. For crop diseases, all of these factors as listed by Agrios (2005) are critical. Weed diseases are no exception to the factors in the tetrahedron. Nevertheless, for CBC of weeds with pathogens, human interference is limited to the transport and release of the agent. Normally, little is done in terms of additional interventions after the pathogen has been released into a new area. This strategy relies almost completely on the innate ability of the pathogen to disperse, infect and survive in the new habitat of introduction.

11.2.3 Procedures

Balciunas (2000, 2004) proposed a voluntary 'Code of Best Practices for classical biological control of weeds', which should be followed by biocontrol practitioners. The 12 guidelines are listed below:

1. Ensure the target weed's potential impact justifies the release of non-endemic agents.
2. Obtain multiagency approval for the target.
3. Select agents with potential to control the target.
4. Release safe and approved agents.
5. Ensure that only the intended agent is released.
6. Use appropriate protocols for release and documentation.
7. Monitor the impact on the target.
8. Stop releases of ineffective agents, or when control is achieved.
9. Monitor impacts on potential non-targets.
10. Encourage assessment of changes in plant and animal communities.
11. Monitor interactions among agents.
12. Communicate the results to the public.

Suitability of target weed

The choice of a plant species as a target for a CBC programme is not made by biocontrol scientists, who usually only become involved when attempts to tackle an intractable weed problem by other means have already failed. Typically, farmers, environmentalists or government authorities

often appeal to them as a 'last resource'. Nevertheless, attempts to objectively assess that a weed is suitable as a target for CBC have been made by biocontrol scientists, most notably by Peschken and McClay (1995). One of the most important challenges when determining the suitability of a weed as a CBC target is trying to avoid conflicts of interest. However, this is a major and constant issue in CBC, as the situation where an introduced weedy plant species is acknowledged as only having negative aspects is the exception rather than the rule. Stanley and Fowler (2004) have reviewed this subject and recognized four categories of conflicts of interest: (i) where one or more groups value the target plant for economic and/or cultural use; (ii) potential non-target effects of biological control; (iii) where biocontrol programmes target native plants; and (iv) factors related to the impact of successful biocontrol as harming the native biota that uses the weed.

Suitable agents

One of the most important steps for the success of any CBC programme is the selection of the most effective agents from the list of potential candidates. Nevertheless, in practice, weed biological control has sometimes been compared with a lottery where many control organisms are released in order to find the few that are effective (McEvoy, 2004). Berner and Bruckart (2005) devised a decision tree for the better selection of exotic plant pathogens as CBC agents based mainly on pathogenicity and specificity studies. However, agent selection is often more subjective and based on observations of damage in the field, candidate biology and taxonomy (Puliafico *et al.*, 2008), or sometimes piggy-backing on previous programmes that have been successful elsewhere. Morin *et al.* (2006a) have introduced a framework to facilitate streamlining of the selection process for CBC agents. They divided the process of obtaining a suitable agent into two series of steps that were enumerated and described in detail. The first step is identification of a suitable pathogen by: (i) elucidation of host taxonomy and diversity; (ii) literature searches; (iii) field surveys and climatic matching; (iv) prioritization of candidate pathogens; and (v) collection of suitable strains. The second step is assessment of the prioritized pathogen by assessment of: (i) pathogenicity tests; (ii) host–pathogen matching; (iii) pathogen genetic structure; (iv) evaluation of multiple pathogen strains; (v) pathogen taxonomy and life cycle; (vi) pathogen impact and aggressiveness of strains; and (vii) preliminary host-specificity testing.

This represents an objective form of organizing the selection of suitable agents, which in practice is already routinely followed for most current weed biocontrol programmes. Unfortunately, CBC is not an exact science: successful agents that have worked well in one region of the world have sometimes failed in another area. An iconic example is that of the rust *P. chondrillina*, which, as already mentioned, produced spectacular results in Australia but then failed to deliver after it was introduced into Argentina (Julien and Griffiths, 1998) and had less impact than obtained in Australia in the USA. There is little information about the introduction that was made in Argentina but, for the USA, indications are that a combination of attack on the rust by mycoparasites and host resistance to or incompatibility of the strain of the rust that was introduced, together with the heterogeneity of the habitats that were invaded in the USA, are considered to have contributed to the inadequate control levels that were achieved (Parsons *et al.*, 2008). The search for a matching pathotype within the population of a pathogen under evaluation for use in CBC is increasingly being recognized as an issue of critical importance. This has been shown to be of particular relevance with highly specialized or obligate pathogens, such as the rust fungi. For example, Ellison *et al.* (2004) showed clearly that identifying rust pathotypes that are physiologically compatible with and virulent to the weed population in the target area can be crucial for success.

Surveys

Various types of survey can be undertaken:

- Literature and herbarium surveys, online databases such as the US Department of Agriculture (USDA)'s database on fungal–host distributions (Farr and Rossman, 2011) or Embrapa's Brazilian list of fungi on plants (Embrapa Recursos Genéticos e Biotecnologia, 2011), as well as the many published national or regional check-lists of plant pathogens in general, are a very useful source of information on the known occurrence of potentially exploitable pathogens of weeds, and also as indicators of possible survey areas. Additionally, information obtained from herbarium records of fungi on target weeds have been proven to be very useful for selecting localities within survey areas, and examination of deposited plant material often provides useful information on the damage caused by pathogens to their hosts. An example of such a work, which provided the basis for many later weed biocontrol projects, can be found in Evans (1987a). Care should be taken, nevertheless, with the interpretation of such records, as the information available may be strongly influenced by the intensity of research activities of mycologists and plant pathologists in different areas of the world. Ironically, it often happens that a much larger mycobiota is known for a weed in a country where it is a noxious alien than where it is a native, simply because of its abundance. Clearly, the centre of origin of the target weed is the correct source for the potentially useful, specific (coevolved) biocontrol organisms. Those pathogens already existing in the areas where the plant has become a weed are likely to be either polyphagous organisms that already occurred in the exotic situations, or natural enemies that accompanied the weed at the occasion of its introduction or have reached the new areas later. In either case, such pathogens are likely to be of no interest for biological control, as these are not having enough impact on weed populations to mitigate the ongoing invasions. That is the case for weeds such as *L. camara*, which has a much larger mycobiota described from India – where it is ranked as one of the worst weeds – than for Brazil, where it is native and of relatively minor importance as a weed (Barreto *et al.*, 1995).
- Surveys in the area where the weed problem occurs may reveal a considerable diversity of plant pathogens already occurring in the new area of introduction of a weed. This was the case, for instance, of the survey of fungi associated with purple nutsedge (*Cyperus rotundus*) in Brazil. Although this sedge is native to the Old World, a total of seven fungal pathogens were collected in Brazil, including a previously undescribed species, *Ascochyta cyperiphthora* (Pomella and Barreto, 1997). Such surveys, although clearly of no relevance for searching for CBC agents, are valuable and fully justifiable to assess the overall mycobiota of the target weed, but there are few published examples (Barreto and Evans, 1995a; Winks *et al.*, 2003; Soares and Barreto, 2008b; Waipara *et al.*, 2008). These can help to avoid a later superfluous (and also wasteful and potentially embarrassing) introduction of natural enemies that may be already present but were ineffective, and hence remained unnoticed in the target area.
- Surveys can be carried out in the centre of origin. Stals *et al.* (2004) were of the opinion that taxonomists residing in the weed's country of origin know local conditions (biologically, geographically and politically) and are acquainted with the indigenous biota, and therefore are likely to possess in-depth knowledge valuable to biocontrol surveying. Unfortunately, for many of the regions of the world that are important sources of natural enemies for CBC, there are no such experts available for cooperation and the work needs to be performed entirely by teams from the country where the invasion is taking place, or by international organizations.
- Trap gardens, sentinel plants and similar

approaches have increasingly been used for a range of target weeds. Some recent examples are: blackberry (Scott *et al.*, 2004; Morin *et al.*, 2011), wandering Jew (Pereira *et al.*, 2008) and miconia weed (Seixas *et al.*, 2004). The principle is simple and consists, with variations, of cultivating and maintaining plants of the target-weed species (of the same biotype that is invasive in exotic situations) in selected areas in the centre of origin, and thus exposed to natural sources of arthropods or pathogens throughout a designated period of time. Such plants are then continuously observed and any natural enemies are collected for evaluation and further studies.

Taxonomy and life cycle

The taxonomic status of a CBC agent intended for introduction in an exotic situation is a basic requirement that should be met by any candidate before a request for importation is filed. Pathogens of dubious identity are, understandably, regarded as unacceptable for introduction into a country by quarantine authorities, as they may belong to a taxon known to have a broad host range and hence be unsafe for introduction. Modern classification of organisms based on phylogenetic information allows for generalizations that can be useful when a CBC agent candidate is being evaluated. For instance, members of the rust fungi Pucciniales are known to be specialized biotrophs that are highly host specific (although there are known exceptions), and thus are usually regarded as inherently safe for use in CBC, whereas oomycetes, such as members of the Peronosporales and Pythiales, are normally polyphagous pathogens that are regarded as unsafe for use in CBC. The level of detail in the characterization that is required for any pathogen chosen for use in CBC depends on many factors, such as the occurrence or not of significant genetic differences in virulence to the weed population in its area of introduction. If a CBC programme involves introducing new strains of a species of pathogen that is already associated with the weed in the exotic situation – for instance, more virulent, more prolific, better adapted to the environmental conditions or having another desirable trait – then it is critical that molecular tools are used in order to follow the fate of the new strain (Morin and Hartley, 2008).

One critical issue that needs to be clarified before the introduction of a CBC agent is its life cycle. The life cycle of a fungal pathogen can be relatively simple, many species being limited to their asexual stage (anamorph). At the other extreme, there are important weed biocontrol agents such as rust fungi (Pucciniales) that may have up to five different spore types and sometimes are heteroecious, requiring two unrelated host species to complete their cycle. Unravelling rust life cycles, therefore, can be challenging, particularly when spore stages appear to have become vestigial or redundant, and no longer have a function in the life cycle. To complicate things even further, it is not uncommon for rust species to have dormancy factors that are extremely difficult to break. For example, the sexual spores (teliospores) of the rust *Diabole cubensis*, released against the giant sensitive plant (*Mimosa pigra*) in Australia (Seier and Evans, 1996; Seier, 1998), rapidly enter a dormancy phase when dried, which has proven difficult to break and therefore must be processed when fresh if infective inoculum is required for host-range studies. This is also the case with rusts attacking the grassy weed *Nassella neesiana* (Anderson *et al.*, 2006, 2008a,b). Evans (1987b) found that the dormancy of teliospores of the rust *Puccinia abrupta* var. *partheniicola*, a pathogen of *Parthenium hysterophorus*, could only be broken by prolonged bleaching in concentrated hydrogen peroxide. Similar difficulties have also emerged during a study aimed at evaluating the ascomycete *Coccodiella miconiae*, a potential CBC agent for use against *Miconia calvescens* in Hawaii. Years of frustrating attempts were necessary until a method for harvesting viable infective inoculum of this fungus was finally developed (Alves, 2008).

Ecology

The validity of the assumption that the best geographical source of biocontrol agents for a weed is the region with the best climate match to the introduced range has been confirmed for insect agents on a number of occasions. Climate matching ranges in sophistication from intuition, to comparing climate graphs, to more quantitative comparisons using techniques such as the climate-matching function in CLIMEX (van Klinken *et al.*, 2002; Byrne *et al.*, 2004). Recent demonstrations of this approach have been provided by Grevstad *et al.* (2008) and Robertson *et al.* (2008). The latter used distribution records of *C. odorata* in South Africa and Swaziland to generate climatic similarity maps using CLIMEX (Sutherst and Maywald, 1999) and FloraMap. They then identified areas of the Neotropics with the most climatically similar pattern to the southern areas of Africa invaded by this composite weed. Nevertheless, as discussed by van Klinken *et al.* (2002), climate matching can be unreliable for widely distributed weeds such as *Prosopis* spp., and they concluded that new 'predictive tools are required because simple climatic matching that do not account for the unique climatic requirements of individual species are an unreliable predictor of where agents will do best'.

The contribution of ecology to biological control has been modest in the past. However, the expectations for the future are that better monitoring at each stage in the development of a programme, the use of appropriate mathematical and experimental models and a closer alliance between basic biocontrol research and implementation will lead to models that more faithfully attend to the details of life histories and the mechanisms governing encounters between natural enemies and their hosts (McEvoy, 2004).

For fungal pathogens, emphasis is usually placed on matching of fungal isolates or pathotypes from the centre of origin with biotypes of the weed in the introduced area. This was first highlighted when the skeleton weed rust was released in Australia and successfully controlled the target weed (Cullen *et al.*, 1973). However, it soon came to light that other cryptic biotypes were also present, which became dominant because of their resistance to the introduced rust pathotype, and which originated from a different native range in the drier, more eastern region of the Mediterranean (Hasan, 1981). Similar experiences have also been reported during the initial release phase of the rubber vine rust (*Maravalia cryptostegiae*) when the 'wrong' pathotype was imported into Australia (Evans, 2000).

An important aspect that needs to be considered, particularly for CBC of aquatic weeds, is the effect of excess nutrients in the water bodies where the target populations are occurring. High levels of nitrogen and phosphate may lead to abnormally high growth rates and increased vigour, leading to decreased effectiveness of otherwise good agents, as demonstrated for insects released to control water hyacinth growing in nutrient-rich ecosystems (Brudvig *et al.*, 2008). In such situations, it is unrealistic to expect that biological control will mitigate weed infestations without prior control of the main source of imbalance, which is water pollution.

Potential effectiveness

Cullen (1995) discussed the feasibility of predicting successful control for any particular agent by establishing rules and scoring systems, but this does not account for the importance of exceptions, which cannot be predicted on the basis of available data. He concluded that, for complex systems such as agent–weed interactions, a better approach is to address the questions relating to agent effectiveness, which are the damage produced by the agent and the ecology of both the agent and the target weed. However, even this does not take into account the potential impact of the release of the invasive species from coevolved endophytes (Evans, 2008), nor the release of the selected agent from its own suite of natural enemies. Since the first system of

ranking potential biocontrol candidates based on their attributes was proposed – for insects by Harris (1973) – little has been achieved. Previously, Cullen (1992) was pessimistic about such attempts and regarded them as a continuous source of frustration and a waste of resources, and considered that many biocontrol workers regard them as useless and would rather trust agent release as the sole valid method for testing an intended biocontrol organism's potential. For pathogens, an evaluation of CBC potential is, in practice, based on a combination of: (i) observation of disease severity as produced by the pathogen in natural conditions; (ii) demonstration, under controlled conditions, of the pathogenicity of the organism to the biotype(s) of the target weed from the areas being invaded; (iii) knowledge available on the taxonomic group to which the candidate belongs – priority being given to potential agents belonging to groups of pathogens with a good track record in terms of efficacy; and (iv) safety (host specificity). Members of the Pucciniales, when available, are often given top priority (Barreto, 2009).

Host specificity

The inherent safety of coevolved natural enemies has been emphasized by Marohasy (1996) who analysed the case histories of over 600 insect biocontrol agents which have been used for CBC of weeds. Evans (2000) stated that 'in only a few instances was there evidence of host-range expansion, sometimes referred to as host "shift" and of collateral damage to non-target plants; none of which had serious economic or environmental impacts.' All of these 'shifts' were predictable behavioural responses and not the result of proven genetic changes. In comparative terms to chemical control, one of the major advantages of biological control is its evolutionary stability. In coevolved associations, the natural enemy adapts to genetic changes of the host and tends to have a fluctuating but sustainable impact over its host population. On the other hand, Holt and Hochberg (1997) regard chemical pesticides as 'evolutionarily evanescent'. Eventually, resistance to a pesticide emerges, rendering the method non-sustainable. This is readily noted by checking the subject of herbicide resistance in the weed science literature. For example, a search of CAB Abstracts resulted in 1713 references since 2002 that cited herbicide resistance. Evans (2000) discussed the arguments for and against biocontrol that appeared in the literature during the 1990s and concluded that most attacks against CBC of weeds referred to a single case involving the introduction of a weevil agent into North America that resulted in non-target effects on native thistles. However, this is a questionable example to use, as the non-target effects were predicted from previous host-specificity studies. The release was a political rather than a scientific judgement. Nevertheless, the unfortunate media coverage that followed has been negative for CBC, in general. What this example emphasizes is the need to consider non-target effects more stringently. However, a stricter screening by biocontrol scientists would not necessarily prevent ill-founded political decisions by government authorities, or the illegal and deliberate introduction of agents by independent 'stakeholders', which has been problematic for the discipline in the past.

Plant pathogens with wide host ranges are not considered for CBC, as a high level of host specificity is always required. The level of specificity will depend, however, on the flora present in the area of release. For example, the Hawaiian archipelago has no native members of the plant family *Melastomataceae*, while some of the worst environmental weeds in Hawaii belong to this family: *Clidemia hirta*, *M. calvescens* and *Tibouchina herbacea*. Therefore, pathogens specific to melastomes at the family level have been considered for introduction into Hawaii. Similarly, there are no native members of the *Commelinaceae* in New Zealand allowing a less rigorous requirement for host specificity of CBC agents to be introduced against the herbaceous environmental weed *Tradescantia fluminensis*. Barton (2004) closely examined the safety of fungal pathogens used as

biocontrol agents and found not a single example of non-target impact for species introductions that have taken place since fungi started to be used as CBC agents. Subsequently, Barton (2011) re-visited the subject and, of the 28 CBC projects involving the release of fungal pathogens, several non-target impacts were identified. However, all were predicted in the risk assessment (Barton, 2011). This excellent safety record is due to the early application of a protocol developed by entomologists for CBC, which involves host-range screening as a cornerstone for pest risk assessment: the centrifugal phylogenetic host-range test (CPHRT; Wapshere, 1974, 1989).

Growing concerns about the potential risk of non-target effects is leading to more stringent host-specificity testing (Groenteman *et al.*, 2011), as well as to variations in the original design of CPHRT. In the USA, for example, introductions of CBC agents since 1957 have been controlled by the USDA and each introduction has been evaluated by outside agencies before further implementation. This has been progressively broadened and now involves 17 organizations, as well as counterparts in Canada and Mexico. One of the main concerns is the selection of plant species to include in host-specificity tests (Cofrancesco, 2008). In more recent times, there has been a significant improvement thanks to advances in phylogenetic classification of plants, allowing the compilation of more pertinent lists of plant species to be included in CPHRT (Kelch and McClay, 2004).

Charudattan *et al.* (2008) recently validated the centrifugal phylogenetic approach to host-range testing for the first time for a virus, tobacco mild green mosaic virus, which was being tested for use as a bioherbicide against *Solanum viarum*. Their results confirmed that plant pathogenic viruses also obey a host specialization following a centrifugal phylogenetic design.

The scope of the centrifugal phylogenetic tests required by quarantine authorities for the introduction of pathogens has varied over time. For some of the pioneering introductions, relatively few plant species were tested with no real awareness of the centrifugal phylogenetic concept (Trujillo, 1975, 1985; Oehrens and Gonzales, 1975; Oehrens, 1977). In contrast, the introduction of *P. chondrillina* in Australia was undertaken only after it had passed a CPHRT evaluation, and therefore this is probably the first example of a structured approach to screening of weed pathogens for CBC.

Biocontrol scientists have been worried about the possibility of rejection of safe and high-potential weed biocontrol candidates because of expanded host ranges, the so-called false positives, resulting from abnormal laboratory conditions (Wapshere, 1989). Antonini *et al.* (2008) described the novel use of molecular tools to distinguish between morphologically indistinguishable populations of an insect species that are, nevertheless, genetically distinct and have different degrees of host specificity. One recent example of the application of this approach is that of an incorrect rejection of the insect *Ceratapion basicorne*, a potential biocontrol agent for yellow star thistle (*Centaurea solstitiali*) in the USA. Although rejected as unsafe 15 years ago, recent field-based experimental studies have shown that this insect is host specific and not a threat to non-target plants (Smith *et al.*, 2008). Such tools may also be used for pathogens where difficulties in morphological distinction are even more common, as was done for the rust complex associated with the same host (Luster *et al.*, 2004).

Complementary observations of plant–pathogen interactions at the microscopic level, in addition to the traditional macroscopic assessment, is now becoming standard in programmes involving fungi. For example, based on an interpretation of fungal–plant interactions – both on and within host and non-host tissues, using clear staining and scanning electron microscopy (Evans and Tomley, 1994) – Australian authorities did allow the rust *M. cryptostegiae* to be released for the control of rubber vine weed (*Cryptostegia grandiflora*), despite the fact that it was shown to infect (albeit mildly) a rare native asclepiad in greenhouse tests. Predictions

that the rust would not attack this species in the field situation – based on wind-tunnel tests for better simulation of natural infection – have proved to be correct, and the rust has been restricted to and has successfully controlled its target host (Evans and Tomley, 1994, 1996; Evans, 1995, 2000; Tomley and Evans, 2004).

Importation – paperwork

The following documentation is usually required for the importation and export of CBC agents:

- *Dossier on candidate agent*. This contains all the information accrued on an agent.
- *Export document*. In line with the Convention on Biological Diversity of 1992 (Convention on Biological Diversity, 2011), an agreement from the agent source country must be obtained.
- *Phytosanitary certificate*. This states that the shipment is pure and contaminant free.
- *Record keeping and voucher specimens*. All agents used in CBC must have an official record that the agent has been imported from the source country and a voucher specimen deposited in an official collection. With pathogens, it is usually possible to preserve this specimen alive. This can act as a source for any necessary re-establishment of an agent in the field or additional introductions into new countries, and can aid the settling of any possible disputes if the genome is sequenced. Preferably, the organism should also be deposited in an official collection in the country of origin.

The shipment box should have the following documentation securely attached to the outside, but accessible, and also a copy of each must be placed within the outer packaging:

- *Import permit*. This must contain the following information: exact identity of agent, reason for import, quantity to be imported, source of material, details of certificates of purity, information on packaging, instructions on routing, instructions on procedures on arrival and any special precautions to follow, and details of contact persons in exporting and importing countries.
- *Phytosanitary certificate*. Ironically, this may state that the consignment is free from infectious organisms. In effect, it is a guarantee that the consignment is free from contaminants, such as hyperparasites.
- *Labelling consignments*. The outside of the shipment container must have the following information clearly visible: the address of the recipient and sender of the package, instructions on handling and storage in transit, a warning not to open except in quarantine and any additional labels required by the receiving authority.

Release and establishment

Although the rate of establishment of CBC agents is presently ranked as being as high as 80–90%, Fowler *et al.* (2008) acknowledged that there is a general lack of fundamental experimental science following weed biocontrol releases. These authors suggested that initial releases should be treated as opportune experiments where predictions from ecological theory and retrospective studies could be tested. Although the authors were referring to arthropods, the situation for plant pathogens is no different. Methodologies for release vary for each project and depend on the biology of the agent and the resources available. Obligate biotrophic pathogens, such as rust fungi, are sometimes introduced through susceptible individual plants that are previously infected with the pathogen and taken to selected areas in the field, particularly where dense infestations of the weed are present. Culturable pathogens, and some biotrophs that produce large quantities of infective powdery spores *in vivo* (e.g. urediniospores of certain rust fungi), are sometimes introduced inundatively by spraying of spores or other

propagule suspensions on to target plants in selected areas, particularly where there is a need for urgent action against the weed and thus speeding up of the release phase (Tomley and Evans, 2004). Timing is an important factor for success, as, ideally, release of a pathogen should be made when susceptible tissue of the host is available for inoculation and the environment is also favourable for infection. Tomley *et al.* (2004) discussed the release strategies and factors affecting the establishment of four rust fungi introduced into Australia as CBC agents against weeds, namely: (i) *P. abrupta* var. *partheniicola* and *Puccinia melampodii* against *P. hysterophorus*; (ii) *M. cryptostegiae* against *C. grandiflora*; and (iii) *Prospodium tuberculatum* against *L. camara*. These authors also highlighted the difficulties imposed by exceptionally dry weather or unseasonal drought that followed the release of the rusts against *L. camara* and *P. hysterophorus*. A recent example of inundative release of a CBC agent followed by successful establishment was that of *Mycovellosiella* (= *Passalora*) *lantanae* var. *lantanae* in South Africa against *L. camara* (Den Breeyen, 2004). In this case, a combination of isolates that were virulent to a range of *L. camara* biotypes were applied as oil-based sprays.

Impact assessment

Standardized monitoring protocols that evaluate the impact of biocontrol agents on target weed and associated plant and animal communities are important but underutilized tools to improve the scientific basis of CBC. We need to understand: (i) the interactions of control agents, target weeds and native plant communities; and (ii) the contribution of species attacking different plant parts and the impact of single and multiple species releases on plant performance and weed populations, in order to improve the success rate and to increase the predictability of biocontrol programmes (Blossey and Skinner, 2000). The investment in post-release evaluation in CBC programmes is known to be minimal as, paradoxically, funding agencies tend to show little interest in monitoring the effectiveness of biocontrol agents. As a result, few published evaluations are available, and these are often limited to subjective anecdotal assessments (Crawley, 1989). There are, nevertheless, protocols that allow an objective evaluation of the effect that the release of a CBC agent has on a weed population. Well-established methods have been developed by plant ecologists, such as transect sampling, which combines evaluations of weed density and biomass (above and below ground) and seed production (Blossey and Skinner, 2000). Nevertheless, such traditional methods can be time-consuming, costly and labour-intensive. There are now modern alternatives, however, which combine global positioning system (GPS) and geographic information system (GIS) analysis, for example, as described by Carney *et al.* (2008), which will tend to replace traditional methods and so improve the precision, while significantly reducing costs and hopefully therefore favouring the funding of such post-release studies.

Hoffmann and Moran (2008) recently discussed the mistaken expectations commonly surrounding CBC of weeds, which have led to the evaluation of any introduction that did not lead to almost complete extermination of the target as a failure. Although there are well-documented examples of agents that did cause extreme reductions in their host – as in the case of the rusts used against skeleton weed, bridal creeper, rubber vine and golden wreath wattle – often referred to as 'silver bullets', the majority of agents have not had this level of impact. Hoffmann and Moran (2008) suggested that one way of ensuring that agents are not discredited unnecessarily is to ask, 'What would the situation have been without biological control?' rather than 'What has been achieved?' They provided evidence from a South African programme against the cactus weed *Opuntia stricta* which showed that prolonged monitoring can reveal cryptic but real benefits from CBC. The case of the biocontrol

programme against *P. hysterophorus* in Australia represents a good example of a poorly recognized partial success of CBC. Dhileepan (2007) demonstrated through experiments involving the exclusion of biocontrol agents (insects and two rust species), which were introduced into Australia, that the impact of parthenium weed infestations on grass production was significantly more severe when insects and rusts were controlled. Both insects (particularly, the weevil *Zygogramma bicolorata*) and the rust *P. melampodii* contributed to parthenium weed control. Even experienced biocontrol scientists tend to expect a 'silver-bullet' effect from their agent releases and tend to become frustrated if highly significant control is not obviously evident, often within an unfeasibly short period of time. Tomley *et al.* (2004), for instance, regarded the impact resulting from the introduction of *P. melampodii* into Australia as uncertain, but subsequent monitoring is revealing the real benefits (Dhileepan, 2007).

Few economic impact assessments of biocontrol projects have been undertaken in over 100 years of CBC of weeds and all have been on highly successful programmes. For Australia, a recent economic impact assessment of weed biocontrol since 1903 yielded an annual benefit of AU$95.3 million against an annual investment of AU$4.3 million, a cost:benefit ratio of 1:22. The analysis showed the great economic benefit of 14 highly successful programmes, but, surprisingly, indicated that two other programmes usually regarded as failures were in fact highly beneficial in economic terms because the apparent low impact on these important weed populations was sufficient to result in significant savings (McFadyen, 2008). The author went on to recommend that advice from economists should be sought routinely at all stages of a biocontrol programme.

For environmental weeds, economic evaluation of benefits is notoriously difficult to establish. However, an assessment of ecological benefits, in terms of reduction in biomass of the weed and increased species richness in infested areas where the agent was introduced, should be normal practice. Examples where this has been undertaken for weed biocontrol programmes include: (i) mistflower (*Ageratina riparia*) in New Zealand (Barton *et al.*, 2007); (ii) rubber vine (*C. grandiflora*) in Australia (Vogler and Lindsay, 2002; Tomley and Evans, 2004); and (iii) bridal creeper (*Asparagus asparagoides*) in Australia (Morin *et al.*, 2002; Reid and Morin, 2008). In the latter programme, evidence is already emerging from post-release studies of the recovery of two Australian plant species that are highly endangered – *Pimelea spicata*, a small native shrub, and the rare orchid *Pterostylis arenicola* – partly because of ecosystem invasion by this South African weed (Willis *et al.*, 2004). Similarly, a detailed study on the impact of the release of a fungus against *M. calvescens* in Tahiti on populations of *Ophiorrhiza subumbellata*, a rare endemic plant that is threatened by miconia infestations, have demonstrated that defoliation of miconia caused by the fungus was accompanied by an increase in fertility and the number of seedlings and juvenile individuals of the native species (Meyer and Fourdrigniez, 2011). A recent economic assessment of the contribution of weed CBC on ecosystem services was undertaken in South Africa. Rather than looking at the effect of individual invasive alien weeds, the authors attempted a more holistic approach by evaluating the benefits of controlling groups of weed species that exert a similar impact on and can substitute for each other in ecosystems (de Lange and van Wilgen, 2010). They estimated that the general savings for South Africa as a result of past weed biocontrol programmes amounted to around US$21 billion. The cost:benefit ratios for CBC of individual weeds ranged from 1:50 for invasive subtropical shrubs up to 1:3726 for invasive Australian trees. The authors concluded, therefore, that CBC plays an important role in the protection of ecosystem services.

11.2.4 Progress in CBC of weeds with plant pathogens

Existing programmes

An updated list of weed CBC projects involving the use of pathogens since the pioneering projects in the early 1970s is provided in Table 11.2. Twenty-eight plant species (with one species complex included, in the case of *Rubus fruticosus*; Morin *et al.*, 2006b) have been targeted in programmes that actually led to the release of a pathogen into an exotic situation. Several others were conducted but were interrupted before an introduction took place or have not yet reached the stage of introduction. Although some nematode species have been studied in detail, and indeed show potential for use in CBC of weeds, until now the only group of pathogens to be introduced have been the fungi. Twenty-seven species have been introduced into 12 countries in all inhabited continents, with the stark exception of Europe, as well as in several archipelagos. The distribution of introduction of fungal CBC agents among countries, in order of frequency, is: Australia (12 species), South Africa (five species) and the USA – seven species in Hawaii compared with three species in continental USA. This reflects the early lead that these countries had in the history of CBC. In contrast, eight of the other countries had only one or two introductions. It is worth noting the important part played by organizations such as CAB International (on a worldwide basis), Commonwealth Scientific and Industrial Research Organization (CSIRO) and Queensland Department of Lands (Australia), PPRI (South Africa), HDOA (Hawaii, USA), APHIS (continental USA) and Landcare Research (New Zealand) in the development of the programmes that led to these fungal introductions. In some instances, individual scientists are to be credited for their leadership and initiative in the discipline. By way of example, E. Oehrens (Universidad Austral de Chile) conducted – almost single-handedly – two of the three introductions of fungi for CBC of weeds that took place in Latin America, while E. Trujillo (University of Hawaii) led the introduction of four of the seven introductions that took place in Hawaii, showing that this is a field where both institutional involvement and individual determination can make a difference. There is still plenty of room for expansion in the use of CBC of weeds throughout the world. Most countries are not familiar with this approach, and inaugurating the discipline, as well as educating both the public and the authorities, may be challenging. The recent example of successfully piggy-backing on the experiences of the introduction of a rust fungus into India, followed by its subsequent introduction into PR China, Fiji, Papua New Guinea and Taiwan against the highly invasive mikania weed (Ellison and Day, 2011), is an elegant example of how the discipline could be expanded at minimal cost on a worldwide scale.

Recent examples and updates of ongoing programmes

BRAZILIAN PEPPER TREE The Brazilian pepper tree (*Schinus terebinthifolius*, *Anacardiaceae*) is a small tree native to southern South America. In Brazil, this species is generally not regarded as a noxious weed. In contrast, it is valued for its culinary and medicinal uses (Lorenzi, 1992; Lorenzi and Matos, 2002). The situation is very different in many regions of the world where it has been introduced, particularly in oceanic islands and the USA (mostly Hawaii and Florida) (Cronk and Fuller, 1995), where it invades natural habitats, displacing native herbaceous communities and leading to loss of local biodiversity (Cuda *et al.*, 2006). Biological control was recognized at an early stage as the ideal strategy for managing *S. terebithifolius* and the initial searches for insect natural enemies were led by US entomologists who conducted surveys in Brazil and Argentina (Hight *et al.*, 2002). Several promising insects were found and eventually introduced into Hawaii (Yoshioka and Markin, 1991), but problems related to the existence of highly variable

Table 11.2. Classical biological control programmes of weeds involving the introduction of exotic pathogens.

Target weed	Pathogen species	Country/ continent of origin	Country of introduction	Results attained[a]	Reference(s)
Acacia saligna	*Uromycladium tepperianum*	Australia	South Africa	Sig.	Morris (1987); Lennox *et al.* (2004)
Ageratina adenophora	*Phaeoramularia eupatorii-odorati*	Mexico	South Africa	Part./EI	Morris (1989); A.K. Wood (2008, personal communication)
Ageratina riparia	*Entyloma ageratinae*	Jamaica	USA (Hawaii)	Sig.	Morin *et al.* (1997); Fröhlich *et al.* (1999); Trujillo (2005); A.K. Wood (2008, personal communication); Barton and Fowler (2008)
			South Africa	EI	
			New Zealand	Sig.	
Asparagus asparagoides	*Puccinia myrsiphylli*	Brazil	Australia	Sig.	Morin *et al.* (2002, 2004, 2006c)
Baccharis halimifolia	*Puccinia evadens*	USA	Australia	Sig.	Verma *et al.* (1996); Tomley and Willsher (2002)
Carduus nutans	*Puccinia carduorum*	Turkey	USA (continental)	Sig.	Politis *et al.* (1984); Baudoin *et al.* (1993); Bruckart (2005)
Carduus pycnocephalus/ Carduus tenuiflorus	*Puccinia cardui-pycnocephali*	Italy, France	Australia	Sig.	Burdon and Thrall (2002)
Centaurea solstitialis	*Puccinia jaceae* var. *solstitialis*	France	USA (continental)	Rec.	Fisher *et al.* (2007); California Department of Food and Agriculture (2011)
Chondrilla juncea	*Puccinia chondrillina*	Italy	Australia/USA (continental)/ Argentina	Sig./Part./ EI	Hasan (1972); Cullen *et al.* (1973); Emge *et al.* (1981); Supkoff *et al.* (1988); Julien and Griffiths (1998)
Clematis vitalba	*Phoma clematidina*	USA	New Zealand	NE	Gourlay *et al.* (2000); S. Dodd (2008, personal communication)
Clidemia hirta	*Colletotrichum gloeosporioides* f. sp. *clidemiae*	Panama	USA (Hawaii)	Part.	Trujillo *et al.* (1986); Trujillo (2005)
Cryptostegia grandiflora	*Maravalia cryptostegiae*	Madagascar	Australia	Sig.	Tomley and Evans (2004)
Eichhornia crassipes	*Cercospora piaropi*	United Arab Emirates	South Africa	Part.	Conway and Freeman (1977); Morris *et al.* (1999)
Galega officinalis	*Uromyces galegae*	France	Chile	EI	Oehrens and Gonzales (1975)
Heliotropium europaeum	*Uromyces helitropii*	Turkey	Australia	EI	Hasan *et al.* (1992, 1995)
Hieracium pilosella	*Puccinia hieracci* var. *piloselloidarum*	Ireland (via Australia)	New Zealand	L[b]	S. Dodd (2008, personal communication)

Table 11.2. Continued.

Target weed	Pathogen species	Country/ continent of origin	Country of introduction	Results attained[a]	Reference(s)
Lantana camara	*Septoria* sp.	Ecuador	USA (Hawaii)	Part.	Barreto *et al.* (1995); Trujillo and Norman (1995); Tomley and Riding (2002); Den Breeÿen and Morris (2003); Den Breeÿen (2004)
	Prospodium tuberculatum	Brazil	Australia	EI	
	Mycovellosiella lantanae var. *lantanae*	South America	South Africa	Rec.	
Miconia calvescens	*Colletotrichum gloeosporioides* f. sp. *miconiae*	Brazil	USA (Hawaii)	Part.	Killgore *et al.* (1999); Meyer and Killgore (2000); Meyer *et al.* (2008); Meyer and Fourdrigniez (2011)
			French Polynesia	Part.	
Mikania micrantha	*Puccinia spegazzinii*	Trinidad	India, China, Fiji, Taiwan, Papua New Guinea	Rec.	Ellison *et al.* (2008); Sankaran *et al.* (2008); Day *et al.* (2010)
Mimosa pigra	*Diabole cubensis*	Mexico	Australia	EI	Seier and Evans (1996); Evans (2000)
Mimosa pigra	*Sphaerulina mimosae-pigrae*	Mexico	Australia	EI	Forno *et al.* (1996); Seier and Evans (1996); Evans (2000)
Myrica faya	*Septoria hodgessi*	USA (continental – North Carolina)	USA (Hawaii)	NE	Gardner *et al.* (1999); E. Killgore (2008, personal communication)
Parthenium hysterophorus	*Puccinia abrupta* var. *partheniicola*	Mexico	Australia	EI	Parker *et al.* (1994); Evans (2000)
	Puccinia melampodii	Mexico	Australia	EI	Evans (2000); Dhileepan *et al.* (2006)
Passiflora tarminiana	*Septoria passiflorae*	Colombia	USA (Hawaii)	Sig.	Trujillo *et al.* (1994); Trujillo (2005)
Rubus constrictus/ Rubus ulmifolius	*Phragmidium violaceum*	Germany	Chile	Sig., EI	Oehrens and Gonzales (1977)
Rubus fruticosus agg.	*Phragmidium violaceum*	France	Australia	Part.	Bruzzese and Hasan (1986); Scott *et al.* (2002); Evans *et al.* (2004)
Ulex europaeus	*Uromyces genistae-tinctoriae* f. sp. *ulicis*, *Uromyces pisi* f. sp. *europaei*	UK	USA (Hawaii)	NE	Gardner *et al.* (1996); E. Killgore (2008, personal communication)

[a]Sig., Significant levels of control; Part., partial control; EI, established but levels of control achieved insignificant; NE, agent not established; Rec., recent introduction (evaluation still regarded as impossible at the present stage); L, information lacking or contradictory.
[b]Agent spread spontaneously from Australia without known human interference.

weed populations or even cryptic species, particularly in Florida, broadened the search for CBC agents to include pathogens. A diverse pathogenic mycobiota was unravelled during the subsequent surveys (Faria *et al.*, 2008; Macedo *et al.*, 2010). These are still being documented and include several new fungal species, two of which (*Septoria* sp. and *Corynespora* sp.) are of particular interest. Both cause leaf spots and lead to intense defoliation of infected trees in the field. Additionally, a complex of dieback fungi has been documented. Although pathogenicity tests showed *Septoria* sp. to be infective to the biotypes of *S. terebinthifolius* that are present in Florida, host-range studies showed later that it infected a rare native plant in the USA (*Rhus michauxii*). Although this was regarded as negative for the introduction of the fungus, it remains to be determined whether, under natural conditions, *R. michauxii* would become infected.

BRIDAL CREEPER Bridal creeper (*A. asparagoides*, *Asparagaceae*) is a climbing vine that is particularly problematic environmentally, as it can significantly disrupt the ecology of natural ecosystems (Morin *et al.*, 2006c). In a reverse situation to Port Jackson willow (discussed below), bridal creeper is a South African endemic that has invaded Australia. The coevolved natural enemies introduced so far from South Africa, especially the rust *Puccinia myrsiphylli*, have established widely and are rapidly reducing the density of weed infestations (Morin and Edwards, 2006), with evidence of synergism between pathogen and insect agents (Turner *et al.*, 2010). An economic impact assessment is not available thus far, although there is a long-term, before-and-after monitoring system in place to generate such data (Palmer *et al.*, 2010).

GIANT HOGWEED Giant hogweed (*Heracleum mantegazzianum*, *Apiaceae*), which is native to the Caucasus and is invasive in many parts of Europe and the USA, poses a risk not only to biodiversity but also to public health due to its photosensitizing sap, which causes burns to affected skin upon UV exposure (Dodd *et al.*, 1994; Tiley *et al.*, 1996). The plant was assessed as a target for biological control under the framework of a European Union-funded multidisciplinary project in 2002–2005. The fungal pathogens *Phloeospora heraclei* and *Septoria heracleicola*, as well as species of *Phomopsis* and *Ramulariopsis*, associated with giant hogweed in its native range, were considered to have potential as CBC agents. An initial host-specificity assessment under greenhouse conditions revealed that each of the pathogens infected and sporulated on selected commercial varieties of parsnip (*Pastinaca sativa*). *P. heraclei*, perhaps the most promising agent, as it is especially aggressive to the seedling stage, also infected commercial varieties of coriander (*Coriandrum sativum*) (Seier and Evans, 2007). However, the European native common hogweed (*Heracleum sphondylium*), a reported host of these pathogens, was not attacked (Allescher, 1903; Adamska, 2001; Seier and Evans, 2007). It was postulated that distinct strains or pathotypes of these fungal species exist that are adapted and specific to different *Heracleum* spp. This hypothesis is supported by results of a recent cross-inoculation study using distinct accessions of *P. heraclei* collected from *H. mantegazzianum* and *H. sphondylium* (M.K. Seier, unpublished observations). None the less, the ability to attack commercial varieties of certain garden crops precludes any of the assessed pathogens from immediate consideration as a CBC agent for giant hogweed, given Europe's current lack of experience with this management strategy (Cock and Seier, 2007).

HIMALAYAN BALSAM Himalayan balsam (*Impatiens glandulifera*, *Balsaminaceae*) is an invasive annual herb native to the western Himalayas, which was introduced into Europe in the mid-1800s as an ornamental plant and has now extensively invaded riparian habitats and damp woodlands throughout the UK and parts of mainland Europe. Since 2006, a series of

surveys has been undertaken in the native range in both India and Pakistan to identify suitable natural enemies to introduce into the UK. A number of insect species that might have been potential CBC agents were rejected due to the high levels of non-target feeding in choice tests, and a *Septoria* leaf-spot pathogen was also rejected after it was found to infect some ornamental plant species of *Impatiens*. However, a highly damaging, autoecious rust pathogen *Puccinia* cf. *komarovii* has been selected for further assessment, and the preliminary results are regarded as extremely promising (Tanner *et al*., 2008).

ITCH GRASS Itch grass (*Rottboellia cochinchinensis*, *Poaceae*) is an Old World annual grass, with seeds as the only means of propagation and a short-lived seed bank (3–4 years). It is now pan-tropical in occurrence and is regarded as one of the world's worst agricultural weeds. It is particularly aggressive in Latin America and the Caribbean Islands where it causes serious yield reduction in both perennial and annual crops. The weed was first targeted for biocontrol using a myco-herbicide based on an anthracnose leaf disease (*Colletotrichum* sp.) in the mid-1980s (Ellison, 1992), but it did not show sufficient efficacy under field conditions for commercial development. More recently, work by Alloub *et al.* (2009) using the hyphomycete *Exserohilum prolatum* has shown more promise. This weed has also been targeted for CBC, and two potential fungal agents have been evaluated: (i) the head smut *Sporisorium ophiuri* (Reeder *et al*., 1996; Reeder and Ellison, 1999); and (ii) the leaf rust *Puccinia rottboelliae* (Ellison and Bird, 1996). The smut occurs in Asia and Africa, whereas the rust appears to be restricted to Africa. Both species are highly host specific and exist as a range of special forms, or pathotypes, which have coevolved with and are restricted to specific grass biotypes. Molecular studies have determined the geographical origins of the weed infestations and thus it is possible to match biotype(s) to pathotype(s). The smut is a soil-borne pathogen, infecting itch grass seedlings before they emerge from the soil via germinating teliospores. The infection is systemic, and the fungus is able to invade the flower primordia, resulting in virtually no seed set. Seed heads are converted into columns of powdery black teliospores, which are shed into the soil to infect the next generation of seedlings (Ellison and Evans, 1995). The monocyclic nature of this smut (one disease cycle a year) could restrict the potential efficacy of this pathogen (Smith *et al*., 1997). However, Smith *et al.* (2001) later demonstrated that, under integrated pest management, the smut could have a significant impact with only 50% infection of a weed population. Reeder and Ellison (1999) proposed the introduction of a strain of the smut from Madagascar into Costa Rica following the successful completion of a Department for International Development (DIFID, UK)-funded, collaborative programme between Centro Agronomico Tropical de Investigacion y Enseñanza (CATIE) in Costa Rica and CAB International and the Natural Resources Institute (NRI, UK) (Sánchez-Garita, 1999). Although the smut was cleared for introduction into Costa Rica by the quarantine authorities (Sanidad Vegetal), due to an unfortunate combination of institutional and funding issues, the smut was not released. This would have been the pioneering CBC project for Central America. It is unfortunate that, despite the declared interest of the Costa Rican government in release of the smut, the programme is still stalled. Potentially, however, it could be resuscitated by other Latin American countries affected by this increasingly important invasive weed, and at relatively little cost.

Investigation of the rust *P. rottboelliae* has been less extensive than the smut (Ellison and Bird, 1996). Two rust isolates from Kenya were assessed, and seedlings were found to be highly susceptible and were often killed after only one application. A limited host-specificity test of a number of graminaceous crops indicated that the rust is specific to *R. cochinchinensis.* There was variation in the susceptibility of the 33 different biotypes of itch grass tested.

Unfortunately, although nearly 80% of these were susceptible to at least one of the two Kenyan isolates, the rust only moderately infected biotypes of the weed from Latin America and the Caribbean Islands. However, it was interesting to note that there was significant variation between the two isolates in the susceptibility score of the weed biotypes. This suggests that it would be valuable to test a broader range of rust isolates in order to find one or more strains that might be highly virulent to those biotypes established in Latin America.

JAPANESE KNOTWEED Japanese knotweed (*Fallopia japonica*, *Polygonaceae*) was introduced into Europe as an ornamental in the early 19th century and, following an initial lag period, has spread exponentially during recent decades (Djeddour *et al.*, 2008; Shaw *et al.*, 2009). Currently, the plant is widely considered as the most troublesome invasive alien weed, impacting severely on native biodiversity, as well as on local economies in the UK (Bailey and Conolly, 2000; Gerber *et al.*, 2008). *F. japonica* is an equally problematic alien invader in the USA (Forman and Kesseli, 2003). A CBC programme against this weed, funded by a UK consortium, was initiated in 2002, and surveys for natural enemies were undertaken throughout Japan from 2002 to 2005 (Kurose *et al.*, 2006). Besides several arthropods, three damaging fungal pathogens were identified as potential CBC agents: (i) an aecidial rust that is prevalent early in the season; (ii) a highly damaging uredinial/telial rust present later in the season; and (iii) a ubiquitous, highly damaging *Mycosphaerella* leaf-spot fungus (Kurose *et al.*, 2006). Initially, it was assumed that the observed rust infections constitute different stages of one autoecious species. However, more detailed field observations and experimental data revealed that, in fact, they represent two distinct heteroecious taxa. A search of the Japanese literature revealed the aecial rust to be *Puccinia phragmitis*, while the uredinial rust was identified as *Puccinia polygoni-amphibii* var. *tovariae*. Although questions about their life cycles remain, both rusts were dismissed as biocontrol agents, first, because they require alternative hosts and, secondly, because their ability to survive the winter period in the UK is questionable (Kurose *et al.*, 2006). Furthermore, urediniospores of *P. polygoni-amphibii* var. *tovariae* were shown to infect and produce viable inoculum on the UK native *Rumex longifolius* and the ornamental *Fallopia baldschuanica* or Russian vine (Kurose *et al.*, 2006; Djeddour *et al.*, 2008). The causal agent of the damaging leaf-spot disease observed in the field in Japan was identified as *Mycosphaerella polygoni-cuspidati* (Kurose *et al.*, 2009). This hemibiotrophic pathogen cycles only through its sexual stage, with ascospores being the infective propagules. Infection remains symptomless for up to 15 days, when discrete chocolate-brown spots start to develop leading to widespread leaf necrosis, leaf distortion and premature defoliation (Kurose *et al.*, 2009). Field observations indicated a high host specificity of *M. polygoni-cuspidati*, and this assumption has been supported by the results of greenhouse host-range studies (Djeddour *et al.*, 2008). This pathogen is a highly promising candidate for biocontrol of *F. japonica* and, following future approval of a mandatory pest-risk assessment by the relevant authorities, could become the first exotic fungus deliberately released for weed biocontrol in Europe. In terms of CBC for Japanese knotweed, *M. polygoni-cuspidati* would thereby follow in the footsteps of a recently released exotic insect agent against this weed in the UK (the psyllid, *Aphalara itadori*; Shaw *et al.*, 2009; Morelle, 2010).

LANTANA Lantana (*Lantana camara*, *Verbenaceae*) is a spiny and toxic shrub, common throughout its native range in the Americas, which was distributed throughout the tropics as an ornamental and quickly escaped to become one of the world's ten worst weeds (Holm and Herberger, 1969). It is of limited importance as a weed in its native range, where it is attacked by a plethora of natural enemies. It has been the target of CBC for over 100 years, but it was only more recently

that pathogens have been investigated for use as CBC agents (Barreto *et al.*, 1995). There have been more than 40 natural enemies released over the last century to try and achieve control of lantana in its invasive, paleotropic range (Day and Zalucki, 2009). None of the natural enemies have had a significant impact, but many have led to some suppression of the different genotypes of this pernicious weed in the variety of environmental conditions under which it can thrive. Thus far, three pathogens have been intensively studied and released, although another (*Puccinia lantanae*) is currently being assessed (Renteria and Ellison, 2004). The first pathogen to be released was a *Septoria* sp. in Hawaii in 1997 (Trujillo and Norman, 1995; Trujillo, 2005). In 2001, the second, the leaf-spot fungus *M. lantanae* var. *lantanae*, was released in South Africa. This pathogen was assessed under quarantine in South Africa, and was found to have a restricted host range (Den Breeÿen and Morris, 2003). However, a number of strains were required to achieve infection of an acceptable number of lantana genotypes (Den Breeÿen, 2004). Although it was expected that the fungus would reduce the vigour and reproductive potential of *L. camara*, this has not been borne out in the field. Initial establishment was observed within the first 3 months but did not persist, presumably due to climatic limitations; possibly, the fungus could not survive the dry winters (Retief, 2010). Around the same time, a highly host-specific rust pathogen, *P. tuberculatum*, collected from Brazil, was introduced into Australia and was quick to establish at a number of release sites (Tomley and Riding, 2002; Ellison *et al.*, 2006; Thomas *et al.*, 2006). Unfortunately, due in part to the prolonged drought in Australia, the rust only occurred at a low density. However, after the extensive rains of 2008, growth of the rust suddenly took off and it increased in abundance, causing widespread leaf fall (Taylor *et al.*, 2008; Palmer *et al.*, 2010). This isolate infects only some pink biotypes of lantana, and requires subtropical summer conditions for infection (Ellison *et al.*, 2006). Strains or pathotypes of *P. tuberculatum* matching the other weed biotypes are likely to exist in the weed's native range and might be explored, particularly for additional introductions into subtropical areas. Another rust species, *P. lantanae*, collected from the Amazonian region of Peru, was found to be pathogenic to a wider range of weedy lantana biotypes than *P. tuberclatum*. It also causes more damage to plants, attacking stems as well as leaves (Renteria and Ellison, 2004). This isolate is currently being assessed for potential release in Australia, New Zealand and South Africa (C.A. Ellison, unpublished data).

MICONIA Miconia (*M. calvescens*, *Melastomataceae*) is a neotropical shrub or small tree with attractive foliage, which led it to be introduced as an ornamental in many regions in the tropics. It became a highly aggressive invader in Tahiti in 1937, Hawaii in 1960 and other Pacific islands in the 1990s (Meyer, 1996, 1998). In 1995, a search for pathogens of native *M. calvescens* that might have potential as CBC agents was initiated. Selected areas in Brazil, Costa Rica and Ecuador were surveyed and a wide range of pathogens was found, including: (i) a phytoplasma causing witches' broom disease; (ii) two species of nematodes, *Ditylenchus drepanocercus* causing angular leaf spots (Seixas *et al.*, 2004) and a new undescribed species of *Ditylenchus* causing galls of above-ground parts; and (iii) an oomycete, *Pythium* sp., causing root rot. Fungi collected over the intervening years have been described in two recent publications (Seixas *et al.*, 2007; Alves *et al.*, 2010). Currently, only one agent has been involved in a CBC introduction against *M. calvescens*: *Colletotrichum gloeosporioides* f. sp. *miconiae* (Cgm), introduced into Hawaii in 1997 (Seixas *et al.*, 2007) and into French Polynesia in 2000 (Meyer *et al.*, 2008). Although the fungus has established in the two archipelagoes, the opinion of biocontrol scientists in Hawaii is that forest invasions by *M. calvescens* remains unchecked and that more biocontrol agents are needed in order to achieve adequate levels of control for this target weed. The

results of the introduction of Cgm into French Polynesia were more favourable, however, as indicated by recent accounts of a 6-year series of post-release studies (Meyer *et al.*, 2008) and its beneficial impact on an endangered plant species, threatened by miconia invasion in Tahiti (Meyer and Fourdrigniez, 2011). Recent studies on *Coccodiella miconiae* have paved the way for its complete evaluation and have confirmed its potential for use in CBC (Alves, 2008). Additionally, a highly damaging species of gall-forming nematode (*Ditylenchus* sp.) was also evaluated and is regarded as having a high CBC potential (A. Santin, unpublished data). Unfortunately, emphasis (in terms of financial resources) in Hawaii has been biased towards attempted mechanical and herbicide control aimed at eradication of miconia. Substantial resources and significant human effort have been invested in such an ambitious – and possibly futile – task. It has involved eradication blitzes: hand-pulling of plants from inaccessible areas by mountaineers and herbicide application from helicopters. Although undoubtedly this is an impressive effort, it is a questionable option from a sustainability perspective, particularly considering the large seed bank produced by miconia and its broad distribution in Hawaii. It is likely that, once funding for eradication becomes scarce, the contribution from Cgm in Hawaii will become more visible, as the fungus is now ubiquitous in all places where miconia occurs (R.W. Barreto, personal observation). Paradoxically, now it has become widely recognized that additional introductions of CBC agents are needed against miconia, and that such agents (both arthropods and pathogens) have now been documented in Latin America (Alves, 2008; Alves *et al.*, 2010), funding for CBC has stopped and work has been suspended.

MILE-A-MINUTE Mile-a-minute (*Mikania micrantha*, *Asteraceae*) or mikania weed is a perennial vine from the Neotropics, with a native range from Mexico to Argentina. It has become a major invasive weed in many countries within the moist tropical and subtropical zones of Asia and the Pacific, and is still invading new areas, such as northern Australia. Mile-a-minute is able to smother plants in agricultural ecosystems – especially tea in Assam – and agroforestry, seriously affecting livelihoods by increasing the time farmers and plantation workers need to spend weeding (Ellison, 2004). However, it is also a serious weed of native habitats, affecting biodiversity: for example, it has invaded Chitwan National Park in Nepal, where it is over-growing the *Imperata cylindrica* ecosystem (Peet *et al.*, 1999), which provides forage for the rare and endangered one-horned rhino (Khadka, 2010). Surveys for fungal pathogens to be used as CBC agents were started in 1988 (Barreto and Evans, 1995b) and confirmed the potential of *Puccinia spegazzinii* for classical introductions. This is a damaging rust fungus found throughout the natural range of *M. micrantha* in Central and South America (Evans and Ellison, 2005). Subsequently, the rust was successfully assessed and screened for specificity to this weed host (Ellison *et al.*, 2008). It has now been released as a CBC agent in India (Sankaran *et al.*, 2008), mainland China and Taiwan, Papua New Guinea and Fiji (Day *et al.*, 2010). Reports from Taiwan are that the rust has established well in the wetter areas and is spreading (S.S. Tzean, 2009, personal communication), although in India and mainland China, the rust apparently failed to persist in the field. In Papua New Guinea, *P. spegazzinii* has been widely released in over 500 sites in 15 provinces, by placing potted, rust-infected plants in among young infestations of mile-a-minute weed. To date, nearly 4000 pots have been distributed and, so far, the rust has established in approximately 100 sites in seven provinces. Spread of the rust has been extremely encouraging: for example, at one site it was found to have dispersed 37 km in 18 months. In Fiji, the rust has also established and is spreading well at 20 sites on the islands of Viti Levu and Vanua Levu. Comparative growth studies and field monitoring in Papua New Guinea show that the rust can significantly reduce the growth and density of *M. micrantha* and offers

great potential for control of this weed. These results show that the rust is adapted to and thrives in the wet tropics, and also that the release strategy (applying the rust in high doses over a range of climatic conditions where *M. micrantha* is invasive) is critical to its successful establishment and subsequent spread (Day *et al.*, 2010). Ellison and Day (2011) consider that future work should focus on screening other biocontrol agents of *M. micrantha* that are adapted to drier areas in the native range.

PARTHENIUM WEED Parthenium weed (*P. hysterophorus*, *Asteraceae*) is a highly allergenic plant from the Americas that has become a serious pan-tropical invasive weed through accidental introduction into numerous tropical and subtropical countries. As a result of an extensive CBC programme, two rust fungi originating from Mexico have been released as control agents in Australia: (i) *P. abrupta* var. *partheniicola*, the so-called 'winter rust' based on its infection requirements of lower temperatures and longer dew periods, introduced in 1991 (Parker *et al.*, 1994; Evans, 2000); and (ii) *Puccinia xanthii* var. *parthenii-hysterophorae* (previously *Puccinia melampodii*; Seier *et al.*, 2009), the 'summer rust' according to its adaptation to a hotter and drier climate, introduced in 1999 (Evans, 1997a,b, 2000). As anticipated, the winter rust showed establishment in localized cooler areas in central Queensland but failed to establish under the higher temperatures prevailing in northern Queensland (Dhileepan and McFadyen, 1997; Fauzi *et al.*, 1999; Dhileepan *et al.*, 2006). In contrast, *P. xanthii* var. *parthenii-hysterophorae* established and persisted well, with an overall higher prevalence in northern Queensland and a disease incidence of more than 60% (Dhileepan *et al.*, 2006; Dhileepan, 2007). Together with another CBC agent, the stem-boring moth *Epiblema strenuana*, the summer rust has a synergistic negative effect on parthenium weed. However, its impact to date has been limited, possibly due to inadequate rainfall (Dhileepan *et al.*, 2006; Dhileepan, 2007). *P. abrupta* var. *partheniicola* has also been recorded on *P. hysterophorus* from parts of Africa as well as from India, most probably due to accidental introduction with contaminated seed material; however, the rust has shown little impact on the weed in these regions (Evans, 1997a,b; Kumar and Evans, 2005; A.R. Wood, personal communication). *P. xanthii* var. *parthenii-hysterophorae* has recently been given permission to be released in South Africa, and efforts for mass release of the rust are currently under way (A.R. Wood, personal communication). An introduction into India has not been considered due to the predicted non-target effects of this rust on selected commercial sunflower varieties, as well as on marigold (*Calendula officinalis*) a socio-economically important plant in Indian culture (Seier, 2005).

PORT JACKSON WILLOW Port Jackson willow (*A. saligna*, *Mimosaceae*) is a shrub or small tree that was introduced into many regions of the world from Australia as a useful plant for soil stabilization, as source of firewood and for other purposes but became an environmental weed in many areas, particularly in South Africa (Henderson, 2001), where it became the target of a CBC programme involving the use of fungal pathogens. Evidence has now been gathered and assessed on the impact of the gall-forming rust *Uromycladium tepperianum* on *A. saligna* in South Africa. Since its release in 1990, its impact has been monitored on five selected populations, where there was an estimated annual tree mortality of 18% as the rust galls proliferated leading to tree collapse and death (Morris *et al.*, 1999). A high reduction in tree biomass and seed production was also recorded. These data have been confirmed recently, and therefore it is concluded that this has been a highly successful CBC programme (Wood, 2008).

RUBBER VINE WEED Rubber vine weed (*C. grandiflora*, *Asclepiadaceae*) is a woody liana or shrub with showy flowers and toxic milky sap that is native to Madagascar (Evans, 1993). It has been introduced and spread into other tropical regions of the

world for ornamental purposes, or as an experimental source of rubber, among others. In northern Australia, it caused devastating invasions of natural ecosystems and has been recognized as the biggest threat to tropical ecosystems in Australia (McFadyen and Harvey, 1990). Invasions of this weed threatening the natural ecosystems are also recorded in the Caribbean Netherlands Antilles (Tomley and Evans, 2004; H.C. Evans, personal observation), and more recently in Mexico and the USA (Rodriguez-Estrella *et al.*, 2010). A biocontrol programme funded by the Queensland Department of Lands was initiated in the 1980s. Two agents from the Madagascan centre of origin – the pyralid moth *Euclasta whalleyi* and the rust *M. cryptostegiae* – were released in the 1990s in the target area of northern Queensland (McFadyen and Marohasy, 1990; Tomley and Hardwick, 1996). Although the moth larvae can periodically cause substantial defoliation (Mo *et al.*, 2000), the main impact has been from the rust. Independent long-term monitoring by agronomists and ecologists – so absent from many programmes – has shown a greater than 40% reduction in weed populations with virtually no seedling recruitment (Vogler and Lindsay, 2002). A recent economic impact assessment put the net benefit of the programme in 2004/2005 at over AU$230 million, with a cost:benefit ratio of 1:108 (Page and Lacey, 2006), making this one of the most successful CBC weed programmes in Australia (Palmer *et al.*, 2010). There are recent reports of rubber vine infestations in the Neotropics, notably in north-east Brazil, with major impacts on an indigenous and emblematic palm species (Herrera and Major, 2006). The invasive weed involved was later clarified as being the closely related species *Cryptostegia madagascariensis* (Alves *et al.*, 2008). The increasing impact of this weed on local biodiversity and the urgency for a solution to the problem suggests that piggy-backing on the success of the Australian project would be a sensible option.

WANDERING JEW Wandering Jew (*Tradescantia fluminensis*, *Commelinaceae*) is a herbaceous plant native to South America and is particularly common along the coast in south-eastern and southern Brazil where it forms small patches in humid rocky habitats, such as along creek margins. It never forms dense extensive populations and is not regarded as a weed of importance, failing to appear in the regional weed lists (Kissmann, 2000; Lorenzi, 2000). Conversely, where it was introduced into exotic tropical and subtropical regions of the world, it has become a serious invader of native ecosystems, particularly in forest ecosystems in New Zealand where it has no significant natural enemies (Winks *et al.*, 2003). Searches for natural enemies (both arthropods and pathogens) were started in 2004 in Brazil and yielded a diverse list of potential CBC agents, including fungi (Pereira *et al.*, 2008). A new species of white smut (*Kordyana*) was selected for more detailed evaluation and has proven to be host specific and capable of causing severe disease symptoms on the biotype of the weed established in New Zealand. Its potential is regarded as being high, and permission for its introduction into New Zealand has now been approved (R.W. Barreto, personal communication).

Newly initiated programmes

BELLYACHE BUSH Bellyache bush (*Jatropha gossypiifolia*, *Euphorbiaceae*) is a shrub that can grow up to 4 m high and is native to the Americas, where it occurs from Brazil to Mexico. It was introduced as a botanical curiosity in many areas of the world and escaped to become a highly invasive weed. In Australia, bellyache bush populations can replace native plant communities, forming dense monospecific stands, which can impact on ecological functioning and restrict access. The situation in the Northern Territories is particularly serious, and this weed is presently the target of an eradication programme (Anon., 2010). Heard *et al.* (2002) discussed the potential for CBC of bellyache bush, and the rust *Phakopsora jatrophicola* is currently undergoing assessment in Australia. In the native range, this

rust species is recorded from a number of *Jatropha* spp., including the biofuel crop species *Jatropha curcas* (Farr and Rossman, 2011). Cross-inoculation studies, undertaken with accessions from *J. gossypiifolia* and *J. curcas*, have clearly shown that the rust comprises distinct strains or pathotypes adapted to either of the two hosts, although some degree of cross-infection also occurred. Full host-range testing will be undertaken with a strain of *P. jatrophicola* ex. *J. gossypiifolia*, which is most virulent to the target weed while having the least impact on *J. curcas* (M.K. Seier, unpublished data).

PRICKLY ACACIA Prickly acacia (*A. nilotica* subsp. *indica*, *Mimosaceae*) is a weed of national significance in Australia and, as such, this Afro-Asian tree has been the subject of a CBC programme since the early 1980s (Dhileepan, 2009). More recently, the initial focus on arthropods has been expanded to include fungal pathogens as potential CBC agents. The rust *Ravenelia acaciae-arabicae*, collected from prickly acacia in India, is currently being evaluated for host specificity. Given the importance of the Mimosaceae plant family in Australia, and the numerous native *Acacia* spp., host specificity of the rust will be critical, but initial results look promising (M.K. Seier, unpublished data).

11.2.5 Limitations and future prospects

Ellison and Barreto (2004) discussed CBC from a Latin American perspective and recognized that the majority of CBC implementation programmes against weeds have been in the USA, Australia, South Africa, Canada and New Zealand, and that, although an increasing number of programmes are being developed in several Asian and African countries, there are still very few examples in Latin America (Julien and Griffiths, 1998). Pathogens have been introduced in only two Latin American countries: three in Chile and one in Argentina (Julien and Griffiths, 1998). The reasons for this paucity of weed biocontrol programmes in that part of the world have been listed (Ellison and Barreto, 2004): underinvestment and a lack of recognition among authorities dealing with conservation and agricultural issues in Latin American – and even by weed scientists – of the problems caused by invasive alien weeds, as well as a common belief in the fallacy of introduced species being unable to compete with the native flora. Fifty-nine examples of invasive alien weeds in Latin America were also given that could be targeted in CBC programmes (Ellison and Barreto, 2004). Later, Barreto (2008) discussed the challenges for this discipline in Latin America and listed all the major groups of scientists or individuals involved in this research area in the region, and considered that conditions for re-inaugurating CBC in Latin America are favourable, although successful examples of application of its principles are urgently needed to help consolidate the discipline, as well as educating the public and the authorities about the potential and safety of this approach. However, Europe is equally lagging behind in embracing CBC as part of an invasive weed-management strategy. Sheppard *et al.* (2006) gave possible reasons for this as: (i) a less visible impact of invasive weeds in Europe; (ii) more intensive land management; and (iii) potentially, a lower susceptibility to alien invasions of European habitats shaped by agriculture and human disturbance.

Recent efforts to research CBC as a control strategy for important pan-European alien weeds – as detailed above for giant hogweed, Himalayan balsam and Japanese knotweed – have led to the recent release of a sap-sucking psyllid as a CBC agent against Japanese knotweed in the UK (Morelle, 2010). Fungal pathogens, however, are still awaiting their turn, as the release of such agents is, and will continue to be, more problematic (see discussion of pathophobia).

Ironically, there is also the possibility that some of these invasive alien weeds could disappear from their native ranges before their natural enemies (and, of course, potential CBC agents) have ever been documented. For instance, humid submontane forest ecosystems are listed as among the most threatened habitats in the

world, and weeds such as wild gingers (*Hedychium* spp.) are listed as endangered in their native East Himalayan ranges (Chadha, 2005; Anon., 2007). Similarly, the highly invasive grass *I. cylindrica* has long been considered to be one of the worst invasive weeds in the world (Holm and Herberger, 1969). However, in its homeland of Nepal, threatened *I. cylindrica* grasslands are now being managed and conserved in National Parks in the ecologically sensitive Terai region (Peet *et al.*, 1999).

Prospects for expansion of CBC of weeds with pathogens in India now appear promising, after the first initiative to release the rust pathogen *P. spegazzinii* for the control of mile-a-minute weed (see above) was implemented in Assam and the Western Ghats. Encouragingly, other weeds of neotropical origin are presently being considered as potential targets for new programmes in India (Kumar *et al.*, 2008). Most of the weeds indicated by these authors are, coincidentally, species for which a body of published information on pathogenic fungi is already available, namely: *C. odorata*, *Eichhornia crassipes*, *L. camara* and *P. hysterophorus.*

Perspectives have changed radically over the past four decades, and new examples of CBC successes have emerged as well as some new procedures and ideas, but, sadly, one of the closing statements in a weed CBC review chapter 20 years ago, that 'biological control is normally thought of as an alternative to other methods of control when these other methods have failed to control a particular troublesome, dominant weed' (Watson, 1991), still remains as true now as it did before. This is particularly worrying, as, in several instances, whole island ecosystems – such as the native forests of Mauritius, Hawaii or French Polynesia, as well as the World Heritage Galapagos Isles – with their endemic species are increasingly threatened by monotypic invasions of exotic plants. The only economically and environmentally viable tool for managing these invasions is CBC. However, this resource remains largely untapped because of limited funding, lack of personnel, inherent costs (need for quarantine infrastructure, foreign collaboration and exploration), the long length of time taken from accomplishing the introduction of an agent and, increasingly, strict risk-analysis studies based on host-specificity tests (Groenteman *et al.*, 2011). Basically, little has changed since these constraints were first highlighted two decades ago (Watson, 1991).

Despite the overall excellent track record of safety for CBC as a strategy, and the impeccable results in terms of limited non-target impact of pathogens (Barton, 2004, 2011) and the general adherence of biocontrol practitioners to the FAO Code of Conduct for CBC (Schulten, 1997), biological control has been under attack and subject to criticism by ecologists, and sometimes the ill-informed press. An unreasonable attitude of fear and rejection of CBC and of pathogens, in particular, or pathophobia (Freeman and Charudattan, 1985; Warner, 2011), could be said to be harming the principle alternative for effective, sustainable, safe and economic (compared with other methods) control of many critically important invasive alien weeds. Although there have been records of non-target impacts of some insects introduced as CBC agents of alien weeds, the true facts behind these much-cited 'mistakes' have already been addressed (Marohasy, 1996; Evans, 2000). It was demonstrated that either all these introductions would be rejected under the protocols that have been used in the discipline for over 30 years, or they were not recommended by the scientists involved who were aware of the potential problems that might result. Nevertheless, political decisions taken by non-scientists have unfortunately led to questioning of the safety of the CBC strategy for weed management by scientists (Simberloff and Stiling, 1996; Strong, 1997). Charudattan (2001) included in the final section of his overview paper on the discipline, the following statement: 'It is unimaginable, both from economic and ecological standpoints, to think that invasive weeds can be managed by regulations (exclusion and quarantine) or physical and chemical

controls. Biological control, in all of its aspects, should be the center piece of a global strategy to tackle invasive weeds.'

Difficulties in obtaining funding for relatively expensive CBC programmes are due in part to life in a world dominated by 'minimal government ideas', as well as to their long-term nature in which any credits accrued will be a long way down the political pipeline. Conversely, the risks involved will be short term and will have immediate political impact on decision makers. As CBC is not commercial and thus requires funding that comes indirectly or directly from government sources, decisions to proceed with programmes or to release agents are made in a different political climate from that when the programmes were initiated: in effect, the 'rules of engagement' may have changed (see discussion of itch grass above). It is also necessary to acknowledge that, historically, biocontrol scientists have been poor advocates of their discipline and have not given enough attention to the need for public awareness and involvement. A study undertaken recently in North America revealed that the general public remains ill-informed about CBC and its potential benefits (Warner *et al.*, 2008; Warner, 2011). The authors provided key considerations about the public and elected officials that are relevant to biocontrol researchers as they undertake any CBC initiative. The combination of increasing environmental awareness, the popularity of organic products and increasing costs for the development of new chemical herbicides – coupled with increasing restrictions to existing products – make up a scenario in both developed and developing countries that appears to favour weed biocontrol (Vurro and Evans, 2008).

11.3 Inundative Biological Control

11.3.1 Introduction

The term inundative biological control (IBC) is used most often to describe the bioherbicide approach to weed management in which a product – based on a living pathogen – is manufactured, commercialized and applied in much the same way as a conventional chemical herbicide. In direct contrast to CBC, the target is usually a native weed, while the pathogen of choice is an indigenous fungal necrotroph that can readily be grown *in vitro*, and not a coevolved exotic biotroph that can only be cultured *in planta*. However, there may also be overlap in these seemingly radically different approaches, as well as considerable variation in the type of agent used and the degree of sophistication of the final product.

Wilson (1969) signalled four decades ago that the time was right for the use of bioherbicides and that this could be achieved through technology by selecting and formulating virulent pathogens, and even increasing pathogenicity through genetic modification, or by harnessing their phytotoxic secondary metabolites. Thus, while CBC was led by plant pathologists, IBC was pioneered predominantly by biochemists and microbiologists. Both strategies for weed management were, in fact, implemented around the same time in the early 1970s, when the potential of bioherbicides was first identified in the USA (Daniel *et al.*, 1973; Burnett *et al.*, 1974), although commercialization of the first products was delayed for over a decade (Bowers, 1986; Kenney, 1986), due to both technical and regulatory hurdles (Templeton, 1992). If we look at the initial promise, aspirations and overarching strategy of IBC and compare these four decades on, the changes have been considerable. This early optimism has been tempered over the years by pragmatism, especially economic constraints, while the strategy has virtually been turned on its head, and some have even attempted to change the terminology used, with the introduction of new terms such as bio- or mycoherbistat, for example (Crump *et al.*, 1999; Jahromi *et al.*, 2002). Fortunately, for the sake of clarity, such emendations to the weed pathology nomenclature have not been universally adopted.

The pioneering and subsequent development history of IBC over the

decades has been much reviewed (Templeton *et al.*, 1979; Bowers, 1982; TeBeest, 1984; TeBeest and Templeton, 1985; Charudattan, 1988, 1991; TeBeest, 1991; Auld and Morin, 1995; Greaves, 1996; Zidak and Quimby, 1998; Pilgeram and Sands, 1999; Boyette, 2000; Charudattan, 2001), alongside more comparative overviews on biological control of weeds using pathogens (Cullen and Hasan, 1988; TeBeest, 1993; Mortensen, 1997; Evans *et al.*, 2001a,b; Evans, 2002; Yandoc-Ables *et al.*, 2006a,b) so it is not necessary to repeat this here. However, a list of bioherbicides that were developed up to the point of being registered or patented, or otherwise having reached the user (updated from Barton, 2005), is included in Table 11.3. The salient points are summarized below on the overall strategy and expectations of IBC of weeds during the formative decades.

A variety of agents were used:

- For most of the period, and certainly for many of the weed targets, fungal pathogens were considered, almost exclusively.
- The focus was on selecting agents based on indigenous necrotrophs, because these were amenable to *in vitro* culture.
- The selected agents were expected to be host specific.

A number of different strategies were also employed:

- The rigid host specificity limited the market size and, in effect, targeted single crop weeds with limited scope therefore for commercialization by agrochemical companies.
- Predominantly, with a single exception (that of water hyacinth – work in the University of Florida under the initiative of R. Charudattan), the target weeds chosen were of limited relevance, either agriculturally or environmentally. Most were weeds of importance regionally on a single crop, resulting in limited markets.
- Initial research and development were carried out, typically at the university level with limited public funding.
- Formulation was usually crude, with expertise often borrowed from the food industry.
- Expectations – and the first optimistic reports/publications – were based on limited, small-scale trials at the greenhouse or small-plot level, with little thought given to the problems of scaling up, environmental constraints and the consistency levels demanded of commercial herbicides for agriculture.
- Only at a later stage was the prototype product offered for commercialization, when significantly larger financial inputs were necessary for product development and safety testing.
- Invariably, profitability had not been researched adequately, or even at all, and thus many of these projects failed to attract investment.

In short, the initial euphoria and high expectations were generated largely by committed or even ideological university researchers who saw IBC as a viable and preferable answer to the increasing and often indiscriminate use of chemical herbicides. In contrast, the agrochemical industry viewed this as a somewhat esoteric, ivory-tower approach to weed control: hard-nosed pragmatism versus blue-sky thinking, perhaps. Nevertheless, it is probably true to say that the industry has always kept a wary eye on the happenings in IBC, or has sat on the fence, and has even dabbled in the 'dark arts', just in case something unexpected turns up.

So, who are the winners and losers, four decades on? As intimated above, the early optimism and flood of publications claiming that mycoherbicide X based on fungus Y is the answer for control of weed Z – 'Sprays of living fungi can help Florida in its $360 million war with weeds', as one press release from an experimental station announced in 1980 – have been toned down, while industry has been trying to keep pace with the ever-increasing number of banned or failed products as legislation tightens and herbicide-resistant weeds evolve. It would seem, however, that there will never be a bioherbicide to replace the

Table 11.3. Bioherbicides at an advanced stage of development, registered or that have reached use in the field.

Product and biocontrol agent	Target species	Target areas	Countries	Status
Biochon™: *Chondrostereum purpureum*	*Prunus serotina* and other invasive trees	Forestry and natural reserve areas	Holland	Manufactured (by Koppert) until the end of 2000 but a combination of factors (see text) led to removal from market
BioMal®: *Colletotrichum gloeosporioides* f. sp. *malvae*	*Malva pusilla*	Lentils, linseed and wheat	Canada	Production interrupted for a long time. Registration renewed by Encore Technologies LLC but seemingly unavailable in the market
Camperico™: *Xanthomonas campestris* pv. *poae*	*Poa annua*	Golf fields	Japan	Present status uncertain. It was manufactured for some time by Japan Tobacco
CASST™: *Alternaria cassiae*	*Cassia* spp.	Groundnuts and soybean	USA	Registered but not available commercially
Chontrol Paste™ = Ecoclear™: *Chondrostereum purpureum*	Invasive woody plants	Paths in natural areas and forestry	Canada	Available in the market (manufacturer MycoLogic)
DeVine®: *Phytophthora palmivora*	*Morrenia odorata*	Citrus	USA	Uncertain status. Manufactured in the past under special request by Abbot Laboratories
Dr BioSedge: *Puccinia canaliculata*	*Cyperus esculentus*	Cotton, potatoes, sugarcane, maize and soybean	USA	Registered but never reached the market because of technical problems in mass production and because of the presence in the field of resistant weed biotypes
Hakatak: *Colletotrichum acutatum*	*Hakea gummosis* and *Hakea sericea*	Native vegetation	South Africa	Not registered but available under special request
LockDown ™ (formerly Collego™) *Colletotrichum gloeosporioides* f. sp. *aeschynomene*	*Aeschynomene virginica*	Rice and soybean	USA	Recently re-registered and manufactured by Natural Industries Inc.
Lubao: *Colletotrichum gloeosporioides* f. sp. *cuscutae*	*Cuscuta* spp.	Soybean	China	Uncertain but possibly available locally (small-scale cottage production)

Continued

Table 11.3. Continued.

Product and biocontrol agent	Target species	Target areas	Countries	Status
Myco-herb® *Lewia chlamidosporiformans*	*Euphorbia heterophylla* and other euphorbiaceous weeds	Soybean and other crops	Brazil	Recently developed, patent filed and market name registered. Not available yet
Myco-Tech™ paste: *Chondrostereum purpureum*	Deciduous woody invasives	Paths in natural areas and forestry	Canada	Available in the market (manufacturer Myco-Forestis Corp.)
Smolder: *Alternaria destruens*	*Cuscuta* spp.	Several crops and ornamental nurseries	USA	Registered in 2005. Plans were announced by the manufacturer (Platte Chemical Co.) for its release to the market in 2007. No updates on its status are available
Stumpout™: *Cylindrobasidium laeve*	Several species of *Acacia*	Native vegetation	South Africa	Last news on its availability is from 2005, but demand was then said to be small
Woad Warrior: *Puccinia thlaspeos*	*Isatis tinctoria* in farms, rangeland, waste areas and roadsides	Cultivated areas, pastures, derelict areas and roadsides	USA	Registered but never reached the market because of lack of commercial interest. Later the fungus was widely distributed in nature by the scientists involved in its development

ubiquitous glyphosate, for example, because no pathogen has the host range coupled with the efficacy of this chemical. Thus, in summary, agriculture is still reliant for large-scale weed management on chemical products – albeit a diminishing choice – while IBC has remained at a cottage-industry level, now targeting mainly niche-market, amenity weeds rather than those of crops.

It is worthwhile, however, revisiting the pioneering and much quoted mycoherbicides, which provided the early impetus for IBC: namely, Collego (TeBeest and Templeton, 1985; Bowers, 1986) and DeVine (Ridings, 1986; Charudattan, 1987) for use in rice and citrus in the southern USA, respectively. The early success of the former was due to its low production cost, based on a simple formulation, and the ability for rapid and efficient secondary spread within the crop ecosystem of the pathogen, *C. gloeosporioides*. Details are still hard to come by as to why Collego gradually lost favour and seemingly disappeared from the marketplace for a number of years. However, according to Weaver *et al.* (2007), with changing agricultural practices, there is now a renewed interest in this product. In fact, this mycoherbicide has now re-emerged, under the new name LockDown, and is being manufactured by Natural Industries Inc. (R. Charudattan, personal com-

munication). Likewise, DeVine targeted milkweed vine, an invasive weed that was locally important in Florida citrus plantations. The product was based on liquid cultures of chlamydospores of *Phytophthora palmivora*, which was cheap to produce and was more or less delivered on demand straight from the production unit. With its short shelf life and logistical problems in producing and distributing it, as well as the small specialist market, DeVine was a commercial failure (Ridings, 1986). In fact, it has been suggested that DeVine was a victim of its own success, as once the product was applied the pathogen persisted in the soil and milkweed vine ceased to be a problem. Additionally, the shifting of citrus plantations in Florida into new areas, where the target weed was no longer a problem, contributed to the shrinking of an already small market for this product (R. Charudattan, personal communication). Paradoxically, like the concept of the everlasting light bulb and car tyre, a self-sustaining biopesticide can fail commercially because there is no long-term demand.

Nevertheless, the situation is not all doom and gloom, and there have been successes. In addition, some ground-breaking changes have been an expansion in the types of agent selected: (i) to include viruses and bacteria, as well as biotrophic fungi, for example; (ii) to consider the use of pathogens with wide host ranges and therefore to include not just indigenous weeds but also exotics; and (iii) to increase the efficacy of biopesticides through synergism with low-dose chemical herbicides.

11.3.2 Successes in IBC

Fungi are by far the most utilized of the plant pathogens for the IBC strategy, and, invariably, these are necrotrophs that grow readily in culture. Numerous experimental mycoherbicides have been based on species of the genus *Colletotrichum*. However, as outlined above, most of these have failed to reach the commercial stage, and, of the few that have, their present status is difficult to assess. This is not the fault of the science, or even the lack of ingenuity of researchers. For example, great hope was centred on the innovative use of a biotroph – the rust *Puccinia canuliculata* – targeted against one of the world's worst weeds, yellow nutsedge (*Cyperus esculentus*) (Phatak, 1992). The experimental product, Dr BioSedge, if applied early in the season, induced damaging epiphytotics in weed populations, reducing both plant vigour and rhizome size, which had a knock-on effect in the following season. However, it failed to reach the market, and a combination of factors appears to have been responsible, not least problems with mass production of a biotrophic fungus on a living host. Similarly, it was probably a combination of factors that dashed the hopes and put an end to most of the mycoherbicides developed, thus far: (i) economics of mass production (because of fastidious organisms); (ii) lack of investment (because of narrow targets); and (iii) inconsistent performance compared with chemical herbicides (because of formulation problems). Thus, there was, and still is, a vicious circle of poorly funded projects that reach a certain stage but falter because of lack of investment. These are then deemed to be failures. Consequently, we are left with a few (apparent) successes, which, universally, do not involve high tech or large agrochemical companies but are aimed predominantly at troublesome environmental weeds, rather than mainstream agricultural weeds, and are driven by involved scientists funded from the public sector working at the cottage-industry level. Some of these are discussed below.

Stump treatments

Many fast-growing woody weeds, indigenous as well as alien, are especially difficult to manage by traditional cultural techniques – simply felling them, in itself an expensive exercise, leads to re-sprouting and, potentially, a worse-case scenario than without treatment. Stump removal adds immeasurably to the costs involved. Several countries have come up with the same solution to overcome this problem against

different weed targets: the application of an indigenous tree pathogen to kill the stump.

AMERICAN BLACK CHERRY American black cherry (*Prunus serotina*, *Rosaceae*) is a highly invasive species in both natural and plantation forestry in the Netherlands and elsewhere in Europe. The product Biochon has been commercially available for some time to control re-sprouting and effectively kill the tree, although recent reports suggest that it has been withdrawn from the market (Ehlers, 2008). This occurred because of a series of problems with registration, shelf life, quality control and formulation. There is, nevertheless, a growing interest for the product in Germany (K. de Voogd, personal communication). Essentially, it is a mycelial preparation of the basidiomycete fungus *Chondrostereum purpureum* (Meruliaceae), the causal agent of silver leaf of stone fruits and once a notifiable disease in Europe. So why did the 'host-specific mantra' change and how did a plurivorous, even notorious, pathogen manage to prove an acceptable option of control for this weedy tree in the Netherlands? In a series of elegant epidemiological experiments, together with simulation models, it was demonstrated that minimal risks were posed to susceptible fruit crops (de Jong *et al.*, 1990, 1991). The airborne inoculum of the fungus was found to be similar to normal background contamination less than 0.5 km away from treated sites and therefore that it was safe to use outside this buffer zone from a susceptible crop (de Jong, 2000).

A similar approach is now being assessed against a range of woody weed targets in various countries. Most advanced is Canada, where chemical herbicides are now banned from many urban and rights-of-way sectors and this has meant an open market for alternative solutions to control woody invasives. A product based on *C. purpureum* has been evaluated for registration (Evans *et al.*, 2001a), and is now available under the trade name Chontrol Paste (MycoLogic, 2011). Trials are also under way with various formulations of *C. purpureum* and application strategies in the UK and Eire, against *Rhododendron ponticum* (M.K. Seier, unpublished data), as well as in New Zealand, against gorse and broom.

WATTLE Two Australian species of wattle (*Acacia* spp.), *Acacia mearnsii* and *Acacia pycnantha*, have become aggressive invaders in South Africa, especially along watercourses where they impact directly on the access to and availability of water. Chemical herbicides are both economically and environmentally constrained, and weed management is through cultural control by felling as there are no CBC options. However, this is only temporary, as re-growth is rapid and vigorous, creating denser barriers and more water loss than before. However, through a public sector initiative at the cottage-industry level, a crude mycoherbicide based on an indigenous wood-rotting basidiomycete, *Cylindrosporium laeve* (Hyphodermataceae), is helping to solve the problem. This oil-based product (Stumpout), comprising a basidiospore suspension, is applied to the newly cut stump and, over time, kills the shoots as they develop (Morris *et al.*, 1999).

Viruses

It has long been supposed that viruses are not suitable weed biological control agents, for either CBC or IBC, because of the need for a vector and the envisaged problems with safety, genetic stability, mass production and registration. However, Charudattan (2005) discussed their merits and specifically a strain of tobacco mild green mosaic virus (a tobamovirus) obtained from dying tropical soda apple weed (*S. viarum*). This South American plant has become a serious invasive weed in the USA, particularly of rangeland in Florida. The virus, which is transmitted mechanically via wounds, has a relatively restricted host range and, although it can infect peppers and tobacco, symptoms are mild and there is no natural transmission. Solutions containing the virus are power-sprayed on to soda apple infestations, and this is sufficient to induce infection and

subsequent death of the weed through a lethal hypersensitive response. The product has now been approved by the Environmental Protection Agency and is currently being marketed as SolviNix (R. Charudattan, personal communication).

Bacteria

Concerns similar to those for viruses have been voiced about the use of bacteria in biological control of weeds, although there have also been strong advocates for controlling weeds with phytopathogenic bacteria (Johnson *et al.*, 1996). Nevertheless, this optimism has not yielded any major breakthroughs in the commercialization of bacterial herbicides. However, there is one example of a niche-market product that has been developed in Japan, specifically targeted at annual bluegrass (*Poa annua*) in golf courses (Gohbara, 1996), and has also been assessed in the USA (Zhou and Neal, 1995). A host-specific strain, or pathovar, of *Xanthomonas campestris* pv. *poae* in a liquid formulation is applied during or shortly after mowing. The bacterium then invades via the cut surface. This has been marketed under the name Camperico (Masahiro, 2001), but there is uncertainty about the current status of this bioherbicide, although there is still ongoing interest in and investigations on this bacterial agent in the USA (Neal *et al.*, 2008).

11.3.3 Limitations and future prospects

The honeymoon period of IBC is long over, and, as in most new ventures, optimism has given way to pragmatism. In short, bioherbicides have failed to have any measurable impact on the agricultural sector because they cannot compete with chemical herbicides, economically or functionally. Only five among the 15 bioherbicides listed in Table 11.3 appear to be available commercially, and all of them are niche-market products (perhaps with the exception of the *C. purpureum*-based products in Canada). The future prospects, as outlined above, would seem to lie in supplying one-off, specialist products for invasive, predominantly woody, difficult-to-control weeds in non-agricultural situations. Nevertheless, this may change radically depending on the emergence of interest from the agrochemical industries. Their input of financial resources, as well as their expertise in formulation, application technology and marketing strategy, has the potential to change the present scenario. Currently, what seemed like a promising first step and a line to pursue – that of mixing bioherbicides with low-dose chemical herbicides to search for synergy and thus maximize the effects of both, as well as to 'greenify' the product – appears not to have been taken up by the agrochemical companies thus far (Christy *et al.*, 1993; Duke *et al.*, 2007). Similarly, using phytotoxic microbial metabolites, or their analogues – rather than the organism per se – to overcome problems of mass production, efficacy and stability of bioherbicides, offers some promise. However, it is likely that the costs of registration could increase considerably if the products were viewed as chemicals rather than bioherbicides. It could also be argued, of course, that only limited amounts of public funding or venture capital have been invested in the technology, compared with the many millions needed to develop a conventional herbicide (Heiny and Templeton, 1993), and that most of the identified or perceived constraints centred around bioherbicides (Auld and Morin, 1995) could be resolved or overcome with more time and funding. It is no coincidence that the most successful mycoinsecticide – technically, at least – Green Muscle, involved a multidisciplinary, highly funded programme over an extended time period (Shah and Pell, 2003). A later tendency in the discipline, which may prove far reaching, has been towards giving priority to important areas where chemical herbicides have failed or are regarded as an unacceptable option. These are:

- Products for use in aquatic ecosystems and other environmentally sensitive areas.
- Products for use in organic agriculture.

- Products for weeds for which existing chemical herbicides have been lost (because of non-renewal of registration, removal from the market or other reasons).
- Products for herbicide-resistant target weeds.

Other initiatives that are being explored involve the use of bioherbicides with mixtures of pathogens to improve efficacy and, in some cases, extend the target range (Chandramohan and Charudattan, 2003), and through genetic engineering to promote hypervirulence of the pathogen (Amsellem *et al*., 2002; Vurro and Evans, 2008). Both of these, of course, are technically challenging and, in the latter case, politically charged. It is unlikely, therefore, that these approaches will become strategies of choice for the management of weeds in the immediate future.

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12 Virus- and Bacteria-transmitting Arthropod Vectors and their Management

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12.1 Introduction

It is well known that crops can become infected by plant pathogens with serious economic consequences. That plants are stationary yet are subject to disease epidemics indicates the efficient dispersal capacity of plant pathogens. Some plant pathogens are dispersed abiotically (by wind and/or water), while many others utilize mobile vectors that transmit pathogens to the crop. This chapter will explore arthropod vectors of plant-pathogenic viruses and bacteria. Some vectors transmit viruses non-persistently, often by brief feeding probes lasting a few minutes or less. Other vectors transmit the virus semi-persistently (over a few days) or persistently (for weeks or months). As viruses are intracellular parasites and must commandeer the host nucleic acid replication machinery to replicate and multiply, vectors that persistently transmit viruses can be adversely affected in the process. As a general rule, bacterial plant pathogens replicate in the arthropod host and can cause a variety of effects, both positive and negative. Because of this diverse array of transmission pathways, controlling vectors is a complicated and difficult task that requires a thorough understanding of their biology and the underlying pathogen replication mechanisms.

12.2 Vector Competence

Plant bacteria and virus vectors feed in different plant tissues, such as the epidermis and/or mesophyll cells, phloem and xylem, obtaining nutrition from free amino acids and sugars. Plant pathogens are acquired passively by arthropods during feeding on infected plants. The feeding duration necessary to acquire a sufficient titre of the pathogen is the acquisition period (AP), which can be as short as a few seconds, or as long as minutes or hours. In reality, vectors may feed for longer or shorter periods. For persistently transmitted pathogens, the longer the AP, the greater the chance of transmission.

The period of time that elapses from initial acquisition until the pathogen is transmitted is known as the latent period (LP), sometimes also referred to as the incubation period. For non- and semi-persistent pathogens, there is no LP. For most persistent pathogens, during the LP they must cross a number of barriers (e.g.

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epithelial cells of the midgut, muscles lining the midgut, through the haemocoel and into specific regions of the salivary glands) to be egested during feeding (see, for example, Fletcher *et al.*, 1998; Kwon *et al.*, 1999). Any pathogen that can pass through some but not all of the barriers will not be transmitted, even though it may be detected by serological or molecular means (e.g. PCR) within the arthropod's body. Therefore, the only method to determine the competency of a vector is through transmission studies.

Vector competence is a means of describing how often a vector transmits a pathogen and over what period of time. As such, several schemes of categorizing transmission of plant pathogens by arthropod vectors have been developed (reviewed by Nault, 1997; Hogenhout *et al.*, 2008). This chapter will follow the system described by Nault (1997) to discuss transmission of bacterial and viral plant pathogens.

12.2.1 Non-persistent pathogen transmission

The rate of transmission by the arthropod (primarily, if not exclusively, aphids) drops to near zero within a short time (i.e. a matter of hours). Generally, vector acquisition of pathogens increases with time spent feeding on the infected plant source; however, in this case acquisition usually decreases with sustained feeding, indicating that the vector is feeding in vascular tissues (Nault, 1997). Non-persistent pathogens are transmitted primarily by aphids and are stylet-borne. Often they are characterized as having low vector specificity, e.g. potato virus Y (PVY) can be transmitted by more than 30 aphid species. However, certain combinations of vectors with non-persistently transmitted viruses are much more efficient in transmission than others, suggesting that some mechanism of specificity is at work. Non-persistently transmitted viruses can be transmitted immediately after acquisition; there is no LP. The means of transmission is unknown but there are two hypotheses: (i) 'ingestion–egestion' whereby virions are regurgitated from the food canal (Harris, 1977); and (ii) the process of salivation may release virus particles (see Powell, 2005), as the salivary and food canals converge 2–4 μm before the tip of the stylet (Forbes, 1969).

12.2.2 Semi-persistent pathogen transmission

Transmission of the pathogen persists for a few days at most in the arthropod after acquisition. These pathogens are not acquired quickly like the non-persistent ones but rather seem to be ingested by feeding in phloem or xylem tissue (Nault and Styer, 1970). In this case, the pathogen may be retained in the foregut (or midgut) and is gradually lost over time through feeding. As digestive enzymes are excreted, some pathogen particles are washed off the surface and on to the plant.

Although there are hundreds of viruses and bacteria transmitted by arthropod vectors, knowledge about specific host–pathogen interactions and means of pathogen uptake have been derived from only a handful of examples. Recent research has revealed two mechanisms that facilitate the acquisition of non- and semi-persistently transmitted viruses: the capsid strategy and the helper strategy (see the review by Ng and Falk, 2006, and references therein).

The capsid strategy has been studied extensively in the aphid-vectored cucumber mosaic virus (CMV) system. Essentially, specific virus-encoded capsid proteins (CPs) have been found to be critical for aphid acquisition, virus stability and subsequent transmission. Very briefly, there are three essential components involved in aphid transmission of CMV: (i) viral CPs; (ii) virion-associated protein (with its C terminus in the inner shell of the virion and its N terminus out of the capsid); and (iii) aphid transmission factor (which binds to the tip of the maxillary stylet) (Uzest *et al.*, 2007). While this provides an explanation of the binding of the virus within the aphid stylet, the mechanism of release of the virus

is unknown. The CP strategy has also been found to occur in some of the semi-persistent whitefly-transmitted criniviruses.

The helper strategy involves the acquisition of some specific proteins encoded by the virus (termed helper component or HC) before the virus can be acquired and transmitted (Pirone and Blanc, 1996; Froissart *et al.*, 2002). This HC is very complex and is not limited to aphid transmission – it has also been found in mite-vectored, semi-persistently transmitted wheat streak mosaic tritimovirus and leafhopper-vectored, semi-persistent maize chlorotic dwarf waikavirus and tungro disease of rice. The HC strategy seems to allow non-specific acquisition of viruses by a number of vector species.

12.2.3 Persistent pathogen transmission

These pathogens are transmitted over weeks or months – essentially for the life of a vector. In persistently transmitted viruses and bacteria, the pathogens are ingested from the phloem or xylem of infected plants. There are two groups of persistent viruses, those that replicate in the arthropod (propagative) and those that do not (non-propagative). In the latter category of persistent aphid transmission, virions remain active in the haemolymph for the life of the insect but do not replicate (Eskandari *et al.*, 1979).

A factor that may be involved in the persistence of virus in the arthropod body is a protein conferring protection in the haemolymph. Some viruses (primarily luteoviruses and begomoviruses) are protected by symbionin (which is closely related to the heat-shock protein GroEL), which is synthesized and excreted by primary endosymbionts of aphids (van den Heuvel *et al.*, 1997) and whiteflies (Morin *et al.*, 1999). These viruses require the presence of symbionin to pass through the midgut-haemolymph barrier and are not subjected to proteolytic breakdown in the haemolymph. Viruses that are not protected are apparently destroyed over time and cannot be persistently transmitted.

12.3 Groups of Arthropod Vectors

The single most important order of vectors of plant pathogens is the Hemiptera, within which are two important suborders, Sternorrhyncha (aphids, mealybugs, psyllids and whiteflies) and Auchenorrhyncha (leafhoppers and planthoppers). These insects have sucking stylets that allow feeding – and thus direct pathogen transmission – into plant tissues including phloem and xylem, creating systemic infections within the plant. Furthermore, physical damage to the plant is minimal and plants respond uniquely (Thompson and Goggin, 2006). Other groups of vectors (Table 12.1) include Thysanoptera (thrips), Coleopteridae (beetles) and Eriophyidae (mites), which also cause physical damage to the plant.

All arthropods need to moult to increase in size; this process is known as ecdysis. The entire external cuticle is shed along with the fore- and hindgut. Only the midgut is not renewed during ecdysis. This has important implications in terms of pathogen transmission. Pathogens that are retained on the cuticle, in the mouthparts or fore- or hindgut can only be non-persistently or semi-persistently transmitted, until the next moult. Alternatively, those pathogens that are able to enter the haemocoel become circulative and are retained for long periods of time. This generalized moulting scheme is further complicated when considering the differences between hemimetabolous and holometabolous development schemes; the former represents gradual development from the egg stage until the adult stage is reached and all stages are feeding on the same plants, while the latter represent distinct morphological forms (egg, larva, pupae, adult). Holometabolous insects usually occupy different ecological niches and have radically different feeding mechanisms, except for the Coleoptera.

In addition to the above factors, the biology and life cycle of the organism play an important role in the ability to transmit plant pathogens. As such, the biology of each of the major groups of arthropod vectors will be briefly reviewed.

Table 12.1. Arthropod taxa in order of number of pathogens known to be transmitted.

Arthropod	Plant pathogen	Example	Approx. no. of pathogens[a]	Form of transmission
Aphids	Viruses		290	
	e.g. *Bromoviridae*			Non-circulative
	e.g. *Luteoviridae*			Circulative, non-propagative
	e.g. *Rhabdoviridae*			Circulative, propagative
Whiteflies	Viruses		128	
	e.g. *Potyviridae*			Non-circulative
	e.g. *Geminiviridae*			Circulative, non-propagative
Leafhoppers and planthoppers	Bacteria		26	Circulative, propagative
	e.g. *Candidatus* Phytoplasma			
	e.g. *Xylella*			Non-circulative, propagative
	Viruses		44	
	e.g. *Caulimoviridae*,			Non-circulative
	e.g. *Bunyaviridae*			Circulative, propagative
	e.g. *Geminiviridae*			Circulative, non-propagative
Beetles (four families)	Viruses		51	
	e.g. *Bromoviridae*			Circulative, non-propagative
	e.g. *Astroviridae*			Non-circulative
Mealybugs and soft scales	Viruses		21	
	e.g. *Caulimoviridae*			Non-circulative semi-persistent
	e.g. *Closteroviridae*			Circulative
Thrips	Viruses		16	
	e.g. *Bunyaviridae*			Circulative, propagative
Psyllid	Viruses		5	
	e.g. unidentified			Circulative, propagative
	Bacteria		6	
	e.g. *Candidatus* Phytoplasma			Circulative, propagative
	e.g. *Candidatus* Liberibacter			Circulative, propagative
Eriophyid mites	Viruses		10	
	e.g. *Bromoviridae*			Non-circulative, propagative
	e.g. *Potyviridae*			Non-circulative

[a]Note that the number of pathogens is not the same as the number of diseases, i.e. one pathogen may be called different disease names in various crops. There are hundreds of unidentified bacteria and viruses whose vector status is unknown.

12.3.1 Aphids

Of all of the arthropod vectors, aphids transmit more plant pathogens, particularly viruses, than any other single group. There are 227 species known to transmit viruses (Fereres and Moreno, 2009). Aphid mouthparts are typical of most Hemiptera: paired maxillary stylets fuse to form the food canal and separate salivary canal and these are surrounded by the paired mandibulary stylets, all of which are held together within the labium. Feeding behaviours have been described (reviewed by Backus, 1988). During feeding, hemipterans often secrete a small amount of gelling saliva, which forms a proteinaceous sheath to protect the delicate stylets as they move through different cells penetrating deeper into the plant, or an enzyme-laden watery saliva (Carolan *et al.*, 2009) to taste the contents of cells and determine if they are feeding in the correct tissue. Plant pathogens are introduced into plants during the secretion of saliva.

In aphids, there are no chemosensory structures on the labia. Instead, they must ingest some cell contents (epidermis, mesophyll or parenchyma) to determine whether they are feeding on an acceptable host plant. In species that vector non-persistent viruses, it is during these short probing periods that viruses are transmitted. When aphids have determined that the host plant is acceptable and they have chosen to feed, their stylets move between cells until they reach the phloem, in which they may feed continuously for days (Tagu *et al.*, 2008). It is during phloem feeding that persistent viruses are transmitted.

All immature and adult stages can transmit viruses. The behaviour of aphids in a colony often dictates the efficacy of transmission; many species tend to form colonies and remain apterous for a number of generations before alate forms are produced. As wingless individuals, apterae have only a limited capacity to move among plants; therefore, once on an infected plant, there is minimal transmission to surrounding plants. In contrast, alatae forms have a greater potential as vectors as they actively move by flight into fields and between plants in a field. Many species transmit in a non-persistent manner (the majority of virus transmission; Pirone and Perry, 2002), while fewer transmit in a semi-persistent or persistent manner (Reavy and Mayo, 2002).

12.3.2 Whiteflies

Whiteflies are small insects; the first stage is termed a crawler and is capable of moving up to several centimetres after hatching from the egg. Later stages are physically attached, usually to the underside of leaves, where they develop through four nymphal instars, pupate and emerge as winged, feeding adults. Although there are only four species known to transmit viruses, the primary group of viruses that they transmit, begomoviruses, is economically very important. Whiteflies have typical hemipteran mouthparts. Unlike aphids, at least some species possess chemosensory sensilla on their labium, meaning that they do not have to imbibe cell contents to determine whether a plant is an acceptable host (Walker and Gordh, 1989). Perhaps because of the presence of these chemoreceptors, they do not transmit non-persistent viruses, only persistent circulative and non-circulative viruses (Brown and Czosnek, 2002).

12.3.3 Leafhoppers and planthoppers

Leafhoppers and planthoppers range in size up to a little over 10 mm long, have five nymphal instars and have mouthparts typical of hemipterans. Unlike aphids, they have chemosensilla on the labia and can immediately detect the acceptability of the host plant on which they have landed without imbibing cell contents. Leafhoppers generally complete their life cycle on the plant, while most planthopper species lay eggs at or just below soil level and their immatures often feed on roots. Leafhoppers and planthoppers are capable of transmitting both viruses (Ammar and Nault, 2002) and

bacteria (Weintraub, 2011), in both immature and adult stages. Most hopper-transmitted viruses are found either in mesophyll cells or in phloem: phytoplasma and spiroplasma are found in phloem but *Xylella fastidiosa* is xylem limited. Most of the hopper vectors generally feed in one tissue type; however, some species (e.g. *Nephotettix virescens* and *Cicaduli mbila*) are capable of switching between different plant tissues (phloem and mesophyll, or phloem and xylem) in a host or among different hosts (Khan and Saxena, 1985; Mesfin *et al.*, 1995). *Circulifer tenellus* transmits phytoplasma, *Spiroplasma citri* and hybrigeminiviruses (Weintraub and Beanland, 2006).

12.3.4 Mealybugs and soft scales

Mealybugs are small, oval-shaped insects covered with a whitish, waxy secretion on the dorsal and lateral sides. There are three nymphal instars and pre-adult and adult stages. Central to their ability to transmit viruses is the fact that the first instar is known as a crawler and is capable of rapid movement, moving from plant to plant on air currents and even phoretically on birds' feet. At the end of the first instar, the insect settles down to feed and remains sessile until adulthood is reached. The males are winged but have no mouthparts and are therefore incapable of transmitting viruses. As all stages after the late first instar are non-mobile and females are apterate, they are not effective in transmitting pathogens during these stages. Mealybugs are phloem feeding and may be on any plant part: trunk/cane, leaves or fruit. There are 14 species that can transmit viruses in a semi-persistent manner (Muller, 2008). The virus does not multiply in the mealybugs.

Recently, whole virions of grapevine leafroll-associated virus 3 (GLRaV-3) have been found in salivary glands of *Planococcus citri*, transported from the gut, where it is usually found, via haemolymph (Cid *et al.*, 2007). These authors also found that the highest density of viruses was in the saliva, ready to be transported to plants during feeding. Although these viruses are transported to the salivary glands, they do not replicate in the insect body and are depleted after 96 h (Cid *et al.*, 2006). However, these findings are not applicable to all mealybugs that transmit virus, e.g. GLRaV-1 was not detected in the salivary glands.

12.3.5 Thrips

The relationship between thrips and virus has recently been reviewed extensively (Whitfield *et al.*, 2005, and references therein). There are ten identified thrips species that are vectors of plant viruses, but these species impact on crop plants worldwide as they are often polyphagous. The mouthparts of thrips are asymmetrical, consisting of a single left mandibular stylet that pierces the plant epidermis, after which paired maxillae (fused apically to form the food canal) are inserted. Unlike Hemiptera, which have separate salivary and food canals, all egestion and ingestion is through the one maxillary food canal. Thrips feed by piercing the epidermis, egesting saliva (which may contain virus particles) and then ingesting for either short or long periods. This feeding causes severe plant damage as some cells are emptied, leaving a whitish or silver appearance as the rigid cell is filled with air.

In addition to its asymmetrical mouthpart, Thysanoptera are unique in that they exhibit both holometabolous (first and second instars) and hemimetabolous (so-called pre-pupa and pupal instars, although it is not true pupation and they do not feed) characteristics. To be an effective vector, first-instar thrips must acquire virus from infected plants. Second instars feeding on infected plants yield just over 10% of infective adults. Adult thrips cannot acquire virus. Virus enters the midgut, replicating in the underlying muscle cells and then moves to the primary salivary glands where there is further replication. Once in the salivary glands, it is easily egested with saliva during plant feeding. The virus is persistent and is propagated in the

competent thrips vector, and the LP is sufficiently long that only adults transmit virus (Ullman *et al.*, 2002).

12.3.6 Beetles

Within the Coleoptera, there are 61 known species of beetles that transmit viruses (Mello *et al.*, 2010) and Chrysomelidae is the most important family (transmitting more than 30 viruses), with fewer representatives in the Coccinellidae, Curculionidae and Meloidae (Gergerich, 2002). Beetles are unique among the holometabolous orders because they retain their larval-stage chewing mouthparts (which physically damage the host plants) into adulthood, an important point because it is the winged adults that disseminate plant viruses.

Although our understanding of the mechanisms of transmission are limited (Gergerich, 2002; Mello *et al.*, 2010), some information is available. Virus acquisition is accomplished in as little as 1 min (Lomonossoff, 2008) and transmission is immediate (no LP), although the longer they feed, the greater the efficacy of transmission. Virus does not replicate in the beetles and the levels gradually diminish over time, so they could be considered semi-persistent vectors. Some virus may be retained in the beetles in the haemolymph for days or weeks. It is unknown how the virus moves from the haemolymph, but the gnathal glands may be involved (Gergerich, 2002). For other virus/beetle combinations, the virus does not cross the midgut barrier. In these cases, it is assumed that the virus is retained in the midgut where it is periodically regurgitated during feeding, for transmission to occur. The duration of virus retention varies with different virus/beetle combinations and the activity after feeding. In some cases, diapausing beetles are able to retain virus and immediately transmit it after breaking diapause. The specificity of virus transmission may be due to specific inactivation of most of the viruses in the regurgitated fluid that is produced by the beetles during feeding (Bos, 2008). Virus has not been found to replicate in beetles.

12.3.7 Mites

Eriophyid mites are very small (100–500 μm long), elongated and possess two pairs of legs. They can reproduce with a complete life cycle (two immature stages) within 1–2 weeks depending on the temperature. Because of their small size, they cannot walk great distances, but they are moved by wind or phoretically on insects. Their mouthparts are specifically adapted for piercing plant cells but only to a depth of a few cell layers; *Aceria tulipae* can penetrate about 5 μm (Oldfield, 1970). Typically, they are found in buds and other protected areas. Mite feeding can cause phytotoxic effects in the plant that look very similar to viral symptoms.

Virus is retained in the mite, even after moulting, for up to 10 days (Nault and Styer, 1970), so these mites demonstrate a form of at least semi-persistent transmission. The midgut is composed of a single layer of epithelial cells (Whitmoyer *et al.*, 1972). Virus was found in the midgut of *A. tulipae* and was not found in any other tissue, indicating that it is not circulative. Virus transmission is therefore probably by backflow to the mouthparts from the midgut or by defaecation on disrupted plant tissue. Like thrips, eriophyids must acquire virus at an immature stage if the adult is to be a competent vector (Nault, 1997).

12.3.8 Psyllids

Psyllids are small insects that are becoming increasingly important vectors of plant pathogens. There are generally five nymphal instars, all of which are mobile on the plant. They have typical hemipteran mouthparts. Unfortunately, little is known about their feeding behaviour (Bonani *et al.*, 2010). According to these authors, psyllid stylets move between cells to reach the vascular bundle and can feed on both phloem and xylem tissues, although predominantly on

the former. There are very few records of psyllids transmitting viruses; one such is *Cornegenapsylla sinica*, which transmits longan witches' broom disease virus (Chen and Xu, 2000). Different psyllid species transmit *Candidatus* Liberibacter (*Candidatus* Liberibacter asiaticus (Hung *et al.*, 2004) and *Candidatus* Liberibacter solanacearum (Secor *et al.*, 2009) and *Candidatus Phytoplasma* spp., all of which are phloem-limited and, as the name indicates, fastidious.

12.3.9 True bugs

Heteroptera, or true bugs, are not important virus vectors (reviewed by Mitchell, 2004). In general, they lacerate and flush plant cell contents, thereby acquiring viruses, but, unlike their auchenorrhynchan and sternorrhynchan relatives, they leave the plant visibly damaged. The best-documented example is of *Piesma quadratum* (Fieber), known to transmit beet leaf curl virus in a persistent and propagative manner. The virus can be found in the midgut, haemolymph and salivary glands (Proeseler, 1980). Other known heteropteran vectors of viruses are species of Miridae and Orsillidae. Unlike the majority of heteropteran vectors, the coreid, *Anasa tristis*, feeds directly in the xylem. It is the only known vector of curcubit yellow vine disease (*Serratia marcescens*) and seems to be persistently transmitted (Wayadande *et al.*, 2005).

12.4 Groups of Pathogens

12.4.1 Viruses

There is a diverse array of DNA and RNA plant-pathogenic viruses, but all viruses are intracellular parasites in that they must use the host cellular machinery to replicate (reviewed by Seal *et al.*, 2006; Hogenhout *et al.*, 2008). Because of this, host cells are often ultimately killed through the process of virus replication. In viruses infecting the epidermis or mesophyll, the virus initially replicates and moves from cell to cell, eventually crossing into the vascular system (primarily phloem) where it moves systemically throughout the plant (reviewed by Waigmann *et al.*, 2004). Other viruses are transmitted directly into the phloem by a variety of arthropod vectors. There are only a couple of viruses found in the xylem (Opalka *et al.*, 1998). This is probably due to the physical and biological characteristics of the xylem: the negative tension of xylem sap means that only larger Auchenorrhyncha have sufficient musculature to overcome this physical characteristic, and the nutritionally poor sap means that very large amounts must be consumed by the vectors (Novotny and Wilson, 1997).

12.4.2 Bacteria

There are several groups of bacteria transmitted by arthropods: Mollicutes (*Phytoplasma* and *Spiroplasma*), α- and γ-Proteobacteria and *Rickettsia*. This review will not include bacteria transferred through contamination such as *Pseudomonas* spp. and *Erwinia* spp. Mollicutes are Gram-positive bacteria that have lost their cell wall and have a relatively small genome size. Phytoplasmas are pleiomorphic, non-culturable bacteria causing hundreds of diseases and are transmissible by leafhopper, planthopper and psyllid vectors. Spiroplasmas are a small group (three identified species) of culturable, highly mobile bacteria, transmitted by a few species of leafhopper. There are two groups within the α-Proteobacteria: '*Candidatus* Liberibacter species' (diseases such as citrus greening and zebra chip) and *Rickettsia* (associated with phytoplasma in the phloem and insect vector). The γ-Proteobacteria include xylem-limited leafhopper-vectored *X. fastidiosa* and the phloem-limited planthopper-vectored species '*Candidatus* Phlomobacter fragariae' and the agent of 'basses richesses' syndrome of sugar beet.

12.5 Epidemiological Cycles and Vector Management

The basic cycle for any pathogen in a natural setting is that of a 'closed' system: the pathogen is transmitted to the wild host(s) by a vector species. This cycles at a low level because in nature plants do not occur in monoculture and vector populations are held in check by natural enemies. The alternative epidemiological cycle is that of an 'open' system: either new vectors or host species are introduced, resulting in a temporal or spatial spread of the pathogen. The new vector or host may arrive through human activities or range spreading due to climate changes. Often in a new environment there are no checks and balances on the species, or in the case of agriculture, vast numbers of new hosts are cultivated in or near an existing 'closed' epidemiological cycle.

While various management tactics may be used, the principal strategy is to reduce crop disease first by starting with clean, certified pathogen-free or pathogen-resistant seeds, planted in a field in which the surrounding area is free of diseased plants. Prevention of vector acquisition and/or transmission of plant pathogens through cultural practices or physical control must also be used. Reviews of general control methods in specific crop/vectors can be found (Hilje *et al.*, 2001; Radcliffe and Ragsdale, 2002; Jarausch and Jarausch, 2010; Weintraub and Wilson, 2010). If these tactics fail, then resorting to chemical applications becomes the *de facto* management tactic. However, as Perring *et al.* (1999) discussed in their excellent review, growers are most familiar with chemical application and, unfortunately, it is usually the first resort.

12.5.1 Chemical intervention

The most common and widespread method of vector control is through the use of insecticides. However, aside from the environmental problems, chemical application is not always effective and, with certain vectors, can actually lead to increased transmission and disease spread. The use of insecticides actually contributes to enhanced PVY transmission in peppers and potatoes (Budnik *et al.*, 1996; Radcliffe and Ragsdale, 2002) and other crops (Perring *et al.*, 1999). The exact mechanism is not clear, but apparently aphids can sense the insecticide, which acts as an irritant, causing them to be more flighty, thus feeding on more plants in a field.

Vector species are becoming increasingly resistant to a wider and wider variety of insecticides (Perring *et al.*, 1999) as a result of misuse/mismanagement. As growers are familiar with pesticides, they are often applied, and are applied prophylactically whether required or not. Because aphids cannot immediately determine whether they have landed on an acceptable host plant, during their initial probing behaviour non-persistent viruses are transmitted.

Persistently and semi-persistently transmitted pathogens require that the potential vector spend more time feeding on the plant; therefore, there is a greater chance of encountering pesticides for a long enough period for the vector to be adversely affected, i.e. for successful chemical management. What must also be taken into consideration is whether primary infective vectors are entering a field or whether secondary plant-to-plant infection is occurring. Pesticides generally do not act fast enough to kill the primary vectors. Mori *et al.* (2002) attempted to manage the primary phytoplasma vector *Hyalesthes obsoletus* by canopy spraying in 18 vineyards but achieved no population reduction and no reduction in disease incidence. Similarly, field tests with systemic (imidacloprid, thiamethoxan) insecticides applied as a seed treatment or spray against maize leafhoppers did not reduce disease incidence (de Oliveira *et al.*, 2007). Pesticides may reduce the number of vectors when secondary transmission is considered; therefore, a clear understanding of the host–vector–pathogen relationship is needed. Recently, based on caged trials mimicking primary and secondary

infections, Saracco *et al.* (2008) proposed developing a two-pronged strategy for the use of insecticides: (i) attempt to protect plants from infectious migratory primary vectors using systemic compounds such as neonicotinoids; and (ii) suppress vectors within a field in secondary infections by using organophosphate insecticides. Indeed, a study of six grape cultivars showed that vineyards treated with two imidacloprid applications had a lower incidence of Pierce's disease after 3 years compared with controls (Krewer *et al.* 2002) and that lower leafhopper vector populations were found in the treated vineyards.

The timing of insecticide applications is also critical. The phytoplasma vector *Cacopsylla melanoneura* has two hosts: (i) in the spring and summer, adults transmit apple proliferation disease to apples and lay eggs; and (ii) the new generation, having fed on infected apple trees, then moves to alternate hosts, primarily pine (Mayer and Gross, 2007). Because of the alternate host, it is only possible to treat when the infective overwintering adults arrive in the spring, and use of chitin synthesis inhibitors or mimics can focus on the developing nymphs (Baldessari *et al.*, 2010) and will not harm beneficials. In fact, because of the dispersal and two-host biology, it would be wise to employ regional management tactics.

Kaolin, a non-abrasive fine-grained aluminosilicate mineral, applied as a particle film, is a new version of a very old type of inorganic chemical control that may prove to be useful. Work by Puterka *et al.* (2003) demonstrated that kaolin protected grape plants from feeding and oviposition by *Homalodisca coagulata* (vector of *X. fastidiosa*) by physically coating the plant. Tubajika *et al.* (2007) showed that grapevines treated with kaolin were less likely to become infected with the bacteria and fewer leafhoppers were found in treated fields.

Spraying of mineral oils is a low-technology form of insecticide, which, like kaolin, creates a physical barrier on the plant and is effective at interfering with aphid feeding, especially for those transmitting non-persistent viruses. While this has proven effective in temperate climes (Wang and Pirone, 1996; Powell *et al.*, 1998; Asjes, 2000), there are problems. Mineral oil photodegrades under ultraviolet light (Hodgkinson *et al.*, 1999), and in Mediterranean Basin countries and other latitudes with intense sunlight, the oil can literally burn the leaves so it cannot be applied frequently.

12.5.2 Plant material

Pathogen-free seeds and seedlings can be produced by a variety of methods and it is beyond the scope of this chapter to detail those methods. However, the production of seedlings through tissue culture involves the use of low concentrations of antibiotics, which may not be enough to kill bacteria (e.g. phytoplasma) but could mask symptoms (Weintraub *et al.*, 2004) and lead to the inadvertent introduction of diseased plants.

A plant that is resistant to a virus has the ability to suppress virus replication and the development of symptoms. Developing plants that are genetically resistant to arthropods or the virus pathogens that they transmit is an arduous challenge, but once achieved represents a very effective management tool. By the same token, it places selection pressure on the pests and pathogens, which in turn may develop resistant races. There are recent reviews of the mechanisms of plant resistance to viruses and lists of collections of genetic resources for a wide range of crops (Lapidot and Friedman, 2002; Kaloshian, 2004; Seal *et al.*, 2006; Vanderschuren *et al.*, 2007; Gomez *et al.*, 2009).

The situation with regard to resistance of arthropod-transmitted bacteria is not as advanced as with the viral pathogens. Phytoplasma-resistant rootstocks have been found for a few trees (Seemuller and Harries, 2010) but not for field crops. There are indications that there is some resistance in some maize to the leafhopper-vectored *Spiroplasma kunkelii* (Silveira *et al.*, 2008), although this is still in the developmental stages.

12.5.3 Cultural methods of control

There are a number of methods, related to agrotechnical activities, that can have a significant effect on vector populations. These include planting time, weed control/habitat management, trap cropping and roguing diseased plants. There are other techniques, such as different types of mulch (e.g. plastics, natural materials) and ground cover that are occasionally but not consistently effective, so they will not be discussed.

Temporal management tactics

Often, when a vector is polyphagous and there are a limited number of pathogen reservoir plant species, almost the only effective means of controlling some viruses is through a crop-free period. If there are abundant weedy hosts, for example, a crop-free period would not produce effective results. To achieve control, all growers in a geographical area (Ucko *et al.*, 1998) must consent to cease growing and completely remove the specific crop. Barring voluntary consent, the crop-free period may need to be mandated legislatively. As reviewed by Hilje *et al.* (2001), crop-free periods have been used since the 1920s as means of reducing virus-transmitting whiteflies. Because of its efficacy, this method continues to be used today (Seal *et al.*, 2006; Nannini *et al.*, 2009).

Another means of limiting virus is by manipulating planting and harvest dates. By planting after large vector populations have left an area or by harvesting before they arrive, virus infestation can be reduced. This has been shown to be effective in controlling aphid-vectored PVY in seed potatoes (Radcliffe and Ragsdale, 2002), planthopper-vectored rice viruses (Zhu *et al.*, 2009) and whitefly-vectored viruses in vegetable crops (Hilje *et al.*, 2001).

Weed control/habitat management

Vectors can be reduced through habitat management. Bressan *et al.* (2007) clearly demonstrated the effect of different weed species on the presence of phytoplasma in grapevines. The majority of individuals of the vector *H. obsoletus* were infected with phytoplasma when weeds that were hosts to the vector and phytoplasma were present in vineyards. In contrast, when weeds that were neither vector nor phytoplasma hosts were present, less than 10% of the planthoppers were infected. Furthermore, herbicide treatment of weed species during the winter significantly reduced vector populations. Weeds have also been shown to be reservoirs for viruses. Cohen *et al.* (1988) showed that *Cynanchum acutum* acts as an overwintering reservoir for tomato yellow leaf curl virus and serves as a plant host upon which whiteflies feed as populations grow in the spring and summer.

As pointed out by Weintraub and Beanland (2006), vegetation composition surrounding a field, orchard or vineyard has a profound effect on the presence and dispersal of phytoplasma vectors. Just as the type of vegetation may mitigate leafhopper populations, a lack of vegetation can also prevent leafhopper movement. As *Scaphoideus titanus* is monophagous and is known to aggregate within a vineyard, plant density could be a potential means of control. Lessio and Alma (2004) studied the effects of grapevine density on the ability of *S. titanus* to disperse within and outside a vineyard, and found that discontinuity of plants strongly affected its movement. In fact, they found that, under normal wind conditions, *S. titanus* could not disperse beyond a 24 m radius. This means that removal of plants can create an effective barrier, protecting vineyards from this phytoplasma vector.

Barrier/trap crops

Plants surrounding a crop can be planted with one of several intentions with respect to the arthropod vector: (i) to act as a barrier that the vector will not penetrate or fly over; (ii) to act as an attractant with the intention of holding the vector within; (iii) to camouflage or mask the primary crop; and (iv) to act as a cleansing mechanism (for general reviews, see Hooks and Fereres,

2006; Shelton and Badenes-Perez, 2006). As a barrier to whiteflies, both sorghum and maize have been used with mixed results (Smith and McSorley, 2000; Hilje *et al.*, 2001). Trap crops such as beans, aubergine, cantaloupe and squash have been suggested for controlling whitefly. While whiteflies are attracted to the trap crop, most research has shown this to be an ineffective means of managing *Bemisia* spp. (Stansly *et al.*, 1998; Smith and McSorley, 2000; Castle, 2006; Lee *et al.*, 2009). There have been very few attempts to use trap crops for leafhopper vectors and only one study met with partial success (Zhou *et al.*, 2002).

The use of barrier crops for controlling aphid-borne non-persistent viruses has recently been reviewed (Hooks and Fereres, 2006, and references therein). As these authors discussed, aphids locate plants by detecting the contrast between soil and foliage at the field edges. Therefore, the greater the percentage of another crop around the primary field, the less the chance of aphids landing in the primary field. Additionally, non-persistently transmitted viruses are lost in a short time with probing behaviour. By providing barrier crops that are not virus hosts, the aphid mouthparts will be cleaned as they move through the barrier plants before arriving in the crop. Assuming that the primary crop is virus free, the 'cleansed' aphids would only cause direct damage in the primary field.

Roguing

Roguing, or the removal of infected plants, is usually only attempted in small plots or orchards, where every plant can be inspected individually. Not only should the infected plant be removed but also those immediately surrounding it (Mowry, 1994) as they may also be infected. Similarly, in orchards, before removing an infected tree, it should be treated with insecticides to kill all vectors so that they do not simply abandon the tree and infect surrounding trees (Uyemoto *et al.*, 1998).

12.5.4 Physical control

Physical methods basically involve the erection or creation of a barrier between the arthropod vector and the crop. This can take the form of fences, covered tunnels of various heights and lengths, and floating row covers.

The most reliable means of controlling vectors is by covering the crop with floating row covers. These physically exclude all arthropods (Orozco-Santos *et al.*, 1995) from the time of sowing/planting. Covers are placed loosely over the soil and as the crop grows it pushes up the fleece. The problems encountered with these coverings are that: (i) woody plants can easily tear the covering; (ii) they cannot be used in large scale open-field cropping systems; (iii) with certain crops they must be opened to allow pollination; and (iv) depending on the climate, some pathogens can be exacerbated by the increased humidity. Notwithstanding these limitations, they have been used successfully to delay the onset of transmitted bacterial and viral diseases (Orozco-Santos *et al.*, 1995; Abou-Jawdah *et al.*, 2000; Bextine *et al.*, 2001; Rekika *et al.*, 2009). Trials on open-field seed potato production have not met with success to date (P.G. Weintraub, unpublished data). Due to the intensity of sunlight in Israel, the 17 and 19 g/m^2 nets become brittle and tear after about 80 days in the field, unfortunately corresponding to increasing populations of PVY-bearing aphids. Work is continuing: earlier planting dates and physical support structures are being investigated.

Insect exclusion screening (usually 50 mesh) is a heavier screening (less subject to tearing) used in greenhouses, tunnels and fences (reviewed by Weintraub and Berlinger, 2004). While these screens are ideal for tunnels and greenhouses when properly maintained, they only exclude larger arthropods; mites, thrips and whiteflies can penetrate these screens, albeit at a reduced rate. Insect exclusion screens can have different spectral properties (Legarrea *et al.*, 2010) through the addition of chemical compounds. As most insects and

mites have photoreceptors sensitive to ultraviolet radiation (McEnroe and Dronka, 1966; Diaz and Fereres, 2007), these screens can be even more effective at reducing insect populations. Their effectiveness in reducing virus incidence and populations of whiteflies and thrips has been clearly demonstrated (Antignus *et al.*, 1998; Costa and Robb, 1999). Research showing the positive effects of covering fruit trees is slowly gaining usage with growers worldwide (e.g. for bananas) and this trend will probably continue. Walsh *et al.* (2006) demonstrated that vectors of phytoplasma-caused papaya diseases could be 100% controlled by covering the trees with screening. Weintraub *et al.* (2008) showed that populations of leafhoppers transmitting phytoplasmas were significantly excluded from entering walk-in tunnels when covered with ultraviolet-absorbing plastic.

Fences are usually erected using 50-mesh screening and can be of various heights, depending on the vector. Gerling and Horowitz (1984) found that whiteflies normally fly at a height of less than 2 m above the ground. In a trial performed on tomatoes evaluating whitefly-transmitted viruses, Holt *et al.* (2008) found that a fence 1.5 m high, with pesticide strips to kill the whiteflies, delayed disease onset by about 2 weeks. However, in a fenced area without pesticide strips, there was actually a higher incidence of viral disease. Through modelling of their results, they concluded that the fencing prevented initial entry of the whitefly, but, at the same time, once the whiteflies had entered, they were contained and did not leave. Other researchers have found inconsistent results with whitefly barriers, which can perhaps be explained by vector migration. Isaacs and Byrne (1998) trapped *Bemisia tabaci* at a height of 7.2 m, and, under the correct conditions, clouds of whiteflies can be observed moving between fields during harvest in south-western USA (P.G. Weintraub, personal observation).

Unlike whiteflies, aphid flight tends to be at higher elevations above ground (Radcliffe and Ragsdale, 2002, and references therein). They generally fly straight up and then horizontal once they reach about 8 m. Although they are weak fliers, they can be carried along by wind currents and stay aloft for hours but actively fly downwards with the proper stimuli. For this reason, fences are not an effective control tactic.

I observed a situation in the field in 2003 where a *Gypsophilia* sp. grower had opened a 500 m^2 greenhouse but left the east and west 1.9 m tall walls intact. Phytoplasma-transmitting leafhoppers were contained by the walls and, while this grower suffered 90% loss, the *Gypsophilia* sp. growing neighbour sustained a loss of less than 3%. In a study by Blua *et al.* (2005), they erected a 5 m tall screen barrier to determine the movement of the *X. fastidiosa* vector *H. coagulata* and found that the leafhopper was repelled by it and was deflected to surrounding vegetation. They concluded that, especially in the case of high-value crops, a physical barrier is an effective management tool.

12.5.5 Biological control

While attractive and environmentally friendly, biological control tactics are often not effective in controlling vectors of plant pathogens for the simple fact that transmission can occur very quickly while biological control is relatively slow. However, whenever possible, broad-spectrum insecticides should not be used to preserve the existing guild of natural enemies as they too contribute overall to control. As is often the case, parasitoids have been found to be highly susceptible to insecticide residues.

12.6 Future Control Methods

There are currently many avenues of research that will, hopefully, bear fruit in the not-too-distant future. Many of the vector arthropods, especially those whose primary food source is nutrient poor (xylem and phloem feeders), carry a diverse assemblage of symbiotic microorganisms that are inherited maternally and that have numerous and major effects on their hosts

(Brownlie and Johnson, 2009). Symbionts found in aphids, leafhoppers, whiteflies, etc. generate essential amino acids and vitamins needed by their hosts. These bacteria can be genetically modified to affect or prevent the transmission of pathogens by (i) reducing vector competence; (ii) expressing a gene product that could kill the pathogen; (iii) inducing cytoplasmic incompatibility causing a high offspring-mortality rate; or (iv) physically competing for space that the pathogenic bacteria would normally occupy (Beard *et al.*, 1998; Ramirez *et al.*, 2008). Other research is also exploring ways of increasing plant defences. Many of the hemipteran vector species attempt to overcome plant defences through chemicals in their saliva (Carolan *et al.*, 2009). Plants respond to invasion in a number of ways, not just at the point of invasion but also systemically. A number of chemical inducers have been elucidated and their use is being explored to activate the plants' natural defences, including systemic acquired resistance (Lherminier *et al.*, 2003; Durrant and Dong, 2004; Hammerschmidt, 2009; Walling, 2009; Wei *et al.*, 2009). Research is also exploring ways to interfere with pathogens directly, such as mutated pathogen genes and coat proteins, and gene silencing (reviewed by Fuchs and Gonsalves, 2007; Vanderschuren *et al.*, 2007). Some of these tactics are still being explored in model plants such as *Arabidopsis* and others have been moved into important crop plants. Other potential areas of research are methods of disrupting pathogen binding (e.g. helper and capsid strategy) and release from the arthropod vector. With these multidimensional approaches to vector and pathogen management, the potential for achieving integrated control and moving away from reliance solely on chemicals seems to be an attainable goal in the not-too-distant future.

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13 Effect of Pesticides on Non-target Sites with Reference to Soil Ecosystems

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13.1 Introduction

Globally, food production has received impetus for more than four decades from the use of synthetic organic pesticides, despite pesticides being known for their negative repercussions on non-target organisms (Atlas *et al.*, 1978; Bharati and Subba Rao, 1986; Bhuniya *et al.*, 1994). Because of the persistent nature of some pesticides, contaminated soil and water may also have long-term deleterious impacts on human health through allergic reactions and mutagenic, teratogenic or carcinogenic effects (Krishnamurthi *et al.*, 2006). Soil harbours an array of microbes responsible for naturally renewing soil fertility, as well as executing other important functions. The conservation of soil biodiversity is therefore essential to enhance and maintain soil fertility for sustainable agricultural productivity (Matson *et al.*, 1997). A lack of understanding of soil life will weaken soil functions, leading to the loss of fertile lands, and can lead to an over-reliance on chemical means for maintaining agricultural production. This necessitates forging collaboration among soil biologists and other stakeholders in promoting improved soil biological management. Knowledge of soil biodiversity is still limited, and there remains a need to explore the impact of pesticides on soil microbial diversity, which will require assessment and evaluation of specific functions of soil biota in relation to various impacts of pesticides, which will affect management practices (Wake, 2001).

13.2 Binding and Persistence of Pesticides in Soil

Soil organic matter contains fulvic and humic acids with a high cation exchange capacity, which can bind to pesticides or transformation products in soil, facilitated by abiotic or biotic agents (microbial or plant enzymes). The processes involved are akin to those responsible for the humification process. Other mechanisms involved in binding of pesticides to organic matter are through van der Waals' forces, hydrogen bonding and hydrophobic bonding resulting in sorption, charge transfer, ion or ligand exchange, covalent bonding or mixture of these reactions. It is possible to isolate and identify cross-coupling products and to elucidate the site and type of binding by understanding the formation of covalent linkages between pesticides and humus constituents such as fulvic and humic acids

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in the presence of phenol oxidases or clay minerals. The binding of chlorinated phenols to humic substances has been determined by using ^{14}C-labelled chemicals, measuring the uptake of radioactivity by the humic material. These experiments paved the way for an understanding of the formation of bound residues in soils and visualizing the toxic potential of immobilized pollutants (Bollag, 1992). Formation of phenol or aromatic amine intermediates occurs through hydrolysis of pesticides to phenol or aromatic intermediates, which becomes a part of the fabric of humic material through covalent bonding catalysed by oxido-reductive enzymes or abiotic agents (Bollag, 1991). Further investigations by Bollag (1991) showed that the release of xenobiotics through biochemical reactions in soil is slow and that they are further degraded by microbes, in contrast to the presumption that pesticides were bound to humus and did not pose a threat to the environment. The impact of humus in soil on 17 pesticides was investigated in relation to altitude in an international project on a monitoring network in the Alpine region for persistent and other organic pollutants (MONARPOP) in the German Alps, which revealed that altitude has a considerable influence on the concentration of some organic chemicals in humus (Voigt *et al.*, 2010).

Measurement of the persistence of pesticides in soil ecosystems is conveniently done by calculating the time required for a known concentration of pesticide to diminish by half (half-life), and is a function of biotic and abiotic degradation mechanisms (Calamari and Barg, 1993). Modern pesticides are synthesized to have a shorter half-life, but some pesticides such as parathion are known to take a prolonged time to degrade in the soil ecosystem (Siddaramappa *et al.*, 1973; Spencer *et al.*, 1977). Fishbein (1974) detected dichlorodiphenyltrichloroethane (DDT) in soil as long as 10 years after treatment. Persistence of pesticides for longer periods has an effect on the growth and biomediated processes beneficial for soil fertility (Grossbard, and Davies, 1976; Tu and Miles 1976; Atlas *et al.*, 1978; Cook *et al.*, 1978; Juneja and Dogra, 1978; Wainwright, 1978; Congregado *et al.*, 1979).

13.3 Nutrient Transformation

Pesticides are known to have an impact on the soil biology and related processes as a result of their xenobiotic properties. Some of the deleterious effects are by directly targeting N_2-fixing and phosphorus-solubilizing organisms by disturbing molecular interactions between plants and the N_2-fixing microsymbionts, impairing the biological nitrogen-fixing processes. Important processes in soil, such as nitrogen mineralization, ammonification, redox reactions and enzymatic activity governing the nitrogen cycle, are disturbed by the persistence of pesticides in the soil ecosystem (Nayak and Rao, 1980; Greaves *et al.*, 1981; Ingeborg and Anderson, 1991; Lodhi *et al.*, 1994).

The persistence of the insecticide Baythroid in soil increases immobilization/remineralization of added inorganic N, mineralization of organic N, and nitrification, and the impact was found to be greater with enhanced amounts of insecticide residues (Lodhi *et al.*, 1994), indicating that excessive amounts of Baythroid affect the qualitative and quantitative microbial population. Greaves *et al.* (1981), using *in vitro* studies, attempted to assess the impact of two concentrations of 53 pesticides on nitrogen transformation processes in sand (0.8% C, pH 5.3) and silt (1.6% C, pH 5.7) by measuring the rates of production and quantities of ammonium, nitrate and nitrite A difference of more than 10% between the control and the treatment was considered to be a pesticide-induced effect. Application of 31 of these pesticides at a recommended dose had no long-term effect on nitrogen mineralization. Similarly, at normal strength (2.6 and 26 p.p.m.), dalapon had little impact on soil microbes, but at higher concentrations, it inhibited nitrification in the soil ecosystem. However, in this study, the effect of clay in the soil was not taken into account.

13.4 Biological Nitrification

The process of nitrification in the soils is mediated by two chemoautotrophic bacteria, *Nitrosomonas* and *Nitrobacter*, and the effect of pesticides on these organisms can affect nitrogen release into the soil for plant nutrition. This was studied initially by Lees and Quastel (1946) using a soil perfusion unit. Manometric techniques and perfusion units were also used to monitor the impact of pesticides on these chemoautotrophs to determine the mode of action of several pesticides on growth in aerated cultures of *Nitrobacter agilis*. Winely and SanClemente (1970) investigated eight pesticides and observed no inhibition by aldrin (Fletcher and Bollen, 1954; Shaw and Robinson, 1960; Bartha *et al*., 1967), simazine (Bartha *et al*., 1967) or heptachlor (Shaw and Robinson, 1960). Brown (1954) observed retardation of nitrification with lindane and chlordane with applications of 1000 lb/acre, but Shaw and Robinson (1960) saw no effect with chlordane at 300 lb/acre. Cytochrome *c* and cytochrome *a* components are responsible for nitrite oxidation in *Nitrobacter* (Lees and Simpson, 1957; Aleem and Nason, 1959), but none of the pesticides tested inhibited cytochrome *c* activity (Winely and SanClemente, 1970) except for heptachlor at a concentration of 200 μg/ml, which caused total inhibition of cell-free extract of nitrite oxidase particularly targeting cytochrome *c* oxidase. Straat and Nason (1965) postulated that the enzymatic reduction in nitrate in *Nitrobacter* might be the first step of a sequence for providing nutritional nitrogen, or it could be a means of recycling nitrite for nitrite oxidation. Further investigations carried out by Winely and SanClemente (1970) on the impact of pesticides on the growth of aerated cultures of *N. agilis* observed no inhibitory effect of aldrin and simazine on the growth of bacteria, but five compounds (isopropyl *N*-(3-chlorophenyl)carbamate (CIPC), chlordane, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), heptachlor and lindane) prevented growth when added to the medium at a concentration of 10 μg/ml. Heptachlor and chlordane also caused only partial inhibition of oxidation, but were more toxic with cell-free extract nitrite oxidase. None of the pesticides inhibited the nitrate reductase activity of cell-free extracts, but most caused some repression of cytochrome *c* oxidase activity.

Addition of benomyl, a carbamate fungicide, to alluvial, laterite and two acid sulfate soils resulted in significant increases in N_2 fixation, while carbofuran, a methylcarbamate insecticide, exerted a stimulatory effect on N_2 fixation in alluvial, alterite and acid saline soils. γ-Benzene hexachloride (γ-BHC), a chlorinated hydrocarbon insecticide, stimulated N_2 fixation in alluvial and acid sulphate soils, while considerable inhibition of N_2 fixation was evident in other soils. However, specific groups of N_2 fixers responded to the pesticides in different soil types (Nayak and Rao, 1980). Four insecticides, γ-BHC, phorate, carbofuran and fenvalerate, at rates of 7.5, 1.5, 1.0 and 0.35 kg/ha, respectively, were applied in laterite (Typic Orchragualf) soil to assess their effects on the growth and activities of N_2 fixers and phosphate solubilizers. Insecticides in general, and BHC and phorate in particular, stimulated the proliferation of aerobic non-symbiotic N_2-fixing bacteria and phosphate-solubilizing microorganisms as well as their biochemical activities, which resulted in greater release of available N (NH_4^+ and NO_3^-) and P in soil. All the insecticides were persistent in the soil for a short period of time, and the rate of dissipation was highest for fenvalerate followed by phorate, carbofuran and BHC, with half-lives of 8.8, 9.7, 16.9 and 20.6 days, respectively. The insecticides followed first-order reaction kinetics during their dissipation in the soil (Tu, 1970).

Mineralization of ^{14}C-labelled maize straw in the soil was investigated using denaturing gradient-gel electrophoresis of 16S rRNA gene sequences and ^{13}C nuclear magnetic resonance spectroscopy to monitor the influence of the fungicide dithianon at a concentration of 50 mg/kg on the mineralization and microbial activity

(Liebich *et al.*, 2009). Despite effects on the microbial communities, no significant differences in humification products after 26 weeks of incubation were observed, However, the build up of fungal biomass was inhibited for at least 3 weeks.

13.5 Soil Respiration

To understand the effect of pesticides on soil health, measurement of soil respiration is an effective tool to gauge impairment to soil fertility. Soil respiration also has been used as an indicator of heavy metal toxicity (Brookes, 1995). Zelles *et al.* (1985) used this tool for a 48-day period to assess the impact of various herbicides (atrazine, pentachlorophenol, 4-chloroanile and chloroacetamide), fungicides (zineb and captan) and insecticides (lindane and 4-nitrophenol) on soil respiration. He observed that pesticides induced tangible changes on microbial behaviour. Furthermore, certain microbial variables were monitored for a 27-day period in an Alberta agricultural soil treated with 2,4-dichlorophenoxyacetic acid (2,4-D), picloram and glyphosate at concentrations of 0, 2, 20 and 200 μg/g of soil. All the herbicides at the concentration of 200 μg/g increased the basal respiration for 9 days only. However, substrate-induced respiration was temporarily depressed by picloram and 2,4-D at 200 μg/g but briefly enhanced by glyphosate at 200 μg/g. Tu (1992) applied eight herbicides (atrazine, butylate, ethalfluralin, imazethapyr, linuron, metazachlor, metribuzin and trifluraline) at 10 mg/kg of soil to a loamy sand and observed that soil respiration increased significantly after 96 h incubation with atrazine, suggesting that these herbicides are not deleterious to the soil microbial activity. Haney *et al.* (2002) investigated the effect of isopropylamine salt of glyphosate at concentrations of 47, 94, 140 and 234 μg/g of soil in a Westwood silt loam on microbial activities and microbial biomass. His observation revealed that there was a stimulation of soil C and N mineralization, and microbial biomass was least effected. The C mineralization rate increased from day 1 up to 14 days on application glyphosate. Araújo *et al.* (2003) experimented with the use of glyphosate applied at a rate of 2.16 μg/g of soil, and measured microbial activity by soil respiration (evolution of CO_2) over a period of 32 days in typical Hapludult and Hapludox soils of Brazil and found an increase of 10–15% in the CO_2 evolved in the presence of glyphosate compared with control treatment. Zelles *et al.* (1985) monitored soil microbial activity by determination of ATP content, respiration and fluorescein diacetate hydrolysis for 48 days under the influence of the herbicides atrazine, pentachlorophenol, 4-chloroaniline and chloroacetamide, the fungicides zineb and captan, and the insecticides lindane and 4-nitrophenol, and found that the smallest effects were caused by atrazine, lindane and captan, whereas soil respiration was stimulated by several of the other pesticides at various concentrations. Improvement of soil by the addition of lucerne meal promoted reversibility of the effects caused by the chemicals.

13.6 Soil Enzymes

Soil enzyme activity and microbial biomass are also used as diagnostic tools of soil health affected by different pollutants, agricultural management and land use. Soil enzyme activity provides information on the soil biochemical processes and is regulated by pH and microbial biomass (Dick *et al.*, 1988), as well as soil compaction (Karaca *et al.*, 2000). Some researchers (Bandick and Dick, 1999; Tscherko and Kandeler, 1999) have used these diagnostic tools for measuring environmental stress in relation to soil organic matter, and soil moisture content (Harrison, 1983). Soil enzyme activity is variable over time and is limited by the available substrate supply (Degens, 1998), but may provide a useful link between the microbial community composition and carbon processing (Waldrop *et al.*, 2000). Enzymatic activities during soil microbial processes were used

by González *et al.* (2007) as sensitive indicators to detect alterations in soil health. The importance of enzyme phosphatases in fundamental biochemical processes is indicated by its presence in bacteria in mammals (Posen, 1967). In soil, a group of enzymes that are responsible for the hydrolytic cleavage of a array of ester-phosphate bonds of organic phosphates and anhydrides of orthophosphoric acid (H_3PO_4) into inorganic phosphate are designated phosphatases. The ester bonds binding P to C (C-O-P ester bonds) in organic matter are hydrolysed by acid and alkaline phosphatases, and inorganic P is mineralized from organically bound P in soil organic matter. Evaluation of urease activity in soil helps to develop strategies for nitrogen management. Urease activity is impaired only when the bioavailability in the soil is affected (Nadgórska-Socha *et al.*, 2006). Soil microbes such as fungi, bacteria and actinomycetes may be capable of rapidly degrading pesticides by producing different enzymes in response to different organic substances as the carbon source involved in different metabolic pathways (Degens, 1998). The availability of organic compounds for the organisms present in soil requires specific processes for decomposing pesticide residues, and this is known as co-oxidation (Hill and Wright, 1978). The oxidation processes of the enzymes often make the pesticide more amenable to degradation at the extracellular level and then proceed further at the intracellular level, causing an increase in its water solubility. In an incubation study investigating enzyme activity in soil affected by pesticide, Rahmansyah and Sulistinah (2009) observed that phosphatase activity was lower compared with that of urease. Enzyme activity fluctuated at the start of the 12-week incubation period and finally declined. The first 2 weeks showed an increase in phosphatase and urease levels to 2.45 and 49.25 units/g of soil, respectively. Urease activity was more attenuated in soils with pesticides. CO_2 release observed at 4 weeks with soil with pesticide was 2.55 mg/g/h compared with the soil with no added fresh pesticides, which peaked CO_2 later at 6 weeks' incubation at 1.82 mg/g/h. Deydrogenase activity (triphenyltetrazolium chloride-reduction) in soil is used as another sensitive indicator for effects of pesticides on soil microorganisms for understanding ecological and ecotoxicological influences on soil health (Malkomes, 1991). This enzyme correlates well with several other microbiological variables in soils and has been advocated for testing soil health (Malkomes, 1991). Another enzyme, nitrogenase, responsible for N_2 fixation, was investigated by Vlassak and Livens (1975) to assess the negative effect of pesticides.

13.7 Metagenomics

Metagenomics is used in molecular biology and genetics for quantitative and qualitative analysis of the genetic material from soil samples to understand the impact of pesticides on soil ecosystems, in which microbes in the soil are responsible for various transformations to maintain the fertility of agricultural soils. DNA is one of the most sensitive cellular targets for hazardous chemicals and wastes, which may mutate the bases or cause disruption of the sugar–phosphate backbone (Paterson, 1978; Birnboim and Jevcak, 1981). Yang *et al.* (2000) used randomly amplified polymorphic DNA (RAPD) analysis to evaluate the effect of triadimefon and ammonium bicarbonate and their intermediates on DNA sequence diversities for microbial communities in four soils by using 14 random primers to amplify DNA from four soil microbial communities and generated 134 polymorphic fragments from 155 reliable fragments. The results indicated that chemicals meant for pesticide management affected the soil microbial community diversity at the DNA level. The four soil microbial communities were distinguishable in terms of DNA sequence richness, modified richness, Shannon–Weaver index and coefficient of DNA similarity. By investigating the data, the amounts of organic C and microbial biomass C were found to be low in the soil polluted by

pesticide (mainly triadimefon and its intermediates) but high in the soil polluted by chemical fertilizer (mainly ammonium bicarbonate and its intermediates), and revealed that pesticide contamination decreased soil microbial biomass but maintained high diversity at the DNA level. In contrast, chemical fertilizer pollution caused an increase in the soil biomass but a decrease in the DNA diversity. Analysis of soil microbial biomass appears to be an effective approach for studying the diversity of soil microbial communities. The DNA-damaging potential of pesticide-contaminated soils and the genotoxicity of individual compounds present in the soil were assessed using fluorimetric analysis of DNA unwinding. Krishnamurthi *et al.* (2006), in an experiment with pesticide-contaminated soil samples, showed DNA strand breaks of 79% ($P<0.001$) and 60% ($P<0.02$) damage, respectively, caused by carbaryl and α-napthol, which are the major constituents of the soil sample, analysed by gas chromatography–mass spectrometry (GC-MS). At the subcommunity level, Widenfalk *et al.* (2008) analysed microbial community structure (phospholipid-derived fatty acid composition and bacterial 16S rRNA genotyping, and terminal restriction fragment length polymorphism (TRFLP)) and no impact was seen by pesticide exposure at lower levels of microbial community organization; however, molecular methods revealed treatment-induced changes in community composition. Captan and glyphosate exposure caused significant shifts in bacterial community composition (shown by TRFLP) at environmentally relevant concentrations.

13.8 Soil Invertebrates

Soil invertebrates have a significant role in soil formation along with other microflora and microfauna. Earthworms comprise 80% of total terrestrial invertebrates, which play an important role in perturbation and nutrient cycling. Any change in soil health results in qualitative and quantitative changes in earthworm populations. Therefore, they can be considered a suitable diagnostic tool for measuring the effects of the chemical constituents of soil health and serve as advanced warning of soil health impairment (Culy and Berry, 1995), which is important for protecting soil health and consequently human health (Reinecke and Reinecke, 1999), as well as that of other vertebrates that feed on earthworms (Sandoval *et al.*, 2001). Use of earthworms as a bioindicator of soil contamination is primarily because of their mechanism of digestion of degraded soil organic matter in soil and their responsibility for synthesis of a rich soil horizon (Reinecke and Reinecke, 1999).

Soil macrofauna show elevated metabolism involving excretion of urea and ammonia in pastures treated with carbaryl (Pradhan and Mishra, 1986). Bharti and Subba Rao (1986) observed reduced carbohydrate and glycogen content and elevated phosphorylase 'a' and 'b' activity in the muscle and blood of the earthworm *Lampito mauritii* in contact with even sublethal doses of phosphomid, monocrotophos and dichlorvos. Impaired carbohydrate metabolism was also seen in wall muscle of the earthworm *Octochaetona pattoni* (Varadaraj, 1986). Elevated levels of blood sugar following the use of fenitrothion was observed by Reddy *et al.* (1996). Lord *et al.* (1980) showed that the skin of earthworms serves as a passage for contaminant uptake and suggested that earthworms can be conveniently used as biomass indicator in an ecological risk assessment. Earthworm ecotoxicological tests have been correlated with different physiochemical properties of soil besides their interactions with chemical contaminants in soil in relation to different test species of earthworms. A few species of earthworm have been used as test species of soil health. However, more studies are needed with respect to small metabolites and reproductive parameters in relation to different soil thermal regions and soil types (Shahla and D'Souza, 2010).

13.9 Diagnostic Tools for Measuring the Impact of Pesticides on Soil Health

Soil health is defined as the capacity of soil to sustain biological productivity, regulate water flow, store and cycle nutrients, and filter, buffer and transform organic and inorganic materials. Soil also functions as a habitat and genetic reserve for numerous organisms. Consequently, management strategies that optimize multiple soil functions have a greater potential for improving soil health over management strategies that focus on a single function. There are many types of soil tests suitable for quantifying soil organisms or soil biological activity. Some of the analyses are relatively simple and direct, whereas others are highly complex or indirect. Therefore, greater caution is required when interpreting soil biological tests than is necessary for most soil chemical and physical characteristics. Critical values for 'soil biology' tests need to be developed in relation to climate, soil type and land use. An approach is needed for determining critical values of soil biological tests in tracking changes (monitoring) in selected characteristics in relation to management practice.

13.9.1 Total soil microbial biomass

Both direct and indirect methods have been used for the estimation of microbial biomass in the soil. Direct counting includes the use of staining techniques in conjunction with epifluorescence microscopy or automated image analysis. The most common indirect methods are chloroform fumigation and substrate-induced respiration. In chloroform fumigation, the chloroform vapours kill the microorganisms in the soil and the size of the killed biomass is estimated either by quantification of respired CO_2 or by direct extraction of the soil immediately after the fumigation, followed by quantification of extractable C. Soil biomass gives a general understanding of soil biodiversity covering all biological components inclusive of both soil organisms and roots of a given locality as a measurement of CO_2 evolution rate. Soil microbial activity, on the other hand, expresses the energy capacity in terms of C biomass of soil (Anderson and Ingram, 1993). Methodological problems associated with applying these methods to different soil types and at different times of the year have been researched extensively and the practical aspects are well understood, but tests need to be conducted with caution. If roots and larger animals are removed from the soil prior to assessment, microbial biomass will include mainly microorganisms and smaller soil fauna (such as mites and springtails). This methodology enables estimation of the amount of C, N, P and S in living soil organic matter.

13.9.2 Protozoan biomass

Protozoans in the soil ecosystem comprise both active and inactive (encysted) protozoans. The former are determined by counting directly using an inverted microscope (Foissner, 1994), whereas the latter can be determined using a method described by Rønn *et al.* (1995) that causes protozoa to excyst. A newly developed molecular method, most-probable-number polymerase chain reaction (MPN-PCR), has been used to quantify a specific group of soil flagellates directly (Fredslund *et al.*, 2001) and can be applied to determine soil protozoa. Bioassays based on a 24-h growth response of common ciliates have been developed (Forge *et al.*, 1993; Pratt *et al.*, 1997).

13.9.3 Soil respiration

Respiration metabolism of aerobes in soil involves biological oxidation of organic matter to CO_2 and is correlated with soil organic matter content and microbial activity. Soil respiration can be determined by measuring CO_2 production using

chemical titration, electrical conductivity, gas chromatography or infrared spectroscopy. The metabolic quotient (qCO_2), also called the specific respiratory rate, is defined as the microbial respiration rate per unit of microbial biomass.

13.9.4 Microbial quotient

The microbial quotient provides a measure of soil organic matter dynamics and can be used as an indicator of net C loss or accumulation (Anderson and Domsch, 1990) and can be related to total carbon by the microbial quotient, defined as microbial biomass C/total organic C (C_{micro}/C_{org}).

13.9.5 Potentially mineralizable N test

Determination of potentially mineralizable N (PMN) in soil may be a better way to assess microbial biomass rather than counting methods. This method involves a simple 1-week anoxic incubation of soil, and mineral nitrogen (N_{min}) is then extracted using 2 M KCl by shaking 10 g of soil for 60 min in 50 ml of a KCl solution (2 M). After filtration, extracts are analysed colorimetrically for nitrate and ammonium (N_{min}) by an autoanalyser. A drawback of PMN is that it is a single gross number, expressed as micrograms of NH_4-N/g of soil. This method does not distinguish between, for example, fungal, bacterial, actinomycete and nematode counts. However, until accuracy increases and costs decrease for the counting methods, gross estimates such as PMN or soil respiration may be the best way to go because of their cost-effectiveness. Currently, few commercial laboratories perform PMN tests.

13.9.6 Population studies

Groups of organisms

Identification of groups of soil microbes and alterations in their numbers also serve as indicators of the health of soil. Organisms can be counted individually (e.g. mites or earthworms) or as number per group (e.g. genera or species). For bacteria and fungi, special techniques can be used for particular groups: for example, serological and molecular tests are available for some bacteria (e.g. *Rhizobia*).

Fungal counts

Qualitative and quantitative analysis of soil fungi can be done using suitable media for growth. Measurement of the length of hyphae (km/g of soil) is possible, but it is usually difficult to identify the fungi present. Some fungi can be grown on artificial nutrient media but this represents only 1–5% of the total organisms present.

Bacterial counts

Using the most-probable-number method, it is possible to estimate the bacterial population in soil but this is a very rough estimate. As bacteria occur in small pores within the soil, many are not easily extracted and it was shown that, if bacteria were 'washed' from the soil, then a similar number appeared in the second wash (Bottomley and Maggard, 1990). Another problem with direct counts of bacteria is that it is not possible to distinguish between living and dead bacteria on microscope slides, although this can be overcome using other methods (Bottomley and Maggard, 1990). Although many soil bacteria will grow on agar or in nutrient broth, not all do so, and therefore indirect counts of bacteria based on this type of methodology are often not relevant to the total number of bacteria in the soil.

Nematodes

Another bioindicator of soil health is the composition of nematode communities (plant-parasitic and free-living) because of their involvement in two critical ecological processes – soil nutrient cycling and decomposition. Maturity and trophic diversity indices withstand statistical rigour better than abundances, proportions or

ratios of trophic groups. Maturity indices respond to a variety of land-management practices. Similarity indices may be more useful than diversity indices because they reflect taxon composition. Deborah (2001) suggested that, to enhance the utility of nematodes as bioindicators, it is imperative either to modify existing indices or to form alternative indices refined by a greater understanding of the biology of key taxa. It is important to strike a balance between beneficial and detrimental nematodes. DNA probes are also available for some nematodes.

Termites and enchytraeid worms

These are easily quantified and can be an indicator of soil health in some agricultural environments if calibrated.

Earthworms

Population studies of earthworms can be an indicator of soil health (as discussed above). As the species differ among different soils, measurements need to be calibrated locally.

Microarthropods

Isolation and identification is possible (Bottomley, and Maggard, 1990), and counts can be included in diversity indices.

Abruscular mycorrhizal fungi

Arbuscular mycorrhizal fungi can be assessed by directly scoring colonization of roots using a microscope.

13.9.7 Biosensor bacteria

Biosensor bacteria are environmentally relevant bacteria that can be selected and genetically modified by fusing reporter genes (e.g. bioluminescence). These are then able to respond to certain induced toxicities through the use of reporter genes (Paton *et al.*, 1997), which enables them to give a certain signal to a specific response. Ultimately, fibre optic-linked membrane-bound biosensor probes may facilitate *in situ* ecotoxicity monitoring of soil ecosystems for possible contamination (Paton *et al.*, 1997).

Biosensor bacteria are available that have been evaluated for contamination induced by mercury (Rasmussen *et al.*, 2000), chromate (Peitzsch *et al.*, 1998) and zinc (Paton *et al.*, 1997). Commercial biosensor bacteria products for overall analysis of soil health are now available.

13.9.8 Antibiotic-resistant bacteria

Antibiotic-resistant bacteria in contaminated soil can be enumerated by cultivation and/or by molecular techniques. Methods relying on cultivation of bacteria on selective growth media containing antibiotics are well established, economic and can be easy tools in soil health monitoring. As well as quantitative analysis of resistant bacteria, the minimal inhibitory concentration and breakpoint value may also be determined in the soil ecosystem, which is necessary because the antibody concentration required to distinguish between resistant and sensitive bacteria of one species may not be applicable (Petersen *et al.*, 1997). The difficulty in cultivating some bacteria can be overcome by molecular techniques involving PCR and molecular gene probe analysis for estimating the population sizes of bacteria with the resistance genes. As little is known about the occurrence of antibiotic-resistant bacteria in soil ecosystems, some baseline testing is necessary to investigate the possible differences between contaminated and normal soils. Monitoring of antibiotic-resistant bacteria may be complemented by measurements of bioavailable concentrations of antibiotics using biosensor bacteria (Hansen *et al.*, 2001).

13.9.9 Fatty acids and lipids

It has been estimated that 80–99% of all soil species have not yet been cultured because most soil microorganisms cannot be

characterized by conventional cultivation techniques. Currently, the analysis of phospholipid fatty acids (PLFAs), essential membrane components present in living organisms, can be used to overcome this limitation, thereby providing information on the trophic structure (at the phenotypic level) of the microbial community. Fatty acids are used to compare a fraction of cell components. This group of hydrophobic substances ranges from fatty acids to more complex molecules, such as sterols, terpenes, polynuclear hydrocarbons, chlorophylls, fats, waxes and resins, which constitute the principal group of soil organic matter biomarkers (Killops and Killops, 1993). Extraction of soil lipids is frequently carried out using solvents with variable polarity in a Soxhlet apparatus, although alternative techniques such as supercritical fluid extraction are also available (Bautista *et al.*, 1999). Total lipid extracts can be further fractionated by GC-MS. The use of PLFA patterns for the characterization of microbial communities in soil has been reviewed by Zelles (1999). In general, PLFA analysis is a fast and reliable method for the detection of changes in the structure of soil microbial communities, and the variations detected can be related to changes in soil.

13.9.10 Soil enzymes

Different assays are available for determination of soil enzyme activities based on the substrates used (e.g. cellulase, urease, phosphatase and phenol oxidase) (Dick *et al.*, 1996). Enzyme activities for dehydrogenase (Weaver *et al.*, 1994), phosphatase, β-glucosidase (Gianfreda *et al.*, 1994) and urease (Gianfreda *et al.*, 1994) in soil can be conducted to facilitate detection of changes in the soil ecosystem in response to pesticide contamination in the soil. The assay method for urease activity (Gianfreda *et al.*, 1994) can be performed by taking soil (1 g) and mixing it with 4 ml of 0.1 M phosphate buffer (pH 7.1) and 1 ml of 0.2 M urea and incubating at 37°C for 1 h. After incubation, 10 ml of 2 M KCl is added, and the mixture cooled to 4°C for 10 min to stop the reaction. The mixture is filtered through a Whatman no. 2 filter paper. And the ammonia concentration in an aliquot (1 ml) of the filtrate is determined by the hypochlorite alkaline phenol method (Chaney and Marbach, 1962).

13.9.11 Carbon-based fractions

Soil organic matter and its carbon fractions affect the physical niche of soil organisms. Some fractions may be readily degradable by soil organisms, but others may be inaccessible and protected from breakdown by soil organisms owing to their location in aggregates. A combination of different techniques, both non-destructive and destructive methods appropriate for the study of complex matrices, is used to determine the degree of humification of the different humic substance fractions in soil organic matter. Among the non-destructive methods, nuclear magnetic resonance spectroscopy and gel permeation chromatography have been used to analyse humic acids produced in microcosms, a valuable technique to quantify the different C and N structural groups: aromatic, aliphatic (alkyl: waxes, alkanes, cutins and suberins), *o*-alkyl (carbohydrates, tannins and altered carbohydrates), amide, amine, pyrrolic, etc. Infrared spectroscopy also provides valuable information on oxygen- and nitrogen-containing functionalities, while UV/visible spectroscopy is useful to establish humus maturity and the degree of humic substance aromaticness (Traina *et al.*, 1990). Among the destructive techniques, conventional analytical pyrolysis (Curie point or microfurnace), chemolysis in the presence of alkylating reagents (thermally assisted hydrolysis/methylation) (González-Vila *et al.*, 1996) and wet chemical degradation methods using specific reagents (e.g. CuO/NaOH, $NaBO_3$, $KMnO_4$) (Almendros and González-Vila, 1987) generate fragments amenable to GC-MS analyses, which can be used to unambiguously identify structures present

in the humic substances. Other methods used to characterize the humic substances include isotope ratio monitoring GC-MS, which provides both structural information and insight into the evolution and turnover times of different organic soil fractions (Neunlist *et al.*, 2002). Other emerging techniques are variants of traditional thermal analysis (thermogravimetric analysis differential scanning calorimetry) coupled with isotopic ratio monitoring (Lopez-Capel and Manning, 2004). The carbon management index can be measured indirectly by calculating the change in a treated soil compared with a benchmark soil for labile organic matter (Blair *et al.*, 1995). The result is dependent on defining an adequate reference soil. Catabolic activity can also be measured by using various carbon substrates that can be added to the soil and respiration assessed to indicate the catabolic diversity of the organisms present (Degens, 1998).

13.9.12 Microbial processes

Biomediated processes, such as mineralization immobilization turnover (MIT), of some nutrients serve as indicators of microbial activity. Both abundance and activity measurements of soil organisms are required to serve as potential indicators of the biological state of soil health. Mineralization (including respiration) is relatively easy to determine and can be assessed *in vivo* as well as *in vitro* by determination of ammonium released during mineralization of organic matter, but the amount of ammonium converted to nitrate must also be taken into consideration. Measurement of nitrates in the soil indicates the activity of nitrifiers, but loss of nitrate by leaching, particularly in sandy soils, needs to be taken into consideration for total nitrifying activity to avoid underestimation. Measurement of methanogenesis and denitrification in soil gives an indication of the activity of methanogens and denitrifiers in the soil. The 'potential activity' of soil organisms can be estimated by amending the substrates (e.g. soil) with a carbon source (a sugar/glucose/cellulose) and the amount of CO_2 released is measured by substrate-induced respiration.

13.9.13 Fungal:bacterial ratios

The fungal:bacterial ratio can also be determined by substrate-induced respiration. This method evaluates the ratio of fungi and bacteria in soil on addition of different carbon sources and also their relative quantities. Management of soils involving different farm practices alters the relative abundance of fungi and bacteria in soil, so there is potential to use this as an indicator to evaluate the impact of management practice on soil biological activity. Direct quantification of bacteria and fungi from a particular soil ecosystem can be measured and the ratio of their abundance worked out. The fungal:bacterial ratio is determined using biochemical tests of fungi and bacteria (PLFA analysis) as a basis for estimating the proportion of fungi and bacteria in soil.

13.9.14 Index measurements

The composition of a soil microbial community can also be analysed to evaluate its response to induced stress. Species abundance and diversity measurements can serve as sensitive indicators of soil ecosystems. However, conventional microbiological investigation of community diversity has some drawbacks. It involves extensive analysis of quantification and identification of morphologically similar microbial species and is also time- and labour-intensive. The counting of colony morphotypes has not been tested as an alternative on a broader scale. Simpson's diversity index is a convenient mathematical tool for expressing the species diversity, which takes the number of different species and their total numbers into account. The estimation of the relative abundance of each species of microbes in the soil has paved the way for the determination of the 'equitability index' of numbers of individual

species that is an important estimation of the resilience of a soil. The use of statistical packages such as Phoretix enables quantification of both diversity indices and equitability (Girvan *et al.*, 2003, 2004).

The technology adopted for determination of soil biodiversity will vary with the management practices. Soil systems can be described through geographical information systems (GIS) grids using the data on existing soil biota for future monitoring. Various forms of indices are available, but caution is required in their use because they are not all statistically sound.

13.10 Constraints in Using Bioindicator Tools

There are certain constraints while using bioindicators for soil health, especially soil variability and sampling intensity, as well as financial considerations. The spatial and temporal variation of microbial properties in a soil can be very wide and needs to be considered when choosing indicators for monitoring soil health (Singer and Ewing, 2000). In general, soil attributes that are subject to temporal variation (e.g. soil microbial activity, soil moisture and soluble nutrients) are often also subject to a high spatial variability (Halvorson *et al.*, 1997). This variability poses limitations for accurately quantifying microbial populations and processes in soil. The spatial variability of microbial processes varies with spatial scale (Parkin, 1993). Other important factors at the regional scale are climatic factors, land-use patterns, vegetation including topography, land surface characteristics and soil type. At the plot-scale level are the rhizosphere, application of fertilizers and pesticides, and other soil-management practices. Temporal variations of microbial indicators are non-systematic, periodic, and cyclic or trend changes (Stenberg, 1999). High temporal variability of such measurements suggests higher-frequency soil sample collection. Laboratory measurements can be standardized to exclude the natural changes in temperature and moisture, and would be more appropriate for long-term study of soil bioindicators (Visser and Parkinson, 1992; Halvorson *et al.*, 1997). Sampling methods and pre-treatment of samples are important considerations in the attempt to minimize the variability in soil health assessment. Together with baseline data on spatial and temporal variability of individual microbial indicators, these can help to establish the most appropriate sampling strategies.

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14 Integrated Pest Management in Stored Grains

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14.1 Introduction

Most populations of the world depend for their livelihood on an agro-based economy where a very significant portion of the gross domestic product comes from agriculture. However, in most underdeveloped countries, due to subsistence farming, low education levels and low technology inputs into agriculture, agricultural activities are not advanced, leading to suboptimal production and poor storage of the produce. Most storage losses are due to inadequate and poor storage facilities, and attacks by insect pests and diseases, causing enormous losses annually. Of the many insects that have adapted themselves to a diet of dried vegetable material, a few are primary pests of grain in that they are able to bore into sound kernels. Their initial attack opens the way through the tough seedcoat. It has been estimated that, as a result of their feeding activities, their presence in grain and cereal products and the cost of methods employed to destroy them, this group of insects exacts a yearly toll of at least US$300 million in the USA alone. If we can save these losses, our stockpiles of food grains would grow enabling us to feed millions of hungry people around the world. The losses caused by insect pests and diseases are less apparent than those caused by, for example, erratic monsoons, fluctuations in global weather, improper storage and postharvest procedures. However, much can be done at various levels to reduce and possibly eliminate insect pests in the process of storage.

The introduction of high-yielding varieties in the early 1960s with the advent of the Green Revolution helped India to emerge as one of the leading developing countries, resulting in increased food grain production, touching more than 230 million t/year (Anon., 2009). Despite surplus food production, there is still hunger and poverty, as millions of tonnes of valuable grains worth more than 2000–3000 crores of rupees are either damaged or lost for want of knowledge of scientific methods of storage every year. Furthermore, food grains and their processed products are susceptible to deterioration by a variety of biotic and abiotic factors (Fig. 14.1). Together, they account for the loss of about 25% of food grains worldwide. These include high temperature, moisture, microorganisms, mites, insects and rodents. The losses are highest in tropical and subtropical areas where conditions are relatively conducive for rapid growth and multiplication of the damage-causing organisms to the food

Fig. 14.1. Typical spoilage of food grains due to improper handling and storage.

materials, especially insects. Stored-product insects are serious pests of dried, stored, durable agricultural commodities, and of many value-added food products and non-food derivatives of agricultural products worldwide. Stored-product insects can cause serious postharvest losses, estimated to be from 9% in developed countries to 20% or more in developing countries (Pimentel, 1991), but they also contribute to contamination of food products by the presence of live insects, insect products such as chemical excretions or silk, dead insects and insect body fragments, general infestation of buildings and other storage structures, and accumulation of chemical insecticide residues in food, as well as human exposure to dangerous chemicals as a result of pest-control efforts against them. Worldwide, an annual loss of 8–10% (13 million t of grains lost due to insects and 100 million t due to failure to store properly) is estimated in stored-food commodities. Evidently, reliable pesticides and storage techniques are required to limit the damage to food grains in storage in most developing countries. Insects, pathogens and mites cause the greatest proportion of damage and cause the deterioration of grains by producing entomotoxins and mycotoxins (Morgan and Aldred, 2007). It was estimated that more than 20,000 species of field and storage pests destroy approximately one-third of the world's food production, valued annually at more than US$100 billion (Jacobson, 1982; Ahmed and Grainage, 1986). Most insect pests belong to the orders Coleoptera and Lepidoptera, which account for about 60 and 10%, respectively, of the total number of species of stored-product insect pests (Atwal and Dhaliwal, 2008; FAO, 2009). They feed on grain, boring into the kernel and destroying the germ portion, cause heating and deterioration in stored produce. This causes huge losses as a result of nutritional depletion and a reduction in market value, as well as contamination caused by their excretory products, which can be hazardous to the health of human beings who process and eat the infested grains. Thus, the loss is not merely in terms of quantity but also in quality of food grains. The qualitative loss is attributed to chemical changes in proteins, carbohydrates, amino acids, fatty acids and vitamins, which affect the nutritive value of the grains. In this

chapter, an attempt has been made to acquaint the reader with the insects involved and the most effective methods of controlling them.

14.2 Origin of Stored-grain Insect Pests

The origin of insect pests of stored grain is not well known. Undoubtedly, they lived in the fields, some of them breeding in supplies of seed that escaped the attention of birds and animals, and others feeding on the dried or decaying remains of plant or animal life, while still others bored into the roots, tubers and stems of plants. The custom of storing seeds, roots, herbs and dried meats for food adopted by man in early times provided an easy living for insects that were accidentally brought in with these stores. Ideal conditions for breeding provided by such stores made it unnecessary for these insects to fly long distances in their search for food. Whether or not it is the result of leading such an easy living and the lack of need for flight, it is true that a number of pests of stored grain have completely lost the power to fly. The granary weevil (*Sitophilus granarius* L.) has in fact lost all but the vestiges of its wings, although the closely related rice weevil (*Sitophilus oryzae* L.) is still a powerful flier. Evidence indicates that many of the animals that trouble stores of grain today were prevalent in ancient times.

14.3 Assessment of Loss

Losses can occur at several stages of the postharvest chain, including threshing, storage, transport, milling, wholesale and retail distribution. There has been a tendency to overestimate storage losses mostly based on guesswork rather than on sound empirical testing. Losses to the extent of 30% or more are not uncommon (Greeley, 1987). According to one estimate, post-harvest losses during storage may be as high as 25–30% (Champ, 1985).

By contrast, the results of more detailed field studies suggest that, under traditional storage systems in tropical countries, losses are typically around 5% over a storage season (Tyler and Boxall, 1984), depending on the crop, the ambient conditions, the period of storage and other factors. Somewhat higher levels have been encountered in the wetter parts of West Africa and Central America. Even losses around the 5% level should not be considered insignificant, as the physical losses are usually accompanied by qualitative losses affecting the mass of the grain in store. Furthermore, the losses are mainly experienced during the lean season before the new harvest is ripe, thereby having an adverse effect on the food security of farming families at a particularly critical period. To overcome this problem, farmers in Honduras have adopted metal bins for storage.

Estimation of storage losses is not an easy job and is subject to a number of methodological difficulties (Greeley, 1991). Loss-assessment methods tend to be slow and require skilled field and laboratory staff. They are often undertaken on experimental sites, making it difficult to relate the results to on-farm situations. Another difficulty in using estimates of losses to justify technical change is the problem of assigning a value to the losses that makes sense to the potential user of the technology. The most common form in which losses are expressed is as a percentage weight loss. However, what is important from the farmer's point of view is the use that the grain can be put to or the market price that will be received. Grain intended for sale may instead be consumed, or that intended for consumption used as animal feed. A rapid loss-assessment method for estimating storage losses in maize and cassava has recently been developed in Togo (Compton *et al.*, 1992). The method attempts to incorporate farmers' criteria in defining categories of loss and, as the measurement occurs in the field rather than in a laboratory, the results can be discussed with farmers on the spot. Such methods could usefully be integrated into postharvest technology projects. Technical improvements must be appraised within the context

of a total commodity system. This includes the chain of activities linking farmers and consumers, suppliers of goods and services to the participants in that chain (e.g. banks, equipment suppliers) and government policy and regulatory activity. A four-step approach has been recommended based on an appraisal of the case for bulk handling in Pakistan (Coulter, 1991).

14.4 Storage Losses

Losses caused by insects include not only the direct consumption of kernels but also include accumulations of frass, exuviae, webbing and insect cadavers. High levels of this insect detritus may result in grain that is unfit for human consumption. Insect-induced changes in the storage environment may cause warm, moist 'hotspots' that are suitable for the development of storage fungi causing further losses. Worldwide losses in stored products caused by insects have been estimated to be between 5 and 10%. Heavier losses occurring in the tropics may reach 30%, and the net value of losses in storage in the USA has been placed at over US$200 million annually (Weaver and Petroff, 2004).

Efficient machinery helps to increase productivity by about 30% at the postharvest stages, and maximum benefits will accrue from improvements in storage practices. Chickpea seeds in developing countries suffer heavy qualitative and quantitative losses from attack of the pulse beetle (*Callosobruchus chinensis* L.) (Alam, 1971; Abrol, 1999). Almost 8.5 % of total annual production is lost during postharvest handling and storage (Agrawal *et al.*, 1988).

14.5 Types of Storage Loss Caused by Insects

14.5.1 Quantitative loss

Direct feeding by insects causes loss in weight of the stored grains. For instance, a rice weevil will eat 14 mg of a 20 mg rice kernel during its developmental period. It is not only the loss of weight but commercially whole of the grain is lost. A female weevil, through three generations per year, has the biotic potential to reproduce 1,500,000 offspring, which will consume 1,500,000 kernels of rice (amounting to 30 kg of rice). A gravid female of *Sitotroga cerealella* can destroy 50 g of rice completely in three generations.

14.5.2 Qualitative loss

Direct insect feeding on food grains results in a number of qualitative changes such as chemical changes in grain content. The contamination of grains with moult skin and body parts also makes them more susceptible to the spread of pathogenic microorganisms.

14.5.3 Loss of seed viability

Feeding by insects has been found to cause the loss of viability of seeds by as much as 3.6–41 % in paddy (unmilled rice) (http://agritech.tnau.ac.in/crop_protection/crop_prot_crop_insect%20_storage_importance.html).

14.5.4 Damage to storage structures

As well as causing qualitative and quantitative loss to food grains, some insects, such as the lesser grain borer, also have the ability to destroy wooden storage structures, containers, polythene and lined bags. The direct consequence is loss of food by spillage or consumption by other organisms including insects, while indirect losses result from lowering the food quality to the point where people refuse to eat it. Various estimates of losses of food grains during postharvest operations have been made. According to Lal (1988), postharvest losses were estimated to be 9.33% in India in 1988 (Table 14.1).

In most countries, grains are among the most important staple foods. However, they are produced on a seasonal basis, and in

Table 14.1. Estimates of losses of food grains during postharvest operations.

Postharvest process	Loss (%)
Threshing yard losses	1.68
Transport losses	0.15
Processing	0.92
Storage	
Rodents	2.50
Birds	0.85
Insects	2.55
Moisture	0.68
Total	9.33

Source: Adapted from Lal (1988).

many places there is only one harvest a year, which itself may be subject to failure. This means that, in order to feed the world's population, most of the global production of maize, wheat, rice, sorghum and millet must be held in storage for periods varying from 1 month up to more than 1 year. Protection of food grains through sound scientific storage practices occupies a vital place in the economies of developed and developing countries alike, and is thus a matter warranting urgent attention. The Indian subcontinent has a variety of agro-climates and produces a variety of food grains including cereals, millets, oilseeds and pulses. Whole grains (cereals, millets and pulses with minimal processing, with rice being used in its polished form) and their directly usable forms are the principle food items that are stored in massive quantities in India in stores, markets and households. The whole grains are stored by farmers (small quantities for household use) and in wholesale market warehouses and stores belonging to government agencies. Despite the usage of pesticides and sealable storage bins, food materials continue to suffer considerable damage under storage. Evidently, an integrated approach is required to solve the problem of insect pests in stored grains.

14.6 Detection of Insect Infestation in Stored Commodities

The significance of infestations depends on the species, density and ultimate plans for the grain. Proper sampling and identification during storage help the manager to recognize problems early and thereby prevent further damage (Tables 14.2 and 14.3). Selecting the most appropriate curative or preventative action from the available alternatives is not easily accomplished, especially when the type of insect present is not known. For example, if insects that feed inside kernels

Table 14.2. Detection of infestation.

Methods	Applicability
Physical methods	
Visual inspection	Whole grains and processed foods
Floatation method	Whole grains
Berlese method	Whole grains
Sampling and sieving	Whole grains and milled products
X-ray technique	Whole grains
NMR and near infrared spectroscopy	Whole grains
Chemical methods	
ELISA test	Whole grains, milled products and processed foods
Uric acid analysis	Whole grains, milled products and spices
Analysis for CO_2	Whole grains
Specific gravity method	Whole grains, except oats and maize
Fragment count	Whole grains, milled products and processed foods
Staining technique	
Egg plugs	Whole grains
Ninhydrin method	Whole grains

Source: Rajendran (2005).

Table 14.3. Indicators of insect infestation in stored product commodities.

Indication	Commodities	Insect
Exit holes	Wheat, rice, maize Paddy	*Rhyzopertha dominica*, *Sitophilus* spp. *Sitotroga cerealella*
	Pulses (whole)	*Callosobruchus* spp.
	Whole spices	*Stegobium paniceum*
Eggs on grain surface	Pulses (whole)	*Callosobrucus* spp.
Webbing or silken strands present	Cereals, whole and milled	*Corcyra cephalonica*, *Plodia interpunctella*
	Oilseeds/oilcakes/meals	*Ephestia cautella*, *P. interpunctella*
	Dry fruits	*E. cautella*
	Tree nuts	*E. cautella*
Pupal cases sticking to shells and gunny bags	Groundnuts in shell	*Caryedon serratus*

Source: Rajendran (1999).

are already present in significant numbers, a surface protectant applied as a dust or spray will not be the best management option. Usually, a combination of tactics will provide the most reliable protection.

14.7 Insect Identification in Stored Grain

The identification of stored-grain insects and an understanding of their interaction with the environment are important steps in developing an integrated pest management (IPM) programme. Farmers are generally aware that an infestation of weevils and lesser grain borers can cause significant quality and monetary loss if left uncontrolled. These insects are often classified as 'primary grain pests' because they attack and destroy whole, undamaged grain. The immature stages occur inside the kernels, and thus a 'hidden' infestation may develop. 'Secondary grain pests', the so-called 'bran bug groups', include most other grain-attacking beetles. These insects frequently cause more serious losses where some type of kernel damage precedes their establishment. There are complexes of insect pests that infest grain, and the particular species present will depend on the type of grain. Storage insect pests are categorized into two types: (i) primary pests that are capable of penetrating and infesting intact kernels of grain and have immature stages that can readily develop within a kernel of grain; and (ii) secondary invaders that cannot infest sound grain but feed on broken kernels, debris, higher moisture weed seeds and grain damaged by primary insect pests. In general, the immature stages of these latter species are found external to the grain (Table 14.4).

14.8 Non-insect Pests of Stored Grain

As well as insects, non-insect pests such as rats, bandicoots, gerbils, squirrels and porcupines can cause considerable damage in various countries. They are cunning and clever animals; some feed on grain, while some burrow in the land and floors near the buildings. Table 14.5 lists some of the rodent species causing such damage in India.

14.9 Sources of Infestation

Infestation in stores can come from a variety of sources, including:

Table 14.4. List of important primary and secondary pests of stored grains.

Common name	Pest	Host
Primary pests		
Rice weevil	*Sitophilus oryzae*, *Sitophilus zeamais*, *Sitophilus granarius*	Rice, wheat, sorghum, barley, maize
Khapra beetle	*Trogoderma granarium*	Cereals, groundnut and pulses
Angoumois grain moth	*Rhyzopertha dominica*	Paddy, maize and wheat
Grain moth	*Sitotroga cerealella*	Rice, wheat and maize
Rice moth	*Corcyra cephalonica*	
Lesser grain borer	*R. dominica*	
Pulse beetle	*Callosobruchus chinensis*, *Callosobruchus maculatus*	Pulses, bean and grain
Tamarind/groundnut bruchid	*Caryedon serratus*	Groundnut, tamarind and other legumes
Cigarette beetle	*Lasioderma serricorne*	Wheat flour, cereal bran, groundnuts, cocoa beans, spices, turmeric, chillies, ginger, stored tobacco, cigarettes
Drug store beetle	*Stegobium paniceum*	Turmeric, coriander, ginger, dry vegetable and animal matter
Sweet potato weevil	*Cylas formicarius*	Sweet potato
Potato tuber moth	*Phthorimaea operculella*	Potato
Secondary pests		
Red flour beetle	*Tribolium castaneum*, *Tribolium confusum*	Broken grains, damaged grains, milled products, machinery
Long-headed flour beetle	*Latheticus oryzae*	
Saw toothed grain beetle	*Cryptolestus minutas*, *Laemophloeus pusillus*	Dry fruits, maize, cereals and oilseeds
Red rust grain beetle	*Cryptolestes ferrugineus*	
Flat grain beetle	*Cryptolestes pusillus*	

Source: Ahmad (1983).

Table 14.5. Important rodent species damaging stored grains in India.

Rodent species	Common name	Habitat	Distribution
Rattus rattus	House rat	Rural and urban residential places	All over India
Mus musculus	House mouse	Warehouses and godowns	–
Bandicota bengalensis	Indian mole rat	Stores, warehouses and crop fields	North-east India, South India, West Bengal, Bihar and Maharastra
Tatera indicia	Indian gerbil	Crop fields and grasslands	All over India
Rattus meltada	Soft furred field rat	Crop fields and grasslands	Rajasthan, Gujarat and South India
Mus booduga	Field mouse	Crop fields	Central and southern India
Bandicota indicia	Large bandicoot rat	Rural environment	Southern India

- field infestation (hidden infestation); e.g. pulse beetles and paddy moths infest crops in the field;
- cracks and crevices: insects from old stock infest fresh stock (cross-infestation);
- leftover seed from bins/stores or spilled seeds under the stacks;
- old containers and bags;
- trucks, trollies and bullock carts;
- entry of insects from neighbouring stores;
- carryover of field infestation;
- old infested stores;
- cleaning of the processing plants; and
- Humans.

14.10 Storage Structures

The basic requirement of scientific storage is to provide the maximum possible protection from pests and to allow controlled aeration. Ideal storage structures should be capable of keeping grain cool and free from being rendered airtight, with proper drainage and away from a vicinity that might prove deleterious to a safe storage environment. The storage structure may be earthenware, plastic or wooden bins of different capacities. Indoor structures tend to be circular steel or plastic bins with a capacity of 1–3 t, while outdoor structures may be pre-fabricated steel bins with a hopper bottom or aluminium, roller-compacted concrete, cement masonry or Pusa bins. Some of the different types of storage structure used in India for the protection of stored grains are listed in Table 14.6 and shown in Figs 14.2–14.4. The following points should be considered for safe storage of food grains: (i) proper selection of site (location); (ii) selection of storage structure; (iii) cleaning of storage structures; (iv) cleaning and drying of grains; (v) cleaning of bags; (vi) separate storage of new and old stock; (vii) cleaning of vehicles; (viii) use of dunnage; (ix) proper aeration; and (x) regular inspection.

14.10.1 Traditional storage methods

Traditional methods of storing grain are the product of decades if not centuries of development, perhaps by trial and error but certainly as a result of experience of the users and their ancestors. Farm folk of the

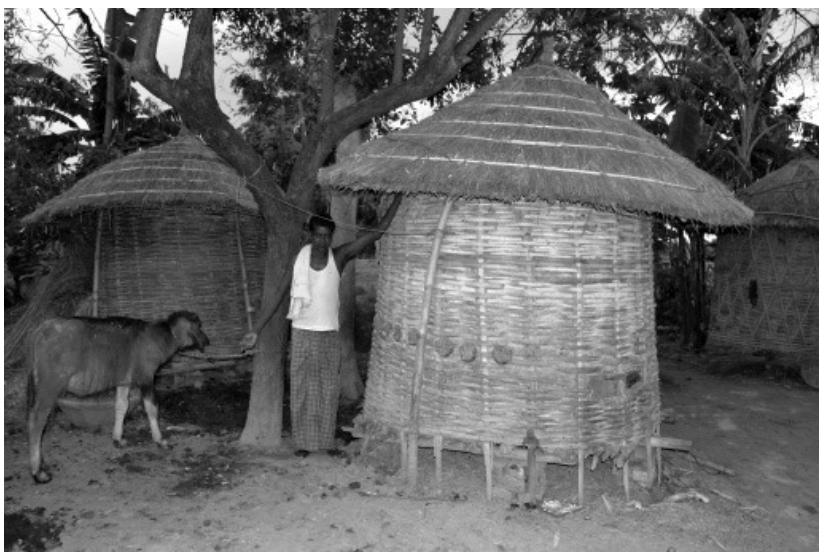

Fig. 14.2. A storage structure made of bamboo stems for the storage of wheat, rice and pulses. Some spices such as chillies, garlic and neem leaves are also added to provide protection against pests.

Table 14.6. Different storage structures used for storage of grains in India.

Structure	Make	Items	Capacity	Remarks
Bamboo structures	Split bamboo woven in the form of a cylinder with a wide base and narrow mouth	Paddy, wheat and sorghum	500 kg	Life 4–5 years. Weight loss due to insect attack is 5% in paddy and 15% in sorghum
Mud and earthen structures	Made of clay, straw and cow dung (3:3:1). The earthen structures are made, sun dried and then burnt in the fire	Paddy, wheat, sorghum, oilseeds and pulses	500–1,000 kg	Life 8–10 years. During rainy season, develop cracks and moisture absorption, followed by insect and mould infestation
Wooden structures	Local wood is painted black. At the top, a 30 × 20 cm inlet and at the bottom a 30 × 15 cm outlet are provided	Paddy	1,000 kg	Life 15–20 years. Not airtight or moisture proof
Brick structures	Rectangular, structures built as part of the house, with brick in cement or lime mortar having a wall thickness of 40–50 cm. At the top 50 x 50 cm inlet and at bottom 15 x15 cm outlet is provided	Paddy, sorghum and wheat	10,000–20,000 kg	Life 25–30 years. High initial cost; not insect or moisture proof
Underground structures	Circular pits vary from 100 to 400 cm in depth, from 50 to 100 cm in diameter at the neck and from 250 to 300 cm at the bottom. For filling and emptying, there is an opening at the top. Before filling, the sides and bottom are packed with straw and husk. After filling, the pit is covered with straw and stone, and finally with mud	Cereals	10,000–20,000 kg	Safe against insects but, loss of seed viability and handling difficulties have made these outdated
Miscellaneous plant materials				
Paddy straw	Paddy straw is wound in the form of rope to varying diameter	Paddy, other cereals and pulses	3,000–10,000 kg	Not insect and rat proof
Stem of *Vitex* and pigeon pea stalks	Stems wound like a bin and both sides are plastered with mud and cow dung	Paddy and other cereals	100–200 kg	Temporary
Bottle gourd shells	Empty shells are used	Pulses, gourd seeds	2–5 kg	Only small quantity of seed lots
Metal corrugated galvanized iron sheets				
	Sheets of about 3 m high are held vertically along one edge and the edges of the other sheets are overlapped and bolted to each other. Thus, a circle of 2–4 m diameter is completed with many such sheets. They are covered on the top with the plain mild steel or galvanized iron sheets	Various types of grains	Vary	Temporary
Hessian cloth bags Gunny bags				

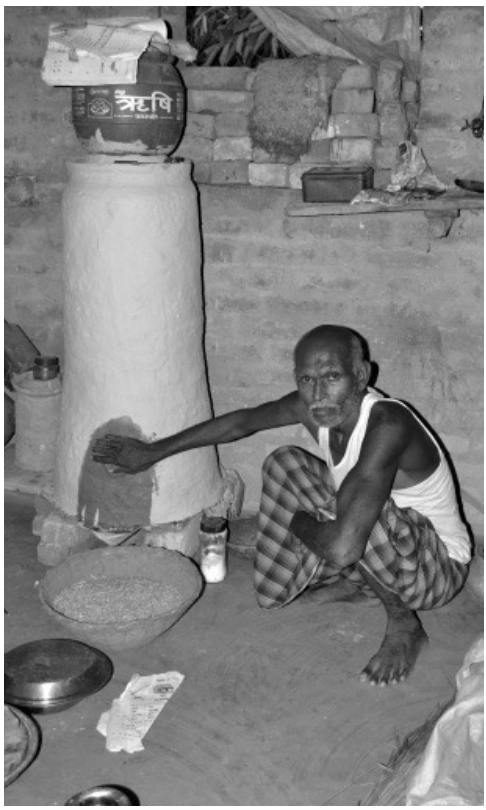

Fig. 14.3. A structure made of mud mixed with sand and straw for storage of food grains.

tropics and subtropics mainly follow indigenous traditional storage methods. A large proportion of harvested food grain is normally retained at the farmer level in traditional storage structures for 6 months to a year (Semple, 1990; Singh, 1993). Resource-poor farmers in developing countries use different physical, chemical and biological methods to protect stored grains against pest infestation by mixing grains with protectant made up of plant products. Although these methods have a strong scientific basis, it is seldom known to the users. These traditional methods have been used for many years with little or no modification and are successful because of the application of scientific principles, despite the farmers being unaware of them. Most of these traditional methods also minimize economic loss and damage to the environment (Table 14.7). In recent years, documenting of traditional wisdom has gained significant attention worldwide because of its importance in developing high-potential, environmentally friendly and sustainable management. The identification and utilization of such indigenous knowledge from the elders in rural and tribal belts may help to bridge the gap between current scientific and age-old practices.

Fig. 14.4. Metal bins used for storage of food grains in rural and urban areas.

Table 14.7. Ethnic practices followed by farmers for managing stored-food pests.

Methods	Effective against
Biological methods	
Neem and *Pongamia* leaves	Stored rice pests
Neem and *Vitex negundo* leaves	Stored maize pests
Leaves of *Annona squamosa*, *Cymbopogon citratus*, *Cymbopogon nardus* and *Erythrina indica*	Pulse pests
Physiochemical methods	
Sun drying	Most food grains
Fly ashes and diatomaceous soil	Pulses, maize
Different coloured polyethene bags	Pulse pest
Match boxes (match sticks have phosphorus, which is anti-feedant and repelent in nature)	Stored wheat pests
Garlic and turmeric powder have pesticidal, insecticidal and antifungal properties	Rice pests
Cloves (the strong flavour and bitterness of cloves protects the grain from spoilage)	Rice pests
Edible oil and salt (oil is an anti-feedent or feeding repellent and lubricating; salt is hygroscopic and insecticidal and thus protects the pulses from spoilage)	Pulse pests
Red chillies (repellent)	Rice pests
Soil topping and mixing	Many stored-food pests

14.11 IPM of Stored Grains

IPM relies on managing insect populations through physical and biological control techniques and, if necessary, chemical insecticides. The IPM approach involves various components (Table 14.8) for efficient management of insect pests in stored grains. These are described in more detail below.

14.11.1 Pest monitoring/sampling

Pest monitoring is an important component in the IPM postharvest practice for stored grain. Inspections should be done frequently, especially after first storage, to enable pest-management decisions to be made (Subramanyam and Hagstrum, 1995). Population density estimates and estimation methods include the following techniques: (i) absolute estimates (e.g. number of insects per kilogram of grain or number of moths per square metre); (ii) indirect estimates (mark-release-recapture methods); and (iii) relative estimates (number of insects caught in a sticky trap, perforated probe trap, food-baited trap, etc.). Trapping relies on insect mobility, which varies by species, environment and trapping period. The capture rate must be adjusted for time and converted to density per kilogram of grain. Traps recover insects from a much larger volume of grain than direct sampling. Sampling should be performed at periodic intervals (sequential sampling) to gather information about population changes over time. Typically, for grain stored above 20°C, sampling should be performed monthly. Grain held below 20°C permits sampling intervals to be longer than a month. Selecting sampling frequency can be based on the time for insects to complete one life cycle. IPM relies heavily on sampling, as the use of physical and biological controls are most effective on low populations. However, if insect populations exceed an economic threshold, fumigant application is recommended. Presently, little information is available concerning the economic thresholds at which fumigants should be administered in stored grain. Monitoring of insect populations and quality deterioration over a particular period of time will be a valuable tool to determine economic thresholds in storage.

Table 14.8. Methods for managing stored grain insect pests.

Type	Method	Details
Ecological method	Temperature control	Temperature below 15°C and above 42°C retards growth and development. Heating of grains to 50–60°C for 10–20 min kills almost all pests
	Moisture control	Grains stored at around 10% moisture content escape attack by insect pests except for khapra beetles
	Oxygen control	Oxygen level >1% is lethal to all insect pests
Mechanical methods	Screening of grains	Regular screening and destruction of infested bags
Physical methods	Heat treatment	Infrared heating of grains
	Controlled atmosphere	Use of low O_2 (2–4%) and high CO_2 (9–9.5%) is lethal to all insects
	Inert dust	Mixing of sand, clay, ash, silica aerosols or activated charcoal with food grains
	Edible oils	Coconut, groundnut or mustard oil at 0.25–0.5% for pulses
Biological methods	Bioagents	Use of *Bt*, parasitoids, predators, etc.
Cultural methods	Airtight storage	Use of airtight sealed structure does not allow insects to survive
	Drying of grains	
	Splitting of pulses	Split pulses escape attack by pulse beetles
Chemical methods	Prophylactic treatment	Malathion 0.5%, pyrethrum with 2% pyrethrin EC, primiphos methyl 0.5% in 1:100 ratio at 3/100 m^3 at 15-day intervals
	Curative treatment	
	Knockdown chemicals	Pyrethrum sprays, lindane smoke generators or fumigation stripes against flying insects
	Grain protectants	Pyrethrum dust or 5% malathion at 250 g/100 kg of seed
	Fumigants	Aluminium phosphide at two tablets (3 g each)/t
		Ethylene bromide at 3 ml/100 kg for wheat and pulses and 3 ml/100 kg for rice and paddy
		Ethylene dichloride/carbon tetrachloride mixture at 55 ml/100 kg stored grains

14.11.2 Preventative measures

Prevention requires good hygiene and sanitation. Typical preventative measures include the following:

- The threshing floor/yard should be clean, free from insect infestation and away from the vicinity of villages/granaries.
- Harvesting and threshing machines should be cleaned before use.
- Trucks, trollies or bullock carts used for transportation of food grains should be free from insect infestation.
- Storage structures should be cleaned before storage of newly harvested crops.
- All dirt, rubbish, sweepings, webbings, etc. should be removed from stores and dumped/destroyed.
- All cracks, cervices and holes in the floors, walls and ceiling should be filled with mud or cement.
- All rat burrows should be closed with a mixture of broken glass pieces and mud and then plastered with mud/cement.

- Stores should be whitewashed before storage of food grains.
- Food grains should be kept in stores that are rat and moisture proof.
- Proper stacking of bags helps in grain protection.
- Before the use of receptacles/stores, they should be disinfested with approved residual insecticides, preferably by spraying malathion 50% effective concentration, at a dilution of 1:100 and applied at a rate of 3 l/100 m^2.

14.11.3 Exclusion and curative measures

Infestations of stored-grain insect pests can be controlled by the following non-chemical methods.

Physical control

The use of physical control measures includes temperature, mechanical methods, moisture and relative humidity control, structural methods (e.g. grain silos, packaging), irradiation and sanitation (Fields and Muir, 1995). The red flour beetle (*Tribolium castaneum* Herbst) has a well-developed chemosensory system (Barrer, 1983) and is able to differentiate changes in the physical environment such as temperature (Saxena *et al.*, 1992; Dowdy, 1999), humidity (Evans, 1983), carbon dioxide levels (Soderstrom *et al.*, 1992) and even different hues immediately around it (Ramos *et al.*, 1983; Viswanathan *et al.*, 1996; Khan *et al.*, 1998). Sheribha *et al.* (2010) assessed possibilities for the management of *T. castaneum* on stored products using coloured lighting systems. They found that red light is not preferred by *T. castaneum* adults. Thus, if storage areas were lit red, *T. castaneum* beetles could be managed without the use of chemical pesticides.

Legal methods

Entry of insects that are not found in a particular area can be prevented by the imposition of laws, such as the Destructive Insects and Pests Act 1914 in India.

Exclusion

Prevention is one critical factor in any effective pest-management programme as prevention of the introduction of pests means prevention of losses of both product and time. Entry of insects into storage facilities can be prevented efficiently if products such as grains, cereals, flour and other packed items are inspected properly. Materials to be stored need to be checked for eggs and insect frass as well as living insects. Any contaminated material should immediately be disinfested or destroyed. Entry of insects can be prevented by using screens over windows and doors. Rodent holes or crevices where insects can enter should be filled. It is also possible to make the area less attractive to insects by using sodium rather than mercury lights.

Environmental modifications

Heat and cold treatments can be used for the management of storage pests, as heat kills some pests while cold blocks their development. It has been reported that a temperature of 15°C prevents storage pests from feeding, while 4°C kills them over a period of time.

COLD TREATMENT A temperature below 4°C results in death, particularly of the immature stages of almost all insect pests. Death occurs rapidly at freezing point. *T. castaneum* and *Oryzaephilus mercator* are highly susceptible to cold, whereas *Trogoderma* spp., *Ephestia* spp. and *Plodia interpunctella* are cold-tolerant species. In most field applications of a cold temperature to control insect pests, the insects are exposed to a temperature of 10–20°C for some days before being exposed to a lethal cold temperature.

HEAT TREATMENT Most stored-grain insect pests die at 50–60°C within a period of 10–20 min. Exposure to a temperature only about 5°C above the optimum for the species will stop development. Exposure to 50°C for 2 h eliminates most insect pests. Grain heating is carried out using a hot-air

fluidized bed, infrared radiation or high-frequency dielectric and microwave heating to achieve a uniform grain temperature throughout the storage structure.

CONTROLLED ATMOSPHERE/HERMETIC STORAGE

Use of a controlled atmosphere for storage of grain involves the use of high CO_2 (9.0–9.5%) and low O_2 (2–4%) levels, conditions that are lethal to all insects. This technology for control of stored pests has been extensively used in the field.

Desiccants

Some desiccants such as earth, silica gel and non-silica and diatomaceous earth can be combined with stored grains to provide protection against insect damage. The desiccants are removed from the grain or stored foods before processing by a cleaning operation that also removes debris. The rate for bulk grain is 100–300 g/t, depending on the insect species and the grain moisture content. The rate for surface treatment is 0.3 mg/g to the top 45.7 cm of the grain mass. Alternative dusting preparations include ash, laterite dust, clay dust or very fine sand. The quantities commonly applied vary considerably and can reach up to 50% by volume. Depending on the type of dust, however, it is also possible to achieve an acceptable protective effect with considerably smaller quantities.

Sorting

Food grains displaying insect infestation, mould, mechanical damage or any other inferior quality must be removed and processed as soon as possible. This will prevent contamination of the healthy grains and maintain the overall quality of the produce.

Dividing the harvest

It is always advisable that produce meant for storage be divided into two parts, one for short-term daily requirements and the other for long-term storage. Generally, no losses are caused by insects for up to 3–4 months. Therefore, food grains intended for consumption during this period need not be treated, whereas those intended for long-term storage require proper treatment.

Treatment with lime dust

Quicklime is one of the most important inexpensive dusts used for food grains stored in their husks. Lime dust has a dehydrating effect on insects and blocks their respiratory orifices.

14.12 Biorational Management

14.12.1 Pheromones

Pheromones are commercially available for approximately 20 species of stored-product insects as slow-release formulations of lures to be used in monitoring traps (Phillips *et al.*, 2000). The most commonly used pheromones are those for *P. interpunctella*, the cigarette beetle (*Lasioderma serricorne* F.; Coleoptera: Anobiidae), the red and confused flour beetles (*T. castaneum* and *Tribolium confusum* Jacquelin du Val, respectively) and the warehouse beetle (*Trogoderma variabile* Ballion; Coleoptera: Dermestidae). The efficacy of pheromone-baited sticky traps varies according to their placement within a building, and other flat landing sites enhance the response of *P. interpunctella* males to pheromone-baited traps (Nansen *et al.*, 2004). For beetles that tend to land and crawl to an odour source, traps are designed to sit on a floor or flat surface and capture insects that walk into the trap as they eventually become stuck to the trapping surface or ensnared inside the trapping receptacle. Barak and Burkholder (1985) developed a trap with horizontal layers of corrugated cardboard in which responding beetles walked through the tunnels of corrugations to reach a cup of oil into which they fell and suffocated.

14.12.2 Natural enemies of insects

A number of natural enemies are associated with stored-product insects, adapted to

human-based habitats, as are their prey and hosts. Detailed information can be found in the reviews by Scholler and Flinn (2000) and Scholler *et al.* (2006). Several species of parasitoid wasps from the Pteromalidae are solitary ectoparasitoids of internal-feeding grain-infesting species of beetles, and similarly there are several common species of Ichneumonidae and Braconidae as ecto-and endoparasitoids associated with stored-product Lepidoptera. Some species of free-living predatory beetles, true bugs (Heteroptera: Anthocoridae), and mites prey on any life stage of numerous species of stored-product insect pests that they can subdue and consume (Abrol *et al.*, 1989, 1994). Populations of parasitoids and predators in storage systems display delayed density dependency in their dynamics that are typical of other predator–prey and parasitoid–host systems in other insect communities, and population declines in stored-product pest species are typically followed by increases in these natural enemy populations.

14.12.3 Microbial pesticides

Spinosad is a commercial bacterial insecticide derived from metabolites of the actinomycete bacterium *Saccharopolyspora spinosa* (Mertz and Yao). It is highly effective in controlling insects associated with stored wheat (Flinn *et al.*, 2004). In field crops, spinosad loses its activity after a week due to breakdown caused by UV radiation from sunlight. However, in farm bins, where most of the wheat is not exposed to sunlight, spinosad degrades very little over 12 months of storage, with no appreciable loss of insecticidal activity against the lesser grain borer (*Rhyzopertha dominica* F.) and the red flour beetle (*T. castaneum* Herbst) (Fang *et al.*, 2002). Laboratory and field tests using stored wheat have shown spinosad to be effective against several stored-product insects. Spinosad applied to wheat at 0.1 and 1.0 mg/kg was effective in killing all adults and preventing population growth of *R. dominica*. A rate of 1.0 mg/kg was necessary for complete control and progeny suppression of the rusty grain beetle (*Cryptolestes ferrugineus* Stephens), the flat grain beetle (*Cryptolestes pusillus* Schonherr) and the confused flour beetle (*T. confusum*) (Toews and Subramanyam, 2003). *Bacillus thuringiensis* (*Bt*) is a registered protectant for use in stored grains in the USA. The larvae of *P. interpunctella* and the dried currant moth (*Ephestia cautella*) show high susceptibility to *Bt*. Nuclear polyhedrosis virus, granulosis virus and cytoplasmic polyhedrosis virus are isolated mainly from lepidopteran insects and have potential for control of these pests.

14.12.4 Biological control

A number of insect predators and parasitic wasps attack insect pests of stored grain and can be used effectively if applied in overwhelming numbers. However, biologicals are generally not used because the US Food and Drug Administration (FDA) and food processors do not accept live insects or insect parts in raw grain. Biological agents have limited commercial availability and are cost prohibitive, except perhaps when used in organic production (Weaver and Petroff, 2004). Controlling insect pests in stored grain and grain products can be very difficult because of the variety of species that can infest grain. Insect parasitoids have been shown to be effective in suppressing a limited number of pest species both in bulk grain storages and in food processing facilities and warehouses. One of the more effective parasitoids is *Theocolax elegans* (Westwood), a small pteromalid wasp (1–2 mm long) that attacks primary grain pests whose immature stages develop inside grain kernels, including the weevils (*Sitophilus* spp.), the lesser grain borer (*R. dominica* F.), the drugstore beetle (*Stegobium paniceum* L.), cowpea weevils (*Callosobruchus* spp.) and the angoumois grain moth (*C. cerealella*) (Flinn *et al.*, 2006). *Trichogramma* spp. have also been evaluated against a variety of stored-product moths in bulk groundnut storage, bulk wheat storage and bakeries, as well as in warehouses and retail stores in Europe (Grieshop *et al.*, 2007). Stored-product moths commonly oviposit on packages and

on shelves holding stored-product packages. *Trichogramma* spp. are especially promising as biological control agents on finished products because they attack the egg stage of the pests, thereby preventing invasion of products by first instars. *Dinarmus* spp. is a larval/pupal parasitoid of *Callosobruchus* spp., *Bruchus* spp., *Bruchidius atrolineatus* and *Acanthoscelides obtectus* in legume seed. Biological control has a limited scope in stored-grain pest management but is becoming an increasingly important part of an IPM approach.

14.13 Chemical Methods

14.13.1 Botanicals

Botanicals are chemicals produced by plants that repel approaching insects, deter feeding and oviposition on the plant, or disrupt the behaviour and physiology of insects in various ways. Various products of plants have been tried recently with a good degree of success as protectants against a number of stored-grain insect pests (Pandey *et al.*, 1986; Yadav and Bhatnagar, 1987; Dixit and Saxena, 1990; Verma and Dubey, 1999; Shukla *et al.*, 2007, Srinivasan, 2008). Botanicals such as neem possess repellent, anti-feedant and feeding deterrent properties against storage insect pests. Neem seed kernel powder at 4.0% (w/w), neem seed oil at 1.0% (v/w) and mahua oil at 1% (v/w) proved repulsive and a potent oviposition inhibitor in checking damage by the pulse beetle, *C. chinensis* for up to 8 months in pigeon pea (Singal and Chouhan, 1997). Wheat grains mixed with neem and dharek (*Melia azedarach*) leaves at 4% (w/w) were found to be less damaged by insects. Essential oils have been widely used as antiparasitic, bactericidal, fungicidal, antivirus and insecticidal agents. Pérez *et al.* (2010) reviewed the activity of essential oils as a biorational alternative to control coleopteran insects in stored grains. The rice grains treated with turmeric powder at 3.25% (w/w) were found to be least infested by rice weevil. Kirubal *et al.* (2008) reported that 0.2% (v/w) ginger grass oil on red gram prevented oviposition and F_1 emergence of *C. chinensis* (L.) for a long period after initial release of the adults. Paranagama *et al.* (2003) reported that damage to grain was lower in *Oryza sativa* treated with the essential oils of *Cymbopogon citratus* (Stapf) and *Cymbopogon nardus* (Rendle) than in the control rice grains. *C. citratus* and *C. nardus* showed deleterious effects on oviposition and F_1 adult emergence of the cowpea bruchid *Callosobruchus maculatus* (F.) compared with the control during no-choice tests. Rajasekharreddy and Usha Rani (2010) evaluated the insecticidal activity against adults of three stored-product pests in a test using a filter paper diffusion method (contact application) and found that *Curcubita maxima* leaf extract showed 100% mortality to *Sarocladium oryzae* and *R. dominica* within 3 days of treatment at a rate of 8.5 mg/cm^2, whereas, only 65% mortality was observed against *T. castaneum* at this dosage. The application of crude plant extracts of *Citrus sinensis* and *Citrus aurantium* in the same concentrations, caused 89 and 76% mortality to *S. oryzae* and *R. dominica*, respectively, by 72 h post-treatment. Among the three insects tested, *T. castaneum* was the most tolerant, having the lowest mortality rate against all the phytochemicals. Antifeedant and ovipositional activities of the essential oils agnuside and viridiflorol, obtained from the leaves of *Vitex negundo* (Lamiaceae) were tested against *C. chinensis* and *S. oryzae*. The essential oils were effective against both species at concentrations of 0.062–0.5%, and had anti-feedant activity against *S. oryzae* at 0.25%, and up to 0.58 and 1.69% seed damage was observed (Rana *et al.*, 2005). The essential oil of *Cymbopogon martini* was an effective repellent against the beetles *C. chinensis* and *T. castaneum*. The oil also affected oviposition, adult development and mortality of *C. chinensis* in cowpeas. The *C. martini* oil used as a fumigant did not affect viability, germination or seedling growth of gram (garbanzo bean) (Rajesh *et al.*, 2007). A list of some of the spices and botanicals used in stored-food protection is given in Table 14.9.

Table 14.9. List of spices and botanicals used in stored-food protection.

Scientific name	Common name	Effect on storage pests	Reference(s)
Spices			
Allium sativum	Garlic	Repels *Tribolium castaneum*. Oil kills *T. castaneum* and *Sitophilus zeamais*. Strong repellent for *T. castaneum* and *S. zeamais*	Mohiuddin *et al.* (1987); Ho (1995)
Curcuma longa	Turmeric	Repels a number of stored insects. A 2% powder mix with rice and wheat can protect from attack by storage pests	Jilani and Su (1983)
	Star anise	Extract kills adults and eggs of *T. castaneum*	Ho (1995)
Piper nigrum	Black pepper	Inhibit development of F_1 of *Callosobruchus chinensis*	Morallo-Rejesus *et al.* (1990)
Syzygium aromaticum	Clove tree	Repels *T. castaneum*. Repels a number of grain insects	Grainge and Ahmed (1988)
Zingiber officinale	Ginger	Causes adult mortality in *C. chinensis* and repels *T. castaneum*	Ho (1995)
Botanicals			
Acorus calamus	Sweet flag	Rhizome powder gave protection to wheat and paddy in storage for 8 months. Oil is toxic to *Sitophilus oryzae* and *Sitotroga cerealella*	Teotia and Tewari (1977)
Adhatoda vasica	'Vasaka'	Resin toxic to stored-grain insects. Leaf powder effective against *S. oryzae* and *R. dominica*	Dastur (1951); Chellappa and Chelliah (1976)
Agave americana	Century plant, American aloe	Leaves are used against stored-grain pests	Grainge and Ahmed (1988)
Annona reticulata, *Annona squamosa*	Bullock's heart, sugar apple, custard apple	Seed is insecticidal to *C. chinensis*. Leaf extract inhibits growth of *S. cerealella*	Hussain and Masood (1975); Grainge and Ahmed (1988)
Artemisia absinthium	Absinthe, wormwood, madderwood	Leaf used as an insecticide against *S. cerealella*, *Sitophilus granarius* and *Tinea granella*	Grainge and Ahmed (1988)
Azadirachta indica	Neem	Almost every part is pesticidal but the seed kernel has maximum pesticidal activity. An example of an insect pest of stored products that is susceptible to neem is *Trogoderma granarium*. Neem oil at 0.5% is the best surface protectant against pulse weevils	Ketkar (1987) and numerous other references
Calophyllum inophyllum	'Undi'	Oil used as surface protectant against pulse weevils	Ketkar (1987)
Cedrus deodaaa	Himalayan cedar	Wood oil is used as grain protectant against rice weevil for 30 days at dosage of 1000 p.p.m.	Singh *et al.* (1989)
Melia azedarach	Chinaberry	Leaf and drupe powders (1 and 4%) protect wheat against *S. cerealella*	Teotia and Tewari (1971)
Pongamia glabra	Pongam oil tree, Indian beech	Leaves have insecticidal property against stored-grain pests	Ahmed and Koppel (1987)
Schleichera trijuga	Lac tree, macassar oil tree	Oil used as surface protectant against pulses weevils. Extract kills adults of *S. zeamais* and eggs of *T. castaneum*. It also repels both species	Ketkar (1987); Ho (1995)
Tephrosia villosa	–	Seeds are insecticidal to *C. chinensis*	Puttarudrian and Bhatta (1955)
Vitex negundo	Indian privet	Leaves have insectcidal property against stored-grain pests	Ahmed and Koppel (1987)

14.13.2 Insect growth regulators

Insect growth regulators (IGRs) are chemicals that affect an insect's ability to develop correctly or pass through the various developmental stages. They have a low toxicity towards humans compared with organophosphate, carbamate and synthetic pyrethroid insecticides. Several IGRs, including hydroprene have been evaluated for efficacy toward stored-product beetle species (Oberlander *et al.*, 1997). Because of the low toxicity of IGRs, they are usually safe to spray directly on to raw products. An IGR should be used where fumigation is not possible or desirable. An IGR is only effective if it directly contacts the target insect so thorough coverage is necessary. IGRs can be applied as a spray to grains, nuts or other foodstuffs during the filling of storage bins. It is necessary to use sufficient spray to protect all of the stored product. The spray should be applied when the insects are at the correct stage of development, as described on the IGR label. Occasionally, application of an IGR extends the larval period, so the larvae may continue feeding for a while before they are destroyed.

14.13.3 Fumigants

Control of stored-product insects in bulk containers, warehouses and other large storage structures/areas requires fumigation. Fumigants help to kill the insects in hidden places that may later become a problem. The area to be fumigated must be properly sealed so that fumigants can reach a lethal concentration and, once the required level is reached, it should be maintained for a specified period for better results. However, small quantities of cereals and other products can be fumigated in small containers subject to the condition that the containers are tightly closed immediately after the treatment. Care must be taken that the containers do not explode as a result of the creation of a vacuum. Thus, the lid is tightened only when the container has warmed up to room temperature.

Of the various available fumigants, methyl bromide has been found to be highly effective as it acts rapidly in less than 48 h killing not only insects but also other pathogens such as microbes and nematodes. However, as this chemical depletes the ozone in the atmosphere, it may become restricted in the near future (Fields and White, 2002). There are many options other than methyl bromide, such as the use of physical control methods such as heat and cold as described above, proper sanitation, and fumigant replacements such as carbonyl sulfide, phosphine and sulfuryl fluoride. Carbon dioxide can be used as a fumigant but has the drawback that it is less toxic to insects than some of the other fumigants and requires a high degree of air tightness to be effective, so is unlikely to find widespread acceptance except in controlled atmospheric storage systems.

14.13.4 Use of residual insecticides

Short-term residual insecticides such as pyrethrins can be used for the rapid knockdown of some types of stored-product insects. These materials can be applied to bulk containers before adding the storage products. They are also used in cupboards and on shelves and areas close to where products are stored, but, if infestation levels are high, frequent re-applications are usually necessary. Residual insecticides, including some persistent pyrethroids, should be used selectively. Residuals are generally applied to the surfaces of empty containers to prevent infestation but are rarely applied directly to foodstuffs (Taylor, 1991).

14.14 Management of Non-insect Pests in Storage

The management of non-insect pests in stored products can be achieved using various chemicals, such as:

- commonly used rodenticides;
- aluminimum phosphide, a fumigant used to kill rats and mice;
- bromodiolone, coumachlor, coumatetralyl and warfarin, anticoagulants that

are mainly stomach poisons and act as rodenticides;
- sodium cyanide insecticide, which has a respiratory action and can also be used as a rodenticide; and
- zinc phosphide, a highly toxic inorganic compound with a garlic-like odour and taste that is commonly used in baits.

Rats can best be controlled by baiting with 2% zinc phosphide. The baits are prepared as 2 g zinc phosphide to 98 g of bait (e.g. wheat flour, grain, cooked rice) along with an edible oil such as mustard, which is added in very small quantities to serve as an attractant. It may take up to 5–7 days for the baiting to be effective.

14.15 Conclusions

It is high time to think about the severity of the problem of storage losses, as it is estimated that different countries throughout the world suffer major losses as a result of attacks by stored-product insects as well as from improper storage conditions. A systems approach rather than a piecemeal one needs to be adopted. The existing postharvest system has to be improved to cut postharvest losses at the farm level where about 70% of grains are stored and consumed as food and feed and for seed purposes.

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15 Role of Integrated Pest Management in Food and Nutritional Security

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15.1 Introduction

Food and nutritional security is an agricultural issue that has political connotations worldwide and more strikingly so in the developing world, where systems of governance have assumed and lost power on this issue. In terms of share in the gross domestic product (GDP) and the proportion of the population engaged in this occupation, agriculture is the mainstay of the Indian economy, which has the second largest population in the world. However, agricultural practices in India do not compare well with the indices obtained in the developed world where only a small proportion of the population is engaged in agriculture. This contrast presents a paradox, requiring further insights to work towards improvements in agricultural productivity to ensure food and nutritional security in India, and poverty alleviation for the most food-insecure and vulnerable populations (FAO, 2008b).

Currently, the world population is growing at an alarming annual rate of 1.2% in countries like India, China, Pakistan, Nigeria, Bangladesh and Indonesia. The population, which was 4.9 billion in 2000, is expected to rise to 8.2 billion by 2050. An adult person needs on average 2900 kcal/day to work productively. In developed countries, the daily average consumption of food provides about 3500 kcal, whereas in poor countries, people may not obtain even 2000 kcal/day and thus suffer from undernourishment. According to the Food and Agriculture Organization of the United Nations (FAO), more than 840 million people all over the world were undernourished in 1998–2000.

At present, the pressing challenge is to produce more food and to ensure food security to alleviate poverty and undernourishment and, at the same time, to improve human health and welfare. Although poverty and undernourishment are also increasing in large cities, more than 80% of the undernourished and poor live in rural areas (FAO, 2005). Enhanced industrialization is unlikely to meet this need; rather, small-scale and sustainable agriculture is the solution to this problem (UN Millennium Project, 2005). Another challenge is to feed the growing world population in the long term (Gilland, 2002). Therefore, the gap between the amount of food produced and that required to feed the global population is likely to increase until at least 2050. The only way to counteract the current undernourishment of many populations and to feed the global

 Integrated Pest Management (eds D.P. Abrol and U. Shankar)

population is to increase agricultural production and to curb the population growth. Despite the conflicting views about the human carrying capacity of the Earth (Cohen, 2005), overpopulation will not help to solve undernutrition. Furthermore, technology may not be able to provide miraculous solutions in time to feed a continuously growing population (Gilland, 2002).

To increase the area of agricultural land use is not an easy task, as the amount of agricultural land per inhabitant is decreasing all over the world. For example, in Latin America and the Caribbean, this area will decrease from 0.40 ha per inhabitant in 1990 to 0.32 ha per inhabitant in 2010, in North Africa and Middle East the decrease is from 0.28 to 0.18 ha per inhabitant in the same period and in South Asia the decrease is from 0.22 to 0.16 ha per inhabitant (Alexandratos, 1999). This is partly due to population growth, but there is also a net loss of agricultural land due to erosion, reductions in fertility, salinization and desertification of soils. New land cannot be found without sacrificing the forest areas, many of which have been classified as ecological reserves and natural parks (Alexandratos, 1999). Another major problem in many countries such as Africa, the Middle East and Asia is the scarcity of water, where there may not even be sufficient for drinking purposes let alone irrigation. One should keep in mind that the production of 100 kg of wheat requires 50,000 l of water, and 200,000 l of water are needed to produce 100 kg of rice.

According to the FAO (1999), the number of hungry people in the world was expected to increase from 825 million in 1997 to an estimated 1.02 billion by the end of 2009 (FAO, 2009a). Food security remains an important issue and a mission for both developed and developing countries. Food security can be defined as the condition when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet both their dietary needs and their food preferences, allowing them to lead active and healthy lives (FAO, 2008a, 2009b). To meet the needs of smallholding, resource-poor farmers, and to reduce the number of hungry people in the world, agricultural development is crucial in rural areas, but non-agricultural improvements can also be important (Diao *et al.*, 2007). Improvements in agricultural productivity that have been stimulated by government investment in rural infrastructure, agricultural research and extension, irrigation and appropriate price incentives have contributed directly to economic growth, poverty reduction and stability (Timmer, 1992, 1995, 2004).

15.2 Food Production and Food Security

Food production and the resultant food security are subject to several constraints such as changes in climate leading to extreme events such as cyclones, floods, hailstorms and droughts (IPCC, 2001, 2007). More than 99% of the world's food supply comes from the land, while less than 1% is from oceans and other aquatic habitats (Pimentel *et al.*, 1994). Of the several factors that affect food loss, pests and pathogens are a major contributor.

15.2.1 Losses caused by insect pests

Crop yields are reduced and destabilized by pests, which also affect the quality of the harvested produce. To keep pace with growing demand, global food production needs to increase by an estimated 70% by 2050 (Bruce, 2010). Insect pests, diseases and weeds inflict enormous losses on potential agricultural production (Tables 15.1 and 15.2). There is some evidence indicating that these losses are rising, despite the increasing use of chemical pesticides. At the same time, there is rising public concern about the potential adverse effects of chemical pesticides on human health, the environment and biodiversity. These negative factors cannot be eliminated altogether; however, their intensity can be minimized through the development, dissemination and promotion of alternative technologies such as the use of biopesticides

Table 15.1. Estimated losses for eight crops (rice, maize, wheat, barley, soybean, coffee, cotton and potato) during 1988–1990.

	Losses due to pests (US$ billion)			
Region	Pathogens	Insects	Weeds	Total
Africa	4.1	4.4	4.3	12.8
Asia	43.8	57.6	43.8	145.2
South America	7.1	7.6	7	21.7

Source: Oerke (2005).

Table 15.2. Production constraints on important food crops.

Food crop	Production constraint
Maize	*Striga hermonthica*, stem borers, phosphorus uptake
Sorghum	*S. hermonthica*, anthracnose, phosphorus uptake
Millet	*S. hermonthica*, head miner, downy mildew
Rice	African rice gall midge, rice yellow mottle virus
Cowpea	Maruca pod borers, bruchids, thrips
Cassava	Root rots, green mite
Banana	Banana weevil, nematodes, black Sigatoka

Source: Blackie and Gibbon (2003).

and bioagents, as well as good agronomic practices, rather than relying solely on chemical pesticides.

Globally, approximately half of the food and fibre produced is lost to field and storage pests (insects, pathogens, weeds, nematodes and vertebrate pests) (Pimentel, 1997). These losses threaten global food security and are a serious economic and nutritional burden to farmers and consumer around the world (Tables 15.3 and 15.4). Evidence indicates that pests cause 25% loss in rice, 5–10% in wheat, 30% in pulses, 35% in oilseeds, 20% in sugarcane and 50% in cotton (Dhaliwal and Arora, 1996). Overall, insect pests inflict crop losses to the tune of 30–40% in vegetable production (Srinivasan, 1993) and in many cases, there is 100% yield loss due to viral diseases vectored by insects (Shivalingaswamy *et al.*, 2002).

A comprehensive study of pest-induced crop losses to date was published by a team of German crop scientists in 1994, with support from the European Crop Protection Association (Oerke *et al.*, 1994). It covers eight crops that together occupy half the world's cropland, with harvests worth US$300 billion in 1988–1990. It did not cover some important developing country food crops, such as cassava, millet and sorghum. The study found that pests accounted for preharvest losses of 42% of the potential value of output over 1988–1990, with 15% attributable to insects and 13% each to weeds and pathogens. Postharvest losses accounted for an additional 10% of the potential value. Among crops, the loss potential of pests worldwide varied from less than 50% (on barley) to more than 80% (on sugarbeet and cotton). Actual losses were estimated at 26–30% for sugarbeet, barley, soybean, wheat and cotton, and 35, 39 and 40% for maize, potatoes and rice, respectively. Overall, weeds had the highest loss potential (32%), with animal pests and pathogens being less important (18 and 15%, respectively). Although viruses cause serious problems in potatoes and sugarbeet in some areas, worldwide losses due to viruses averaged 6–7% on these crops and <1–3% in other crops. The efficacy of crop protection was highest in cash crops (53–68%) and lower (43–50%) in food crops (Oerke and Dehne, 2004).

Table 15.3. Average crop losses attributable to pests of cultivated food crops.

Crop	Pest	Estimated crop loss (%)
Cereals		
Maize	*Busseola fusca*	10–43
Rice	Stem borers	25–30
Sorghum	Weeds	35
Grain legumes		
Cowpea	*Maruca testulalis*	30
	Nezara viridula	25–35
	M. testulalis	80
	Megalurothrips sjostedti	40
Pigeon pea	*Acanthomia tomentosicollis*	15–35
Field beans	*Ophiomyia phaseoli*	30
Groundnuts	*Caryedon* spp., *Serratus* spp.	25
Roots and tubers		
Cassava	*Mononychellus tanajoa*	13–80
	M. tanajoa and *Phenacoccus manihoti*	30
	Nigeria *Zonocerus variegatus*	16–55
Yam	Yam beetles	42
	Amitermes spp.	24
Sweet potato	*Cylas puncticollis*	15–20

Source: Youdeowei (1989).

Table 15.4. Yield losses due to insect pests in vegetables.

Major crops and their relative insect pests	Yield losses (%)
Crucifer crops	
Diamondback moth (*Plutella xylostella*)	16.87–98.83
Cabbage butterfly (*Pieris brassicae*)	68.5
Crucifer leaf webber (*Crocidolomia binotalis*)	28.09–50.88
Cabbage borer (*Hellula undalis*)	30–58
Crucifer aphid (*Lipaphis erysimi*)	36.5
Mustard saw fly (*Athalia lugens proxima*)	36.5
Chillies	
Chilli thrips (*Scirtothrips dorsalis*)	11.8–90
Chilli mites (*Polyphagotarsonemus latus*)	34.14
Brinjal (egg plants)	
Fruit and shoot borer (*Leucinodes orbonalis*)	48–66
Tomato	
Fruit borer (*Helicoverpa armigera*)	22.39–37.79
Okra/lady's finger	
Shoot and fruit borer (*Earias vitella*)	22.79–54.04
Jassids/leafhoppers (*Amrasca bigutulla bigutulla*)	54–66
White flies (*Bemesia tabaci*)	54.04
Fruit borer (*H. armigera*)	22.1
Cucurbit crops/summer vegetables	
Fruit flies (*Bactrocera cucurbitae*)	
Bitter gourd	60–80
Cucumber	20–39
Muskmelon	76–100
Snake gourd	63
Sponge gourd	50

Source: Shivalingaswamy *et al.* (2002).

At the end of 20th century, dependency of rice production on pesticide application in South-east Asia was one of the main factors that, together with population growth, degradation of soil fertility and water resources, created tension in terms of food security and pesticide pollution of the environment (Bull, 1982; Kenmore, 1991; Lampe, 1994). Pesticides are significant inputs to rice production: in 1988 alone, insecticides costing US$910,000,000 were used on rice worldwide, more than for any other crop (Lampe, 1994). The FAO Global Integrated Pest Management (IPM) Facility has helped to start and is currently working with pilot farmer field school (FFS) programmes in over a dozen countries, from Senegal to South Africa.

It has been estimated that 5–40% of crop losses each year are due to pests (Stephensons, 1991). Erinle (1992) also reported that pests account for roughly 30% of the 'untaken harvest'. Pests damage crops at different stages of growth in the field, at harvest, during transportation and in storage. This leads to significant crop losses yearly and has a serious effect on food security for the ever-increasing population. The management of pests in crops to obtain a better yield is paramount for food security. Resource-poor farmers who produce a large percentage of their crop for consumption cannot afford expensive management of pests to meet the yearly target consumption levels for their country. Different methods for the management of pests include cultural, biological and indigenous knowledge systems, the use of resistant varieties, the use of plant extracts, the use of pheromones and the minimal use of chemicals in an IPM system (Alabi *et al*., 2006). According to available data, pests and diseases together cause 30–40% loss in crop production.

15.3 The Role of IPM

It is in this context that the role of IPM needs to be understood. In sustainable agricultural development, IPM can play a key role in a reduction in crop losses, thereby increasing productivity while minimizing environmental contamination and health hazards. In recent years, agricultural research and development partners have pioneered outstanding contributions in IPM, most notably in varietal resistance against pests, biological control of alien invasive species, substitution of inorganic pesticides with biopesticides, new export market opportunities and new tools in biotechnology. Ideally, an IPM programme considers all available pest-control actions, including no action, and evaluates the potential interaction among various control tactics, cultural practices, weather, other pests and the crop to be protected (Flint and van den Bosch, 1981). IPM is a knowledge-intensive, farmer-based approach that encourages natural control of the pest population by anticipating pest problems and preventing the pest from reaching economically damaging levels (Indonesian IPM Secretariat, 1997). Control of the pest population is ideally achieved using techniques such as enhancing natural enemies, planting resistant crops and cultural management, using pesticides only as a last resort (Maredia, 1997; Schillhorn van Veen *et al*., 1997). IPM is a systems approach for design, use and continued evaluation of pest-management procedures that result in favourable socio-economic and environmental consequences (Bird *et al*., 1990). Over the last few decades, many new developments have taken place to reduce chemical input into agriculture, and new knowledge, policies, technologies and strategies have emerged to improve or reduce pesticide usage and to develop alternative strategies to manage pests in an environmentally friendly way.

15.3.1 Essential ingredients of IPM

Successful IPM programmes rely on knowledge of the farmers about the crop, pest and predator biology, use of pest-resistant varieties, crop rotation, improved soil management and encouraging beneficial insects, thereby replacing the dependence on spraying of pesticides. Making farmers aware of non-chemical alternatives is one of

the best solutions to overcome the problem of reliance on pesticides. Governmental support can also help to popularize IPM and its adoption.

15.3.2 Adoption of IPM and success stories

FFSs on IPM in south India have proved very successful strengthening the agricultural knowledge and skills of poor farmers to alleviate their hardship with respect to food and financial security. Jiggins and Mancini (2009) reported that FFSs comprised four principles: (i) conservation of natural enemies; (ii) production of a healthy crop; (iii) performance of regular field observations; and (iv) expertise of farmers in their own field. FFSs involve a group-based learning process to promote IPM, which is utilized by a number of governments, non-governmental organizations and international agencies. In 1989, the FAO organized such FFSs in Indonesia and since then, more than 2 million farmers across Asia have participated in such schools (Bartlett, 2005).

Khan and Pickett (2004) successfully documented the application of push–pull strategies in eastern Africa for the management of maize pests. According to Khan *et al.* (2006), more than 160,000 farmers are now using push–pull strategies to protect their maize and sorghum against stem borer (*Chilo partellus*), based on the combined use of intercrops such as molasses grass (*Melinis minutiflora*) and silverleaf desmodium (*Desmodium unicinatum*), and trap crops such as Napier grass (*Pennisteum purpureum*) or Sudan grass (*Sorghum vulgare sudanense*) that are locally available and exploit natural enemies. The rapid spread of FFSs (Fig. 15.1) and Junior Life Schools (for school-aged children; Fig. 15.2) throughout the world is helping to carry the strategy to an increasing number of farmers. The adoption of the push–pull strategy for stem borers has led to increased crop yields and livestock production, with a significant impact on food security throughout the region.

Fig. 15.1. A typical farmer field school (FFS).

One example of a success story of the establishment of an exotic parasitoid was that of *Diglyphus isaea* (Hymenoptera: Eulophidae). The larval form of this ectoparasitoid of the leaf miner *Liriomyza huidobrensis* was released into the fields in 1997–1998. A post-evaluation survey was carried out in 2000 in six locations of Nuwara Eliya in the district of Sri Lanka where the parasitoid was released. The percentage of parasitism ranged from 1.3 to 65%, and in locations where there was a high level of parasitism, the farmers did not need to use highly toxic insecticides to control these vegetable pests (Nugaliyadde *et al.*, 2001).

15.3.3 The impact of IPM interventions on crop productivity

IPM interventions have been found to minimize pest losses and increase productivity. For instance, IPM intervention in rice reduced pesticide usage by 67% and increased productivity by 25% (Table 15.5). Interestingly, in sugarcane, pesticides have been eliminated altogether, resulting in increased income for farmers. Similarly, in other crops, superior yields have been obtained following IPM interventions. IPM interventions in South Africa are reported to have decreased crop losses by 90% in the case of cassava afflicted by cassava mealybugs (Table 15.6). In other crops, losses dropped by 5% and yield increased by 100%. Pesticide use was reduced considerably from 68% to only 11% for control of rice leaf feeders in the Philippines following IPM interventions.

15.4 Causes for Concern in Food Security

15.4.1 Invasive pests

Invasive pests pose a serious threat to crops and animals in new locations as they thrive well in new areas where their hosts are in abundance and their own natural

Fig. 15.2. A typical IPM awareness programme for school children.

Table 15.5. Impact of IPM interventions in reducing pest losses in India.

Crop	IPM components	Pesticide reduction	Benefits
Rice	Biocontrol insects, subsidy abolished, need-based pesticides	67% quantity, 0–2 versus 4–6 applications, 25% increase in production	Net profit US$ 20/ha. Subsidy abolition. US$1 million/year
Sugarcane	Biocontrol insects, post-harvest burning avoided	No chemicals, aerial applications avoided	US$15/ha/year increase in farmer income
Cotton	Pheromones, Economic Threshold Level (ETL) used, need-based pesticides	40% quantity, 2–4 versus 8–12 applications	US$20/ha
Groundnut	Scouting, need-based pesticides, biocontrol agents	0–1 versus 2–3 applications	Superior crop yields

Source: Shrivastava (2003).

Table 15.6 Impact of IPM interventions in reducing pest losses and increasing productivity.

Pest	Loss/situation at selected locations	Intervention by whom and how?	Benefits
Cassava mealybug	40% loss in 27 countries of sub-Saharan Africa	IITA – biocontrol	90% drop in losses, US$8–20 million
Andean potato weevil	50% loss in Peru	CIP – microbials	Loss drop to 5%, US$12 million
Striga weed in maize	US$13 million loss in Kenya	ICIPE – habitat management	>100% yield gain, >2.5 benefit–cost ratio
Diamondback moth in cabbage	20 sprays needed per season in the Philippines	AVRDC – pesticide use management plus biocontrol	Sprays drop to four, US$10 million/year
Rice leaf feeders	68% of farmers apply insecticides in the Philippines	IRRI – communication	Proportion of farmers spraying dropped to 11%

Source: James *et al.* (2007).

enemies may be reduced or absent. However, in certain situations (Neuenschwander, 2001), the introduction of natural enemies to control an exotic, invasive pest species has shown very good results. For instance, biological control of the cassava mealybug in Africa saved US$8–20 billion and control of cassava green mite saved US$2 billion in South Africa. On the other hand the stem borer, *C. partellus* (Swinhoe), which was accidentally introduced from Asia before the 1930s, became the major pest in the 1990s in eastern and southern Africa resulting in more than 70% loss in yield of cassava in lowland and mid-altitude areas. Evidently, there is a need for stepping up maintenance research to ensure that new strains of pests and pathogens do not cause crop losses and to prevent the introduction of invasive alien species.

15.4.2 Use of chemicals

Use of chemical input has increased agricultural production and productivity. However, negative factors have also been increased, which include damage to the environment, agricultural land and water resources, and pesticide poisoning among agricultural workers, fauna (elimination of natural enemies) and flora. The costs from these negative factors are large and affect farmers' returns. However, despite these high costs, farmers continue to use pesticides

and in increasing quantities (Wilson and Tisdell, 2000). Although farmers in Sri Lanka were willing to pay a higher price to use safer pesticides or adopt IPM strategies that included biological control of pests and diseases, such services are not easily available to farmers in these countries (Wilson, 1998). IPM is practised in many countries but has been carried out on only a small scale for many reasons. As the World Resources Institute has pointed out (WRI, 1994), IPM in developing countries is more the exception than the rule. While the majority of pesticides used in developed countries are herbicides (which are less toxic than insecticides in most instances), the bulk of pesticides used in developing countries are insecticides, which leads to insecticide resistance by pests and causes the most damage to human health (WRI, 1999). Furthermore, the insecticides used in developing countries are often obsolete, belonging to the organophosphates and carbamates, which are noted for their acute toxicity. The initial use of pesticides was very effective in reducing pest infestations and increasing agricultural production and productivity. However, over time, the targeted pests developed resistance to the pesticides, necessitating increasing numbers of applications or resulting in rising populations of pests, or both. After a point, the resistance of the pest may grow to such an extent that application of pesticides is no longer economic. Increases in pesticide use to control pests that easily attack commercially grown high-yielding varieties have led to an increase in the virulence of many species of crop pests due to the destruction of non-target species, which includes natural predators of pests and parasites (Litsinger, 1989; Teng 1990; Pimentel *et al.*, 1992).

15.4.3 Pesticide concerns

Neither developing nor underdeveloped countries can dispense with the use of pesticides in IPM as the demand for food supply is increasing day by day. Pesticides cannot be dispensed with so long as we are required to produce more and more food, and environmental quality will always remain a cause of concern. Accidental deaths from pesticide poisoning worldwide are estimated by the World Health Organization at 20,000 a year, overwhelmingly in developing countries. The International Code of Conduct on the Distribution and Use of Pesticides was agreed by governments and industry in 1985 through the FAO. After nearly 10 years of international efforts to implement the Code, an FAO survey found very limited improvements in health problems, and the effect of pesticides on the environment appeared substantially worse. The corporate agenda is to intensify pesticide use in developing countries. Pesticide sales are increasing, and the industry predicts that developing countries will account for over one-third of all sales by 2000. Pimentel (2005) investigated the complex environmental costs resulting from the USA's dependence on pesticides. The major economic and environmental losses due to the application of pesticides in the USA were: (i) public health, US$1.1 billion/year; (ii) pesticide resistance in pests, US$1.5 billion; (iii) crop losses caused by pesticides, US$1.4 billion; (iv) bird losses due to pesticides, US$2.2 billion; and (v) groundwater contamination, US$2.0 billion. The discovery of neonicotinoids, which act on the central nervous system of insects with low toxicity to mammals, is considered a milestone in insecticide research. Their relatively low risk for non-target organisms and the environment, their high target specificity and the versatility of application methods has enabled this important class of insecticides to be maintained globally for IPM strategies and insect resistance management programmes. Currently, neonicotinoids represent the fastest-growing class of insecticides introduced on to the market since the commercialization of pyrethroids (Nauen and Bretschneider, 2002).

15.4.4 Climate change

Climate change affects agriculture and food production in complex ways. Many weeds,

plant diseases and insect pests benefit from warming (and from elevated CO_2, in the case of most weed plants), sometimes more than crops; as temperatures continue to rise, many weeds, diseases and pests are also likely to expand their ranges (Garrett *et al.*, 2006; CCSP, 2008; Gregory *et al.*, 2009; Lake and Wade, 2009; McDonald *et al.*, 2009). In addition, under higher CO_2 concentrations, some herbicides appear to be less effective (Ziska *et al.*, 1999; Ziska, 2000; CCSP, 2008). In the USA, aggressive weeds such as kudzu, which has already invaded 2.5 million acres of the south-east of the country, is expanding its range into agricultural areas to the north (Frumhoff, 2007). Worldwide, animal diseases and pests are already exhibiting range extensions from low to mid-latitudes as a result of global warming (CCSP, 2008; Diffenbaugh *et al.*, 2008). While these and other changes are expected to have negative impacts on crops, their impact on food production at regional or national scales has not yet been evaluated thoroughly.

Climate change in the western USA is also increasing populations of forest pests such as the spruce beetle, pine beetle, spruce budworm and wooly adelgid (Logan *et al.*, 2003), and expanding their range into forested areas previously protected from insect attack. Climate change, and the higher levels of CO_2 in the atmosphere that help to drive it, also affect the functioning of terrestrial ecosystems and their living communities (Tilman *et al.*, 1997; Loreau *et al.*, 2001); this, in turn, changes how ecosystems influence the atmosphere and climate system (Steffen *et al.*, 2004).

15.5 Strategies for Minimizing Pesticide Use

Different pest-management strategies or practices are being used by farmers to reduce pest infestations. The most important tool for evaluating an insect problem is its economic threshold. The economic threshold is the pest density at which control measures need to be resorted to in order to prevent further damage from the pest reaching economic levels. Cultural practices can help to protect the crop and reduce pest damage to the crops. Some of the most important cultural practices include tillage practices that disrupt the insect's life cycle and destroy crop residue, changing planting dates to minimize insect impact, and crop rotations that include non-susceptible crops. Some cultural practices also help to increase or decrease the population of natural enemies of pests. The utilization of resistant crop varieties is another method for controlling pests (Tables 15.7 and 15.8). The use of resistant varieties also helps considerably to reduce pesticide pressure. In addition, biocontrol agents hold great promise to control pests and minimize pesticide use but often require a long time to establish.

The IPM principle does not preclude chemical pesticide use, but rather uses it as one of the tools in the management package, to be used prudently and integrated with other tools. Thus, the concept of IPM of insects contains three basic elements: (i) maintaining insect populations below levels that cause economic damage; (ii) the use of multiple tactics to manage insect populations; and (iii) the conservation of environmental quality.

Excessive and indiscriminate use of pesticides endangers the health of farm workers and consumers of agricultural products worldwide (Goodell, 1984). People do not want to have reliance on chemicals and look for alternate strategies for pest control such as cultural, biological and biorational methods. According to Rola and Pingali (1993), this has been necessitated as a result of the negative impact of pesticides on biodiversity and food and water quality, and the high cost of pesticides and the development of resistance in pests.

The Government of India adopted IPM as a cardinal principle of plant protection in 1985. Programmes on training of both extension workers and farmers in IPM were started throughout the country. As agricultural pests cause substantial crop losses throughout the world, in the past farmers had to manage this problem in order to secure their basic subsistence needs, and so

Table 15.7 Insect pest-resistant varieties of vegetables.

Crops	Insect pests	Resistant varieties	Sources
Tomato	Fruit borer	Atkinson, Angur Lata, Punjab Chuhara	Gill *et al.* (1980)
		Sabou Prabha, Hisar 1370, No. 128, No.133, No. 135	Kashyap *et al.* (1982)
Aubergine or brinjal	Shoot and fruit borer	Black Pendi, Thorn Pendi, H-165, H-407	Panda *et al.* (1971)
		H-4, Punjab Chamkila, Pusa Purple Cluster, Pusa Purple Long, S-4, S-6	Gill and Chadha (1979)
		PPL, Pusa Kranti, SM- 41, H-4	Raut and Sonone (1980)
		Arka kusmakar, Kalaynpur long green, Muktakeshi, Nimbkar Green, Pusa Kranti, Purple Long, PPL	Mehto and Lall (1981)
		Barahmasi, Gulabi Doria, Pusa Purple Cluster, Pusa Purple Round, PPL, Nurki,Punjab bahar	Kale *et al.* (1986)
		SM 17-4, PBr 129-5, Punjab Barsati, ARV 2-C, Pusa purple round, Punjab Neelam	Singh (1991)
	Aphid, jassid, whitefly	Kalyanpur, Punjab chamkila, GB-1, GB-6	Singh (1991)
Lady's finger (okra)	Jassids	Climpson Spineless, Early Long Green, Pusa Sawani, Crimpson Smooth Long	Sandhu *et al.* (1974); Teli and Dalaya (1981);
	Shoot and fruit borer	Bhindi red-I, Bhindi red-II, Red wonder-I, Red wonder-II	Srinivasan and Narayanaswamy (1961); Mote (1978)
		AE-3, AE-22, AE-52, AE- 57, AE-67 AE-79, Wonderful pink	Kashyap and Verma (1983)
		Parkins long green, Climpson spineless, White snow, Cell round, Climpson spineless, Rashmi	Patil *et al.* (1986)
Louki/ bottle gourd	Fruitfly	MB-29	Nath (1966)
	Red pumpkin beetle	NB-19, NB-25, NB-29, NB.-3	Nath and Thakur (1965)
Melons	Mites	Durgapur cell-1, Early gold, Kocha-4, Hara madhu, MM-1, Punjab Sanchari, Pusa sarbati	Dhooria and Sukhija (1986)
Onion	Thrips	Bombay white, Pusa ratnar, White big round	Saxena (1970); Kaur *et al.* (1978)

they practised and developed cultural and mechanical pest control based on trial and error. Over a period of time, these practices have become a part of their production management system.

15.5.1 Role of push–pull strategies

Lepidopteran pests such as stem borers (*C. partellus* Swinhoe, *Eldana saccharina* Walker, *Busseola fusca* Fuller and *Sesamia calamistis* Hampson) are serious pests of maize (*Zea mays*) and sorghum (*Sorghum bicolor*) throughout eastern and southern Africa causing yield losses of 10–50%. The push–pull strategy has been a blessing for millions of people in South Africa to control these pests (Kfir *et al.*, 2002; Khan and Pickett, 2004). This strategy involves the combined use of intercrops and trap crops, using plants that are appropriate for the farmers and that also exploit natural enemies. Khan and Pickett (2004) reported that this

Table 15.8. Resistant/tolerant varieties of dry land fruit crops against different insect and mite pests in India.

Fruit crop	Resistant/tolerant stock	Insect/mite pest
Citrus	Redblush, Foster, Marsh seedless, Fallglo, Nova mandarin, Star ruby	Whitefly (*Dialeurodes citri* Ashmed)
	Cleopatra, Rubidoux, Orange Michal, Coorgcitron, Deshndo, Nagpur mandarin, Galgal, Kagzi lime	Psylla (*Diaphorina citri* Kuwayama)
	Jatti Khatti, Citrumelo, Carrizo, Troyer, Orange Michal, Coorg citron, Deshndo, Eureka lemon, Savage, Sacaton, Rangatra L. J.	Leaf miner (*Phyllonistis citrella* Stainton)
	Citron, Redblush, Marsh seedless	Lemon caterpillar (*Papilio demoleus* L.)
	Nagpur mandarin, Ikeda Unshiu, Long sport valencia, Campbel valencia, Washington Naval	Black aphids (*Toxoptera aurantii* Boyer de Fonscombe), green aphids (*Aphis gossypii* Glover)
	Washington Naval, Hazara, Jenru tenga	Scales (*Aonidiella aurantii* Maskell and *Coccus hesperidium* L.)
	Chakotra, Blood red, Kagzi lime, Malta, Mosambi, Kinnow, Galgal, Sweet lime, Etrong citron, Coorg, Wilking, Sylhet, Valencia champman	Mites (*Eutetranychus orientalis* Klein and *Panonychus citri* McGreger)
Guava	Nasik, China Surkha, Behat Coconut, Pear shaped, Red flesh, Smooth Green hybrid	Fruit flies (*Bactrocera zonatus* Saunders and *Bactocera dorsalis* Hendel)
	Red flush	Shoot borer, *Indarbela tetraonis* Moore
	Bangalore Round, Bapatala, AC 10, Seedless	Tea mosquito bug, *Helopeltis antonii* Signoret
Mango	Pulhora, Kala Hapus, Keshar Basti, Annanas, Baneshan Bangalora, Chinnarasam and Khander	Leaf hopper (*Amritodes atkinsoni* Lethierry)
	Annanas, Anain, Delicious, Gulabkhas, KO7, KO11, Maharaja of Mysore, Salem, Banglora, Vellakachi	Gall insect (*Procontarinia matteriana* Kieff. & Cecec)
	Toranjo, Monteiro, Manjurad	Fruit fly (*Bactocera dorsalis* Hendel)
	Deshi Malgoba, Alam Baneshan	Leaf gall midge (*Dasyneura mangiferae* Felt)
	Mohandas, Malgoa, Pulliadi	Mites (*Aceria mangiferae* Sayed)
Apple	Malling Merton series (MM)	Woolly apple aphid (*Eriosoma lanigerum* Hausmann)
Peach	Stark Early Giant, Early White Giant and Flavour Crest	Peach leaf-curling aphid (*Brachycaudus helichrysi* Kaltenbach)

Sources: Sharma (2006); Sharma and Singh (2006).

strategy has contributed to increased crop yields and livestock production, resulting in a significant impact on food security and livelihood of farmers in the region.

15.5.2 Location-specific IPM

Many countries of the world have tropical or subtropical climates where the losses caused by pests are most serious, thereby requiring protection of the crop for any significant yield. The management of weeds, insect pests and pathogens is one of the most challenging jobs in these areas, and consequently the use of chemicals for controlling these pests is increasing continuously. The increased use of insecticides not only results in health hazards but also enhances the cost of

production. The use of such chemicals can be minimized by adoption of location-specific IPM techniques, which include the use of natural enemies, varieties with multiple resistance and less toxic chemicals such as biopesticides. For example, in Indonesia, IPM has proved a great success in rice cultivation through a combination of appropriate technology and government support.

15.6 Different Components of IPM for Sustainable Food Productivity

15.6.1 Cultural methods

Since ancient times, farmers have been relying on cultural or physical practices for the management of pests. Cultural practices include making the cropping environment unfavourable or less suitable for pests and more suitable for natural enemies. The best example is the push–pull strategy (Cook *et al.*, 2007) where the crop is made unattractive (push) while another food source is made attractive (pull). In eastern and southern Africa, stem borers in maize and sorghum were repelled by non-host intercrops (*Molasses minutiflora*, *D. uncinatum* and *Desmodium introtum*) (push) and concentrated on attractive trap plants (*Pennisetum purpureum* and *S. vulgare sudanense*) (pull).This was due to the fact that *M. minutiflora* increased parasitism by *Cotesia flavipes* and *Desmodium* suppressed the parasitic weed *Striga hermonthica*. Similar success was achieved in the management of melon bug (*Aspongopus viduatus*) in the Sudan where hand-picking by women and children collected more than 200 t bugs, which were then burned (Bashir *et al.*, 2003). Picking and burning of bolls infested with the pink bollworm (*Pectinophora gossypiella*) at the end of the growing season also proved very successful for the management of this pest (Brader, 1979).

Agroecosystem analysis

In modern agriculture, economic threshold level determination can be replaced with agroecosystem analysis due to the complexity in fixing an arbitrary mean for major insect pests. Pest monitoring is one of the most important components of IPM to take proper decisions to manage the pest problem in plenty of time. Many agroecosystems are unfavourable environments for natural enemies because of the high levels of disturbance. Therefore, understanding biotic interactions in agroecosystems and how they can be utilized to support crop productivity and environmental health is one of the fundamental principals underlying IPM (Shennan, 2008). Important elements for understanding biotic interactions include: (i) consideration of the effects of diversity, species composition and food-web structure on ecosystem processes; (ii) the impacts of timing, frequency and intensity of disturbance; and (iii) the importance of multitrophic interactions.

15.6.2 Behavioural control

Utilization of behavioural attributes of insect pests is one of the best options for their management. Behavioural control can be achieved by a variety of behaviour-modifying chemicals such as pheromones, which are efficient even at low population densities without adversely affecting natural enemies (Fig. 15.3). Pest management is becoming increasingly difficult because of the changing climatic patterns where insecticides are of little help compared with the pheromones and other semiochemicals, which hold great promise (Witzgall *et al.*, 2010). The most important attribute of semiochemicals is their specificity, as most of them are bioactive only towards certain species or groups of insect pests, and as such some efforts have been directed towards the development of reliable controlled-release technologies for semiochemicals (Clarke, 2001).

15.6.3 Host-plant resistance

Host-plant resistance, natural plant products, biopesticides, natural enemies

Fig. 15.3. An example of cultural control.

and agronomic practices offer a potentially viable option for IPM. They are relatively safe for non-target organisms and humans. Host-plant resistance to insect pests is a key component in IPM. It is the most economical method and is compatible with other methods of pest control. Five diseases (blast, bacterial blight, sheath blight, tungro and grassy stunt) and four insects (brown planthopper, green leafhopper, stem borer and gall midge) are of major importance for rice in tropical and subtropical Asia. Most of the modern varieties of rice contain moderate resistance to one or more of these major diseases and insect pests. Resistance to bacterial blight has been achieved by marker-assisted breeding (Singh *et al.*, 2001), and resistance to bacterial blight, sheath blight and stem borer has been achieved by transgene pyramiding (Datta *et al.*, 2002).

15.6.4 Biological control

Like other natural enemies, insect pathogens can exert considerable control over target populations. Spectacular crashes of insect pest populations have been reported to be caused by epizootics (Evans, 1986; McCoy *et al.*, 1988). The natural epizootics produced by nucleopolyhedroviruses (NPVs) of sawflies (*Gilpinia hercyniae* Hartig and *Neodiprion* spp.), gypsy moth (*Lymantria dispar* L.), *Spodoptera litura* NPV of *S. litura* and several other insects are often credited with eliminating the need for further interventions (Kaya, 1976; Evans, 1986; Woods and Elkinton, 1987; Monobrullah and Shankar, 2008).

Management of these pests still relies heavily on the use of pesticides with their associated limitations. If appropriately applied, biological control offers one of the most promising, environmentally sound and sustainable tools for control of arthropod pests (Fig. 15.4) and weeds (van Lenteren *et al.*, 2006; van Driesche *et al.*, 2007). Management of non-indigenous and indigenous pests in many countries of the world has been achieved through public support for the biological control options. There exist significant opportunities for increasing the use and effectiveness of biological control agents.

15.6.5 Biopesticides

The term biopesticide encompasses many aspects of pest control such as entomophagous nematodes, plant-derived

Fig. 15.4. Promotion of natural enemies for protection of crops.

pesticides, secondary metabolites from microorganisms, pheromones and genes used to transform crops to express resistance to pests. India has a rich biodiversity of flora and fauna with the potential for developing into commercial technologies. Nevertheless, the adoption of biopesticides and bioagents remains extremely low because of a number of factors relating to technology, socio-economics, and institutional factors and policies. Some success stories of the successful utilization of biopesticides and biocontrol agents in Indian agriculture include: (i) control of the diamondback moth by *Bacillus thuringiensis*; (ii) mango hoppers, mealybugs and coffee pod borer by *Beauveria bassiana*; (iii) *Helicoverpa armigera* on gram by *H. armigera* NPV; (iv) white fly on cotton by neem products; (v) sugarcane borers by *Trichogramma* sp.; and (vi) various types of rots and wilts in different crops by *Trichoderma*-based products (Kalra and Khanuja, 2007).

In view of consumers' awareness and perception of vegetables without chemical residues, the use of plant products can be an eco-friendly, effective and economical method for production of vegetables that are preferred in local and export markets (Gahukar, 2007). Entomopathogens have become the most preferred method for the management of a variety of invertebrate pests in greenhouses, row crops, orchards, ornamentals, stored products and forestry, and for pests and vector insects of medical and veterinary importance (Burges, 1981; Tanada and Kaya, 1993; Lacey and Kaya, 2000; Lacey *et al.*, 2001).

15.6.6 Botanical pesticides

Botanical pesticides are naturally occurring plant substances used for managing pests (Thacker, 2002). Botanicals are endowed with a spectrum of properties such as insecticidal activity, repellence of pests, insect behaviour modifiers, antifeedent activity, and toxicity to mites, snails, slugs, nematodes and other agricultural pests (Duke, 1990; Narwal *et al.*, 1997). The growing concerns about the use of pesticides has resulted in renewed interest in the use of botanicals in IPM (Crazywacz *et al.*, 2005; Isman, 2006).

15.6.7 Transgenics

The area of land under transgenics or genetically modified (GM) crops is continuing to increase throughout the world

Table 15.9. Countries where genetically modified (GM) crops are grown.

Crop	Trait	Countries commercially producing GM crops
Soybean	Herbicide tolerant	Argentina, Canada, Romania, USA, Uruguay
Maize	Insect resistant and herbicide tolerant	Argentina, Bulgaria, Canada, Germany, Spain, South Africa, USA
Cotton	Insect resistant and herbicide tolerant	Argentina, Australia, Canada, China, Indonesia, Mexico, South Africa, USA
Potato	Virus resistant	Romania, USA

Source: James, 2001; http://www.gmocompass.org/eng/agri_biotechnology/gmo_planting/194.global_growing_area_gm_crops.html.

(Table 15.9). However, production of most of the dominant crops such as soybean, maize, canola and cotton remains concentrated in the USA, Canada and Argentina, followed by Brazil, China, Paraguay, India and South Africa (James, 2004). The coexistence of GM crops and non-GM crops is a myth because the movement of transgenes beyond their intended destinations is a certainty, and this leads to genetic contamination of organic farms and other systems. However, organic agriculture is practised in almost all countries of the world, and its share of agricultural land and farms is growing. In Europe, organic agriculture is increasing rapidly. It is unlikely that transgenes can be retracted once they have escaped and thus the damage to the purity of non-GM seeds is permanent. The dominant GM crops have the potential to reduce biodiversity further by increasing agricultural intensification. There are also potential risks to biodiversity arising from gene flow and toxicity to non-target organisms from herbicide-resistant and insect-resistant (*Bt*) crops (Altieri, 2005).

15.6.8 Chemical control

The use of pesticides is unavoidable once the pest population has built up on the crop (Dhawan, 2001). However, judicious use can overcome the negative impact of pesticides such as resurgence of pests and development of resistance in insects, with management of pesticide residues and conservation of natural enemy complexes and biodiversity in crop ecosystems. Pesticides provide a dependable, rapid, effective and economical means of controlling whole complexes of crop pests. The basis of using pesticides as pest-management options and the consequences of misusing them is carefully analysed in order to obtain maximum benefits from their application, while at the same time preventing and minimizing their possible hazardous effects on non-target organisms and the environment. In most developed countries, the bulk of the pesticides used are herbicides, which are less toxic compared with the insecticides used in developing countries (WRI, 1999). Furthermore, the insecticides used in developing countries are generally obsolete types belonging to the organophosphates and carbamates, which are noted for their acute toxicity. Pesticides that are generally highly toxic and are known to have toxic residual effects are not recommended. To get more profit, farmers often do not wait for the correct period of time after use of the pesticide and harvest the crop to market. This leads to pesticide poisoning, chronic effects and in some cases even death. Thus, more care and cautious is required in applying pest-control practices in field crops.

15.6.9 Biotechnological approaches

The depleting natural resource base coupled with the burgeoning population demands

a quantum jump in our productivity levels to meet the requirements. Biotechnology offers unique opportunities to solve environmental problems, some of which derive from unsustainable agricultural and industrial practices, and has emerged as an important tool in IPM, providing new ways of manipulating plant resistance to pests. Using plant biotechnology, several herbicide-tolerant crops have been developed and commercialized that allow the use of herbicides that are effective, economical and have favourable environmental characteristics. Biotechnology provides the tools to modify performance of important biological elements of pest control, such as natural enemies and plant varieties. New crop cultivars with resistance to insect pests and diseases combined with biocontrol agents should lead to reduced reliance on pesticides, thereby reducing farmers' crop protection costs, while benefiting both the environment and public health (Sharma *et al.*, 2002).

Transgenic resistance to insects has been demonstrated in plants expressing insecticidal genes such as δ-endotoxins from *B. thuringiensis* (*Bt*), protease inhibitors, enzymes, secondary plant metabolites and plant lectins. The protease inhibitor and lectin genes largely affect insect growth and development and, in most instances, do not result in insect mortality. The effective concentrations of these proteins are much greater than the *Bt* toxin proteins. Therefore, the potential of some of the alternative genes can only be realized by deploying them in combination with conventional host-plant resistance and *Bt* genes (Hilder and Boulter, 1999). Genes conferring resistance to insects can also be deployed as multilines or synthetic varieties. Impressive results on the control of *Bt*-susceptible pests have been obtained in the laboratory and in the field, and the first commercial *Bt*-transgenic crops are now in use. The application of biotechnology techniques within the agriculture sector can potentially improve food security by raising crop tolerance to adverse biotic and abiotic conditions by enhancing adaptability of crops to different climates and by improving yields, pest resistance and nutrition, particularly of staple food crops.

15.7 Crop-specific IPM

15.7.1 Cereal crops

Rice is one of the most important staple food crops in the world. Of the total world production of 596,485,000 t, Asia produces 540,621,000 t annually (FAO, 2002). Recent estimates of rice demand indicate that a compound annual growth rate of 1.25% is needed to meet the expected world rice consumption in 2020. In most developing countries, agriculture occupies an important place in national livelihood security systems (World Bank, 2002). IPM has emerged as a science-based approach to minimize the risk associated with the use of pesticides (Nagarajan, 1990; NRI, 1992). IPM is gaining increased attention as a potential means of reducing food and fibre losses to pests, reducing reliance on chemical pest control and therefore fostering the long-term sustainability of agricultural system (World Bank, 1994).

Wheat is another important staple food for more than 4.5 billion people in 94 developing countries of the world (Braun *et al.*, 2010). The improved wheat varieties and their adoption in South and West Asia, China and Latin America have saved millions of lives in these countries (FAOSTAT, 2010: http://faostat.fao.org/). Rosegrant and Agcaoili (2010) stated that the requirement for wheat is projected to increase more than 60% by 2050 while at the same time climate-induced temperature increases are likely to reduce wheat production by 29%, thereby creating a situation of uncertainty and concern. According to an estimate by the World Bank (World Bank, 2008), for every 1% increase in wheat productivity, there is reduction in poverty of 0.5–1.0%. Oerke (2006) reported that under current pest and disease control practices, losses to pathogens, pests and viruses are estimated at 13%, which is equivalent to 45 million t

of wheat/year, valued at US$9 billion in developing countries. There is an increased risk of emergence of new pests and disease outbreaks resulting from ensuing changes in climate. Therefore, coordinated efforts are needed to develop wheat cultivars that are resistant to new races of pests and pathogens that threaten global wheat productivity. The breeding of varieties resistant to pests and diseases offers the most environmentally sustainable approach for their control, thereby allowing farmers to reduce the use of pesticides, increase their profit and help keep wheat prices affordable for consumers. Although host-plant resistance is one of the best options for disease and pest control, a combination of cultural practices and biological control provide economically justified and sound management of pests and diseases.

15.7.2 Vegetable crops

Vegetable production plays a vital role in food security and poverty reduction in developing countries. Besides providing nutritional supplements, vegetable production generates additional employment for the rural poor, many of whom are women. With the advent of globalization and new liberal trade policies, agriculture as a whole in terms of practices and patterns is changing fast. These changes have facilitated and created congenial conditions for vectors and viruses to spread out from natural habitat to new and favourable environments, crossing national boundaries into new geographical areas and resulting in negative social and economic impacts on subsistence agriculture. Crop failure caused by these viruses has created significant financial hardship and food security problems for resource-poor farmers. Proper management practices are required for viruses and their spread between crops, across seasons and between different continents. For example, the western flower thrip *Frankliniella occidentalis* (Thysanoptera: Thripidae) is a major pest and an efficient vector of tomato spotted wilt virus over a wide range of horticultural crops throughout the world (Wijkamp *et al.*, 1996; Kirk and Terry, 2003).

15.7.3 Fruit trees

Among different fruits, citrus occupies the prime position and ranks first internationally as far as its trade is concerned (Norberg, 2008; UNCTAD, 2009). Citrus fruit is produced in more than 140 countries throughout the world. Of these, four countries, namely Brazil (32%), the USA (14%), India (6%) and Mexico (7%), account for about 60% of the orange production (FAOSTAT, 2007: http://www.csrees.usda.gov/fo/pestmanagementalternativessrgp.cfm). Several important national, regional, state and industrial entities are supporting the research on citrus pest management and are encouraging the development and implementation of IPM practices to reduce the use of pesticides. Kaul *et al.* (2009) showed that the repeated application of pesticides has led to the development of resistance in insect pests. This resistance has paved the way for increased application of pesticides and has lead to the collapse of agricultural systems characterized by highly resistant pests, with no natural enemies left to control them. Future tactics such as cultural, mechanical, physical and biological control and the role of host-plant resistance in pest management need to be developed.

15.8 Quarantine and Regulations

In this process, regulatory rules framed by the government are brought into force under which seeds and infested plant materials are not allowed to enter the country or move from one part to other parts of the country. These are known as quarantine methods and are of two types, domestic and exotic quarantine. These regulations help to check the entry of pests into places where they do not naturally exist.

15.9 Implementation and Adoption of IPM

A successful IPM programme takes time, money, patience, short- and long-term planning, flexibility and commitment. The implementing officials need to spend time on self-education and on making contacts with extension and research personnel to discuss farming operations, which vary from location to location. Governments must take the lead in changing the pest-control scenario to make chemical control less attractive and provide incentives to agencies involved in production and promotion of eco-friendly quality inputs essentially required for IPM. However, some vital issues need to be addressed properly.

Indian farmers are used to a chemical pest-management system and consider that alternative approaches pose new risks and create demands for new sources and information. This has mainly been due to low literacy, poor awareness and a positive attitude towards pesticides among small and medium farmers, who constitute the majority. Less than 5% of farmers have followed IPM practices across all agro-ecosytems (Shetty, 2006). At the broadest level, adaptation also includes investment in agricultural research and in institutions to reduce vulnerability. This is because the ability of farmers and others to adapt depends in important ways on the available technology, financial resources and financial risk management instruments, market opportunities, the availability of alternative agricultural practices and, importantly, access to, trust in and use of information such as seasonal forecasts (Cash, 2001; Cash *et al*., 2006). It also depends on specific institutional arrangements, including property rights, social norms, trust, monitoring and sanctions, and agricultural extension institutions that can facilitate diversification (Agrawal and Perrin, 2008). Not all farmers have access to such strategies or support institutions, and smallholders – especially those with substantial debt, often involving loans to invest in commercial crops, and the landless in poor countries – are most likely to suffer negative effects on their livelihood and food security. Smallholder and subsistence farmers will also suffer complex, localized impacts of climate change (Easterling *et al*., 2007).

15.10 Policy Issues

To sustain IPM programmes and disseminate awareness of achievements, trainers and policy makers emphasize the value of involving non-governmental organizations. Extension services and farmers need to be trained, with research linked to on-farm solutions, through pilot programmes. Their participation in planning, training and in some cases delivery of IPM is increasingly important. Governments need to examine policies supporting pesticide use, which can include both price and non-price factors.

15.11 Conclusions and Future Strategies

Although several alternative strategies continue to evolve in pest management, pesticides will continue to be used in agriculture. Enhancing the knowledge base of farmers with modern science, improving their skills and internalizing the inputs at various levels can bring significant changes to the way they carry out agriculture. The Sustainable Agriculture Programme implemented by the Worldwatch Institute focuses on ecologically compatible, economically viable and socially equitable development. IPM makes a thorough case for a comprehensive long-term pest-management programme based on knowledge of an ecosystem that weighs up economic, environmental and social consequences of interventions. Pesticide policies must be effectively linked with appropriate pest-management policies in order to achieve a systematic reduction in the usage of pesticides over time, leading towards the larger goal of agricultural and environmental sustainability. In this context, IPM has been a widely recognized alternative technique towards the develop-

ment of environmentally sustainable agriculture. Access to information and expertise in developing countries, where the need to increase food production is most urgent, will be a key factor for sustained food security. New solutions could include novel resistant cultivars with multiple resistance genes, suitable epigenetic imprints and improved defence responses that are induced by attack. Plant activator agrochemicals could be used to switch on natural plant defences. Habitat manipuations such as push–pull strategies can improve pest management and yields in less intensive systems. Genomic and transcriptomic information will facilitate the development of new resistant crop cultivars once annotation and availability of data on multiple cultivars is improved. Knowledge of the chemical ecology of pest–plant interactions will be better exploited once the genes for biosynthesis of key plant metabolites are discovered. Genetic modification of crops has the potential for speeding up the development of crops with novel resistance.

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16 Role of Information and Communication Technology in Integrated Pest Management

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16.1 Introduction

Information and communication technology (ICT) is the integration of information technology (IT) and communication technology (CT), or the merging of fixed and mobile telephone networks with computer networks. IT has instrumented the shift from an industrial age to a network age. We now live in a society in which the production, acquisition and flow of knowledge drive the economy and in which global information networks represent a key infrastructure (Seruon, 2002). CT has been developing at an increasing pace in the last decade and showing its role in bridging the digital divide. Thus, the emergence of ICT was inevitable. However, ICT has been developed and applied disparately in developed and developing countries.

16.1.1 Disparity of ICT development and applications in different areas

Singer (1970) introduced the concept of international technological dualism, by which he meant essentially unequal developments in the area of science and technology between rich and poor countries. James (2002) used Singer's notion to advance our understanding of the current digital divide between rich and poor countries, and more specifically argued that this divide is essentially another manifestation of the same forces identified by Singer as governing not only the technological relationships between rich and poor countries but also those within the latter. Although some developing countries are undertaking important initiatives to generate indigenous forms of IT, far more research expenditure needs to be devoted to innovations that will benefit the poor in developing nations rather high-income countries.

The global digital divide is a term used to describe these 'great disparities in opportunity to access the Internet and the information and educational/business opportunities tied to this access between developed and developing countries' (Lu, 2001). Unlike the traditional notion of the 'digital divide' between social classes, the global digital divide is essentially a geographical division and is now recognized as an international issue. The quantitative

 Integrated Pest Management (eds D.P. Abrol and U. Shankar)

description of the global digital divide is changing fast year by year. Broadly speaking, the difference is not necessarily determined by access to the Internet but by access to ICT and to media that the different segments of society can use. Nevertheless, to a certain extent, the Internet users can reflect the digital divide.

ICT is any technology that enables communication and the electronic capture, processing and transmission of information. Radio, television and print media are vital in many developing countries. Over the last decade, 'new' ICTs, such as mobile phones and Internet-associated applications such as Voice over Internet Protocol (VoIP), have become available to growing numbers worldwide. The reach of the mobile phone by far outnumbers any other communication device. Today, there are about 4 billion mobile phone subscribers and half of the world's population is estimated to have the ability to access the Internet through mobile phones in 2010. This should be compared with about 1 billion personal computers (PCs) accessing the Internet. In addition, PC penetration is very low in developing countries. In Africa, mobile phone penetration was around 30% by the end of 2007 and was estimated by *Africa & Middle East Telecom-Week* to have passed 50% in 2010. This should be compared with the overall Internet usage penetration in Africa, which, according to Internet World Stats (http://www.internetworldstats.com/), was only 5.6% by the end of 2008. The primary access point to the Internet for the majority of people will be a mobile phone and not a PC. Today, all phones have the capability to receive and send Short Message Service (SMS), giving messaging services a superior reach, but there are clear limitations with SMS compared with Internet services (Vendel, 2010).

Developing countries face challenges when harnessing the potential of ICT for economic development (Michelle and Fong, 2009). What is the best way to make sure that people in the developing world are connected to today's technology? Mobile phones, rather than computers, are the answer. Methods of integrating other information and telecommunication products such as mobile phones, televisions and fixed-line telephones that have been popularized and brought into extensive application with the computer can improve the information services for farmers and provide interactive communication through e-mails, telephone-voice messaging, SMS, voice mail, video conferences and other formats anytime and anywhere.

16.1.2 Integrated pest management as an agricultural system of intensive information

The potential of ICT for the speedy dissemination of information to farmers needs to be realized (Meera *et al.*, 2004). Integrated pest management (IPM) practices have to solve many problems of sensitivity and intractability in the sustainable development of agriculture. As well as professionals, farmers also understand that IPM is a knowledge-dense and technology-intensive area, as described in the other chapters of this book. Therefore, ICT must play an important role.

Theoretically, IPM is based on five disciplines – entomology, plant pathology, weed science, rodent science and pesticide science – covering a large number of species. Practically, IPM is concerned with most constitutional levels of agroecosystems, from populations and communities down to individual viruses or genotypes, and genomes and genes, as well as up to the levels of landscape and global ecosystems. In fact, IPM practices are involved in a complex course responding to climatic change, soil dynamics, vegetation evolution and human activities.

ICT has proved to be a powerful tool in pest forecasting as a prop to giving priority to prevention, as pest forecasting involves data acquisition, processing and information dissemination. ICT can also be very helpful in terms of enforcing IPM. This chapter will present and discuss the development and use of ICT in pest management.

ICT can help developing countries tackle a wide range of health, social and

economic problems. In addition, ICT can play a role in IPM extension. However, the benefits of ICT are not fully realized in many countries: ICT is often out of reach of the poor and those in rural areas. Thus, we take the example of mainland China to show ICT applications in IPM in a developing country with a digital divide from east to west. China is a country with a high frequency of crop pest outbreaks. In 1975, the Ministry of Agriculture of China organized an ad hoc group for the formulation of a pest management policy and brought forward the concept of 'giving priority to prevention and putting IPM into force'. The first concept of 'giving priority to prevention' calls for pest forecasting, while the second, 'putting IPM into force', requires considered measures to conquer plant diseases and insect pests.

Although most examples have been taken from China, we hope this chapter will facilitate the sharing of experiences and knowledge in ICT applications among countries, especially developing countries.

16.2 Personalized Information Services in IPM Practice

Farmers need a personalized information service in order to learn IPM methods and techniques, as well as buying goods such as pesticides, plastic coverage films, fertilizers and seeds of resistant crop varieties. Although information services can be provided efficiently and rapidly by the Internet, Internet-based information systems cannot provide this kind of service to rural people who are not connected to it. This is the so-called 'last mile' problem. In fact, most farmers cannot afford the expenses of buying a computer and accessing the Internet, and most are not proficient in using a computer, even if they do have one.

16.2.1 Technology to eliminate the 'last mile' in rural information service

To solve the 'last mile' problem, IT should be integrated with CT to provide wide coverage over rural areas, for example using mobile communication, telephone lines and cable or satellite television networks. The integrated ICT can provide information services with diversified approaches such as telephone talking, SMS, e-mail, voice mail, video and other formats. Users of the interactive platform can provide and also receive the services by choosing whatever suits their own terminals such as telephone, mobile phone, PC, EduBook, personal digital assistant (PDA) or television with a set-top box (STB); they are not limited to having to operate a computer.

Unified data management technology can build such an ICT platform. This platform has three layers: (i) a database layer; (ii) a business logic layer; and (iii) a user application layer (Fig. 16.1). Beside various databases within the database layer, the protocols and standards useful for the platform are embedded, such as TCP/IP, HTTP, SIP (session initiation protocol), H.264 and H.323. Thus, the business logic layer can unify telecommunication and Internet components, which can implement many communicational functions, for example telephone and mobile phone calls, SMS, e-mails, music and video orders, online games, teleconferences and video conferences. In addition, it unifies the customized database components, to customize a database system with its interface and work flow, statistic tabulation and graphing, and the authority of each user. With the user application layer, various applied systems can be created and run, such as office automation (OA), call centres, monitoring and a thematic geographic information system (GIS) by integrating telecommunication and Internet components.

Pests of fruit trees such as borers cause great damage to fruit growers. Therefore, it is very important to provide an IPM consulting service to growers to help them control these pests. Using unified data management technology, an ICT platform was developed for a national priority project, entitled 'Monitoring, forecasting and controlling of fruit borers in north China' (Fruit Borer project) for 2008–2010. For this IPM information service with

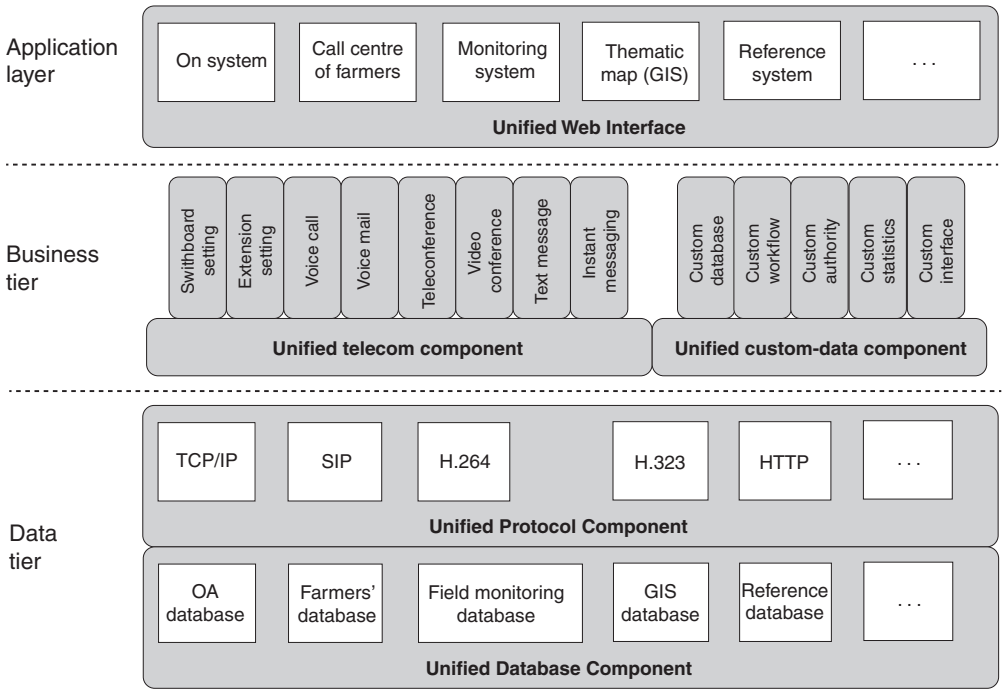

Fig. 16.1. Architecture of a unified data management platform.

individualized solutions, the FruitBorerEx platform has two categories of application: forecasting and consultancy. The following section introduces the latter, the ICT platform for individualized services in IPM extension, while the application of ICT to pest forecasting will be presented in the next section.

16.2.2 The ICT platform for individualized services in IPM extension

The ICT-based IPM information service platform FruitBorerEx consists of four systems: (i) monitoring data acquisition and management system (Monitox); (ii) a forecasting system (Forecastox); (iii) a user database system (Userdatax); and (iv) an IPM expert-response system (Expertox). The FruitBorerEx platform can offer its information service through numerous paths such as telephone, fax, data transmission, radio, television, LED plate screens, video conferences, call centres, podcasts and webcasts.

The Forecastox system can run an interactive voice response (IVR) between farmers and IPM experts. Farmers can use their own terminals, for example, a mobile phone, to ask questions about control of the fruit borer. These questions can be stored immediately as a .wav format file into a database of the FruitBorerEx platform. The voice form also can be transformed into a literal format artificially or automatically and again stored in the database. To respond to questions, a consultation service is started up with the Expertox system. This is a mechanism whereby several experts who can answer the question will be selected artificially or automatically from the Userdatax system, which is a database containing the data of IPM experts, such as their address, telephone number, speciality, teaching of courses and research projects.

In the current system, agricultural students and graduates are engaged as volunteer consultants to answer the questions of farmers, together with professors as consultants. As a result of this

innovative ICT platform, consultants can provide their answers anywhere, anytime, with any type of terminals and in any formats such as VoIP, SMS via mobile phones and even through web page multimedia with pictures and videos about the pest or symptoms caused by the pest. Once any of the consultants has given their answer either verbally or in written form, the Expertox system will send an SMS to the farmer in question, which can be accessed via mobile phone or read from a PC or EduBook, for example. Thus, the enquirer can receive the response anywhere and anytime via any type of terminal. The system can record the ask–answer process using the tracking database. When the process ends, the system manager can collate the questions and answers, and add them to compiled files into the database. Thus, the information content will be enriched and improved for future retrieval.

This interactive communication has thus led to a revolutionary change from instant talk to time-lapsed dialogue. However, the availability of this type of consultancy regime cannot compel farmers to change their habits of acquiring information, and there remains the problem of reducing cost. The FruitBorerEx platform was tested in project sites in Shanxi Province in 2009, and the ICT platform is being improved for more varied and useful functions and will be applied to larger areas in a renewed project for 2011–2015.

16.3 Applications of ICT for Pest Forecasting and Distance Diagnosis

Pest forecasting has been at the forefront of modern computer methods with regard to agricultural science and technology, and distance diagnosis of crop pests has been an achievement of agricultural applications of ICT in the Internet age. Both of these applications of ICT can be incorporated into a universal ICT platform, as presented above. Here, we discuss each individually.

16.3.1 An electronic approach to field surveys for pest forecasting

Forecasts of pest occurrence are perceived as a prerequisite both for the strategy of giving priority to prevention and for the strategy of enforcing IPM. The concept of pest occurrence refers to the pest population density reaching an economic threshold (ET). Thus, forecasting of pest occurrence has two aspects: (i) occurrence time; and (ii) population abundance at the occurrence time. To implement the forecast, data on environmental parameters are useful in modelling pest occurrence, as solar radiation, air and soil temperature, air humidity and soil moisture. These data may be recorded from field monitoring with sensor devices, which can be set to record at specified time intervals and automatically send the data to a remote database through a general packet radio service (GPRS) network.

Monitox was developed using Microsoft Visual Studio and has three functions: (i) collecting data using PDA and GPS as tools; (ii) processing data on its embedded single chip; and (iii) delivering data through the GPRS network. The manually input data in the PDA/GPS device contains information about growth of fruit trees, environment parameters and dynamics of pest populations, as well as the latitude and longitude of the sampling site. All data may be processed in XML form and delivered to the database of the remotely located Forecastox. This significantly improves efficiency and guarantees accuracy in the monitoring and forecasting of fruit pests, much more than the traditional methods where data were recorded manually. In addition to the data coming from PDA/GPS devices, users also can input historical recorded data into the database using the interface of the FruitBorerEx platform.

The Forecastox system can send forecast information to farmers who need such information. Taking the Fruit Borer project as an example, the PDAs of the Monitox system were brought to the project fields of apple orchards in Cangxian county,

Hebei Province. Grass-root technicians and farmers used the PDAs to collect and process the field-sampled data of the fruit borer and surrounding environment parameters and then delivered the data to Forecastox system, which was located remotely in Beijing. Following data accumulation over a period of time, the Forecastox system used the accumulated datasets to produce a forecast using particular modelling calculations. This forecast was then sent back to Cangxian county and issued to farmers of apple orchards for use in IPM implementation.

16.3.2 Distance diagnosis of crop pests to bridge the digital divide

The ICT platform for distance diagnosis of crop pests (DistPestDiag) consists of three parts: (i) an automated system for identification of insect species; (ii) a computer-aided system for identification of insect pests and plant diseases; and (iii) an online diagnosis system for pests, including weeds and rodents (Fig. 16.2). This platform has been working partially for a number of years in Fujian Province in east China and in Xinjiang Province in west China.

When users visit the ICT platform, it is suggested that they first access the automated system for identification of insect species. Many insect species can be identified in terms of morphology and pattern recognition. From images of insect specimens, the system can draw in the geometric parameters: perimeter, area, eccentricity, Euler number, form factor, sphericity, circularity, roundness, rectangularity, location, moments, etc. In addition, the system can quantify colours of the images with four categories of histogram: R, G, B and L, and then give 264 given values for each of the images. These geometric and chromatic parameters can then be used with classification methods, for example by similarity distance, discriminant function and Air Ambulance Network (AAN), for identifying insect species.

The computer-aided system for identification of insect pests and plant diseases is based on the methodology of an expert system. In its reasoning, the multimedia technology allows users to choose a path that approximately leads them towards the correct identification of the insect pest or plant disease. The reasoning can be schemed in user-prefered and understandable ways, rather than for

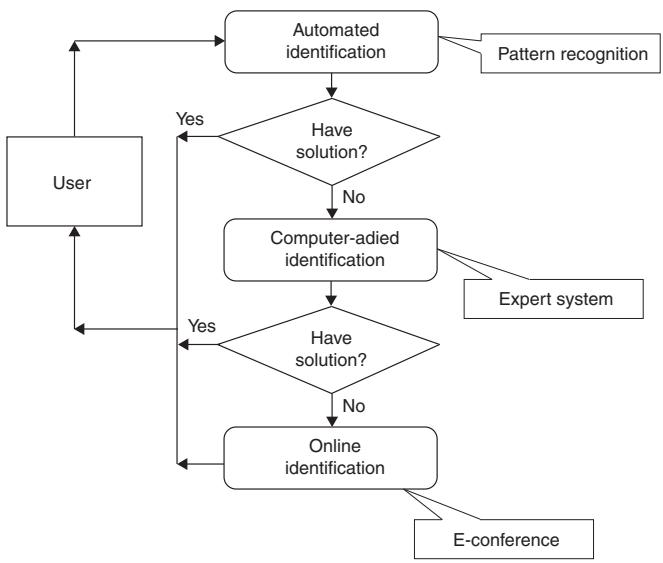

Fig. 16.2. Working flowchart of the DistPestDiag platform for field surveys of crop pests.

academics, for example, starting from the class of host crops (vegetable, fruit, grain or cotton), and possibly giving details at the variety or cultivar level.

Online diagnosis for any pests, including weeds and rodents, can be realized through some e-conference systems, either commercial or self-developed. Following the development of 3G and 4G communication, the e-conference can take place on site and via live broadcasts, for instance, in a grain farm, vegetable field, greenhouse or fruit orchard. Integration of mobile phone communication and broadband Internet will facilitate the distance diagnosis of crop pests.

The ICT platform for distance diagnosis of crop pests will help bridge the digital divide if developing countries build new ICT facilities and adopt these policies. Currently, there is disparity in both ICT applications and knowledge management in different areas of mainland China between the east and west part. Most experts in pest taxonomy and pest management are located in the economically developed east, and those in the poorer west need their help. Therefore, it is hoped that the DistPestDiag platform may go some way towards bridging this digital divide.

16.4 Field Monitoring and Early Warning of Invasive Alien Weeds

A wireless sensor network (WSN) is used in many industrial, agricultural and civilian application areas, generally consisting of spatially distributed autonomous sensors to cooperatively monitor physical or environmental conditions, such as temperature, sound, vibration, pressure, motion or pollutants (Akyildiz *et al.*, 2002; Kay and Mattern, 2004). In addition, the integrated technology mixes sensors, computers and communication devices, changing the ways of precision agriculture (Kitchen, 2008). A sensor network normally constitutes a wireless ad hoc network, meaning that each sensor supports a multi-hop routing algorithm where nodes function as forwarders, relaying data packets to a base station. Here, we will discuss this with regard to applications to field monitoring and early warning of invasive alien weeds.

16.4.1 Biotic invasion as a new critical IPM problem

Biotic invasion is one of the five top drivers for biodiversity loss at the global level, in addition to land use, climate change, overexploitation and pollution (Rhymer and Simberloff, 1996; Copp *et al.*, 2005; Innal and Erk'akan, 2006). There are some local direct drivers such as eutrophication, burning, soil erosion and flooding. Disturbed habitats are prone to invasions that can have adverse effects on local ecosystems, changing ecosystem functions (Mack *et al.*, 2000).

Increasing domination by a few invasive alien species (IAS) increases global homogenization of biodiversity, reducing local diversity and distinctiveness. It is clear that IAS can induce substantial environmental and economic damage, and their negative effects are exacerbated by climate change, pollution, habitat loss and human-induced disturbances. IAS can change the community structure and species composition of native ecosystems directly by outcompeting indigenous species for resources. IAS may also have important indirect effects through changes in nutrient cycling, ecosystem functions and ecological relationships between native species. In addition, IAS can cause cascading effects with other organisms when one species affects another via intermediate species, a shared natural enemy or a shared resource. These chain reactions can be difficult to identify and predict. Furthermore, aggregate effects of multiple IAS can have large and complex impacts in an ecosystem. IAS may also alter the evolutionary pathway of native species by competitive exclusion, niche displacement, hybridization predation and ultimately extinction. IAS themselves may

also evolve due to interactions with native species and with their new environment.

IAS can directly affect human health. Infectious diseases are often IAS imported by travellers or vectored by exotic species of birds, rodents and insects. IAS also have indirect health effects on humans as a result of the use of pesticides and herbicides, which infiltrate water and soil. Alien species have been estimated to cost hundreds of billions of dollars each year (Pimentel *et al.*, 2005).

An IPM problem under the spotlight is that joining the World Trade Organization and increasing tourism has brought more and more IAS into China. Among the current IAS, over 90 are harmful plants and more than 80 others include insects, plant pathogens, molluscs and mammals. Many areas of the mainland have been threatened or affected by IAS to varying degrees. For example, the pine wood nematode extended into Jiangsu, Zhejiang, Anhui, Shandong and Guangdong Provinces in 1999, leading to the death of 15 million trees (Zhang *et al.*, 2004). The IPM problem is to assess the risks and prevent agricultural or ecological catastrophes.

The key principle of IPM for IAS is to recognize the risks and prevent harm before invasion of the IAS. The participation of citizen volunteers plays an important role in the control and monitoring of IAS because of its potentially wide spatial and temporal coverage. A team from the National Institute of Informatics (Tokyo, Japan) reported their capture programme for the IAS *Bombus terrestris* by utilizing an information system to support citizen participation (http://dias.tkl.iis.u-tokyo.ac.jp/seiyou/). Feedback from the participants requested: (i) more communication channels; (ii) scientific evaluation of the activity; (iii) educational content to learn about ecological problems; and (iv) a mobile phone interface to upload capture records by themselves. However, some participants have only a limited interest in the information systems and prefer the traditional style of the activity (Kitamoto *et al.*, 2009).

16.4.2 A WSN for field monitoring of invasive alien weeds

Another ICT platform was developed for a national priority project, entitled 'Preventing and control of yellowtop, *Flaveria bidentis*' (the Yellowtop project) for 2008–2010. The yellowtop was a new weed plant invader into China and originated from South America (Powell, 1978). This project had a need for WSN technology. For field monitoring of these weeds, the relative environmental parameters needed to be measured with various sensors, including for air temperature, air humidity, soil temperature, soil moisture, solar radiation, rainfall, and wind direction and force. The tiny seeds of the yellowtop can be transported great distances by wind.

At the place where yellowtop occurred, a sensor node was set up, and the project WSN consisted of all the nodes, numbering about 100. This meant that the project covered the whole area invaded by yellowtop, mainly within Hebei Province in northern China. In addition to the sensors, each individual node in a sensor network was typically equipped with a radio transceiver or other wireless communications device, a small microcontroller and an energy source, usually a battery charged with a solar-energy plate. The automated field monitoring data could be delivered and stored in a remote database that formed part of a web-based platform, as described below.

16.4.3 An ICT platform for early warning of invasive alien weeds

An ICT platform, YellowtopEx, for monitoring the yellowtop was developed. Compared with the FruitBorerEx platform, which largely served farmers, the YellowtopEx platform was used by governmental agencies, IPM professionals and other key stakeholders. The two main modules of the platform are the data management system (Datamax) and the real-time reporting system (Reportex) for

the event of yellowtop invasion into a new area. In the Datamax system, there are two categories of data, automated field monitoring data, as mentioned above, and artificial recording data. The latter is dynamic data about yellowtop invasion, which are input into the system by local IPM professionals in charge of IAS management. The field monitoring data are used for prediction of yellowtop development and population dynamics.

Using the Reportex system, local people can report their new findings of weed invasion through various approaches such as e-mail, telephone, SMS and other formats. Once the message has been received, the IAS centre can send an order to local professionals, also through the various communication terminals. The local professionals can then go into the field to check whether the event is true and provide their feedback to the IAS centre, which can then make decisions in the IAS management, for example, discussing the emergency and developing specific implementation plans to eliminate the IAS.

16.4.4 Computerized risk analysis for IAS

Many IT software tools can be applied intensively in risk analysis for IAS, such as population modelling, various GIS platforms, artificial intelligence and genetic algorithms, as well as some hardware tools such as remote sensisng and GPS (Kitamoto *et al.*, 2009; Smolik *et al.*, 2010). Some specialized software products have also used, for example, GARP, CLIMEX and DIVA-GIS. For instance, using CLIMEX, potential distribution areas of the yellowtop in China were estimated to cover many provinces: Guangdong, Guangxi, Yunnan, Hainan, Fujian, Taiwan, Jiangxi, Hunan, Guizhou, Sichuan, Chongqing, Hubei, Anhui, Jiangsu and Shanghai (Bai *et al.*, 2009). However, the reliability of the risk analysis clearly depends on the quality of available data and the experience of the risk analyst. There is a need for innovative methods for preprocessing of spatial data, simulation of the IAS-attacked ecosystem and risk analysis thereof.

A risk analysis and alarm system has been developed for IAS management. This system applies general non-linear statistics as a modelling tool in the processing of diverse data related to vegetation, soil type, topography, temperature, humidity, precipitation, solar radiation and geography at the original source site of the target species of the IAS. Results calculated from this system are then compared with the results from commonly used tools such as GARP, CLIMEX, MaxEnt and Bioclim/Domain. Consequently, the colonization risk of the target IAS can be recognized by comparison and modification, and the risk can be represented on a web–GIS platform. The framework of this system is shown in Fig. 16.3. This system has been applied for risk analysis of the red imported fire ant, *Solenopsis invicta* Buren, which invaded Taiwan in October of 2003 and South China the following year (Chen, 2007).

16.5 Remote Sensing and Radar Monitoring for IPM

Remote sensing has been used in entomology for a long time (Riley, 1989). There are two main types of remote sensing: passive and active. Passive remote sensors include film photography, infrared, charge-coupled devices and radiometers, which all detect natural radiation that is sourced commonly from sunlight and emitted or reflected by the object or surrounding area being observed. Active remote sensors mostly refer to radar, which emits energy in order to scan objects and areas, whereupon a sensor then detects and measures the radiation that is reflected or backscattered from the target. In general, remote sensing replaces costly and slow data collection on the ground, ensuring in the process that areas and objects are not disturbed.

In the next section only passive remote sensing will be discussed, and radar technology and its applications in IPM will be presented in the following section. Farming is widely distributed geographically, often varies temporally and is

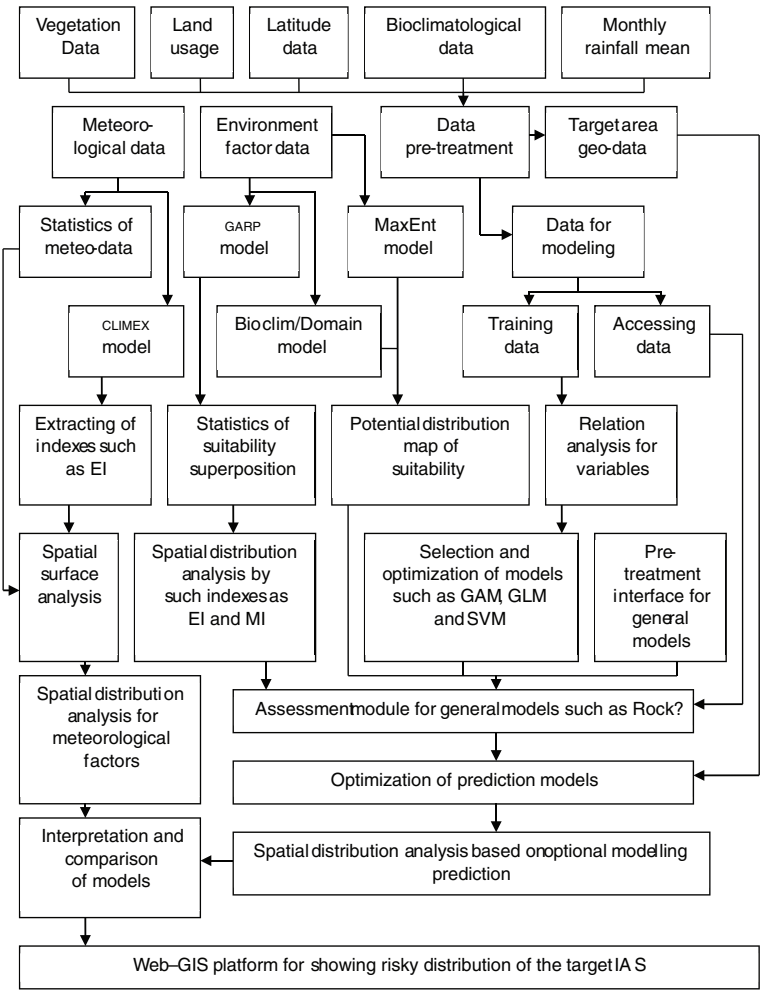

Fig. 16.3. Framework of the risk analysis and alarm system for IAS management.

specialized in the management of different crops. Remote sensing technology provides advanced tools to gather information on IPM in such complex farming practices, especially to carry out macroscopic, integrative, dynamic and fast monitoring of crop pests.

16.5.1 The spectrometer test: from portable to artificial satellites

A handheld or portable spectrometer is a kind of hyperspectral remote sensing tool and can be used to measure the spectral reflectivity of a crop canopy and foliar symptoms of pest harm, indoors and in fields. The data measured are helpful not only to diagnose the type of plant disease or insect pest but also to standardize the analysis of satellite remote sensing data that measure the spectral reflectivity of ground targets on pest-attacked farmland. For instance, a handheld spectrometer can detect a change in chlorophyll content in wheat leaves, and the data can be processed to learn more about the relationship between the chlorophyll content and the number of wheat aphids. This relationship may provide knowledge for airborne remote

monitoring of wheat calamities caused by aphids.

Aerial photographs taken at low altitude are a routine type of aerial remote sensing. A number of research studies have focused on wheat diseases, such as stripe rust and powdery mildew (Huang *et al.*, 2003; Qiao *et al.*, 2006; Cai *et al.*, 2007; Cao *et al.*, 2009; Guo *et al.*, 2009; Zhang *et al.*, 2009). Liu *et al.* (2004) interpreted push-broom hyperspectral imager data and found that, within the wavelength range of 560–670 nm, the reflective overcanopy of winter wheat with stripe rust disease (Rwsr) was higher than that of healthy wheat (Rhww), while within the near-infrared wavelength range, the value for healthy wheat was higher than that for stripe rust diseased wheat. They designed indexes for assessing the severity and monitoring the spatial scale of winter wheat stripe rust disease.

The development of artificial satellites in the latter half of the 20th century allowed remote sensing to progress to a global scale. Instrumentation on board various Earth-observing and weather satellites, such as Landsat, Nimbus and more recent missions such as RADARSAT and UARS, has provided global measurements of various data for civil, research and military purposes. The most successful application of artificial satellite remote sensing for IPM has been to monitor the desert locust (*Schistocerca gregaria* Forska). Outbreaks of the locust relate to blooms of vegetation where the locust dwells and where rainfall places critical pressure. Ji *et al.* (2003) analysed the severity and geographic range of desert locust calamities using MODIS data. Comparing the change in the normalized difference vegetation index (NDVI) of the pasture from pre-calamity to post-calamity, they found that the increase in NDVI indicated the health of the pasture and its decrease responded to the destroyed pasture. Critical values for the different destroyed status of the pasture could be estimated from the NDVI values. When tested in plotted pastures in Hebei Province, the NDVI was 72.97 and 68.35% for heavily and moderately destroyed grasslands, respectively. Recently, Zhang *et al.* (2005) conducted monitoring of *Dendrolimus* spp. damage using National Oceanic and Atmospheric Administration/Advanced Very High Resolution Radiometer (NOAA/AVHRR) data.

16.5.2 Construction of an entomological radar monitoring network

Radar is an example of active remote sensing where the time delay between emission and return is measured, establishing the location, height, speed and direction of an object. Radar entomology has been developing for over 40 years and radar has now become a powerful tool to monitor insect migration (Riley *et al.*, 2007). Radar can actively emit electromagnetic waves that can be reflected by insect bodies, without restriction to day or night time, and without disturbance to insect flying behaviour. The flying behavioural parameters of insect populations, such as migratory azimuth, altitude and moving direction, can all be calculated from radar data, as well as aerial density measurements of the insect troops. In contrast to traditional methods, radar data can provide much larger samples from a wide range of space to monitor flying courses of different insect species at a height of 1 km. The radar provides effective methods for learning the habits of insect migration, their aerial distribution and the movements of insect troops, and for studying the mechanisms of flying off, cruising height, orientation determinant and layer formation, revealed from environmental conditions such as atmospheric structure and movement that influence insect flying behaviour. Weather radar data has also been researched for use in alarm systems for insect migration (Pylkkö *et al.*, 2008).

The Institute of Plant Protection of the Chinese Academy of Agricultural Sciences (CAAS-IPP) is the leader in construction of the entomological radar monitoring network. It built a scanning insect radar in 1998 and a vertical-looking radar in 2004. A millimetric scanning insect radar was first

developed in China by CAAS-IPP, in cooperation with an electronic company, in 2006 (Fig. 16.4) and has been used to track high-flying insects in southern China (Yang *et al.*, 2008). This has been used to monitor migratory pests, for example, the rice leafroller (*Cnaphalocrocis medinalis* Guenée) and some species of Delphacidae, such as the brown planthopper (*Nilaparvata lugens* Stål), the white-backed planthopper (*Sogatella furcifera* Horváth) and the small brown planthopper (*Laodelphax striatellus* Fallén) in Xingan county, Guangxi Province. In 2009, CAAS-IPP made two sets of vertical-looking radars, in cooperation with the Institute of Plant Protection, Beijing Academy of Agricultural Sciences. The two radar sets were located, respectively, in Beihuangcheng Island, Bohai Bay, and Yanqing Region, Beijing Capital. They have been used for long-term monitoring of the flight of the adults of the cotton bollworm (*Helicoverpa armigera* Hübner) and sorghum armyworm (*Mythimna seperata* Walker). The cotton bollworm is well known by the general public in China, because it caused a disaster of tremendous loss in China's cotton production in the early 1990s.

In 2007, a Doppler insect radar was produced at Nanjing Agricultural University, China, that could observe the flying off and landing of target insects, and in particular could collect data from insects flying at a low altitude of 50 m. This Doppler radar has proved useful in monitoring the flying off and emigration of native delphacid insects, and the landing and immigration of alien delphacids. The Nanjing Agricultural University team also used their Doppler insect radar to investigate the migration of the rice leaf roller (*Cnaphalocrocis medinalis*) (Gao *et al.*, 2008). The system utilizes a wide band network for Internet operation and an automated control for the power supply, and is expected to become an important tool in the long-term monitoring and management of rice pests.

In 2008, a centimetric vertical-looking radar with circumrotation and polarization function was assembled by the Institute of Plant Protection, Henan Provincial

Fig. 16.4. The millimetric scanning entomological radar developed by the Institute of Plant Protection of the Chinese Academy of Agricultural Sciences (CAAS-IPP).

Academy of Agricultural Sciences. The research team is developing the data analysis software in cooperation with Australian scientists. Up to now, there have been seven sets of insect radars distributed over mainland China, preliminarily forming an entomological radar network for long-term monitoring and management of diverse crop pests.

16.5.3 Airborne insect migration investigation by radar entomology

One of the most troublesome problems in radar entomology is processing the radar data. Cheng (2001), on publishing his PhD dissertation, became one of the first people with a higher degree among the young generation of radar entomologists in China, and now chairs the Committee of IPM Information Technology, Chinese Society of Plant Protection. During his study for his PhD, he developed a software tool, a migration data acquisition and analysis system for scanning entomological radar data. Some of the content of the dissertation was later published (Cheng *et al.*, 2002), and the system has been improved for public use.

Most species of airborne insects opt to fly downwind. However, whether they fly passively or actively in a free wind has been disputed. Using radar entomological research over a number of years, Wu and his team at CAAS-IPP found that the cotton bollworm can actively utilize wind to aid their flying voyage. Most cotton bollworm individuals fly off at the moment when the wind direction is beneficial for their autumn return, either by going downwind or flying up against the wind first to a certain height and then going downwind (Feng *et al.*, 2009). The ability to select seasonally favourable, high-altitude winds is widespread in large day- and night-flying migrants, and insects adopt optimal flight paths that partially correct for crosswind drift, thus maximizing the distances travelled. Trajectory analyses show that these behaviours increase migration distances by 40% and decrease the degree of drift from seasonally optimal directions (Chapman *et al.*, 2010).

To locate the source of migratory insects is a strong function of radar monitoring techniques. The grassland worm (*Loxostege sticticalis* L.) is one of the most harmful pests in the agropasture area of north China and breaks out periodically putting great pressure on stock raising and crop growing. By using radar in the agropasture area since 2005, Zhang *et al.* (2008) discovered the local source of the migratory grassland worm that had been hitherto unknown (Zhang *et al.*, 2008). The flying trajectory of the grassland worm was analysed and it was found that the worm emigrated from the common boundaries between Mongolia, Russia and China during the years 2007–2010. These researchers also conducted radar research on the clover cutworm (*Scotogramma trifolii* Rottemberg) (Zhang *et al.*, 2007, 2010). Similarly, Qi *et al.* (2010) at Nanjing Agricultural University used their Doppler insect radar to observe the migration of two major paddy pests, *N. lugens* and *S. furcifera*, and also conducted investigations on the pest sources outside China. Through geoclimatological analysis, they found the source locations of this rice pest in Vietnam, Laos, Burma and other areas in the peninsula. Recognition of source and sink locations of migratory pests can provide further research topics, for example, determinant factors such as atmospheric circumfluence, climate and crop breeds in the source and sink locations, which are all involved in the IPM of the pests.

16.6 Further Discussion of the Role of ICT in IPM

Broadly speaking, the role of ICT in IPM is not necessarily determined by the access to the Internet but by access to ICT and the media sources that different sections of society can use. With regard to the Internet, access is only one aspect, and other factors such as the quality of connection and related services must also be considered. Although the most discussed issue today

about the digital divide is the availability of access at an affordable cost, there are other technological aspects, discussed below.

16.6.1 Scaling up of IPM-oriented ICT in developing countries

IPM-oriented ICT needs to be scaled up in developing countries. However, there are a number of issues to address in these countries (Vendel, 2010). Each country has its own ways and traditions, and the solutions may not be the same in the different countries. The problem is often discussed in an international context, indicating that certain countries such as the USA are far more equipped than the developing countries to exploit the benefits of the rapidly expanding Internet. It is imperative that developing nations are able to access and utilize the existing IT infrastructure. The importance of mobile phones has been recognized by organizations and agencies such as the United Nations and the US Agency for International Development (USAID), which support and reward new mobile services suitable to meet the needs of developing countries. In many countries, access to the telephone system is considered such a vital element that governments implement various policies to offer an affordable telephone service. Unfortunately, some countries lack sufficient telephone lines.

The accessibility of rural areas to the Internet is a test of the digital divide. However, nowadays there are different ways to eliminate the digital divide in rural areas. Use of power lines (PLT and PLC) and satellite communications offer new possibilities of universal access to the Internet, and the lack of telephone lines will not limit access. However, lower access prices are also required to bridge the ICT divide.

In industrialized areas such as the USA and Europe, there is debate about LTE (4G), iPhone, app stores and the advanced smartphone (high-end device) applications as the solution to increase the usage of a new generation of advanced mobile services. These new services will not for the foreseeable future be a solution for developing countries. They are far too costly and are often not possible to use independently of a device and operator. What are needed are services that give access to the Internet – the whole Internet and not just the very limited mobile Internet sites – through all mobile phones including the lowest level of Internet-capable phones. There should also be the possibility to publish content directly through a mobile phone. These services need be cost-effective so that they can be offered for free to the end user. The services also have to be able to work on more or less any phone and phone operating system, thus resolving the issue of different mobile phone technologies.

Vendel (2010) has discussed the Universal Mobile Interface concept. Even though it is important that all Internet sites are made available, they still have to be transformed into a format that substantially decreases the data capacity needs, makes it possible to read and browse on a small mobile screen, and takes into account that there are no real keyboards on simple phones. Services that, from many aspects, may be brilliant but can only be used on smartphones or on a limited number of phones will not penetrate these regions. What is needed is a general Internet service platform that can migrate the vast user base from just using SMS to taking advantage of the full Internet, but done in a way that makes it easy to use and gives a decent user experience when using simple low-end phones.

16.6.2 International cooperation of universal ICT facilities for IPM

Even where there is physical access to ICT, many people do not have the technical skills needed to benefit from them, so training is essential.

Poor literacy is a problem with ICT such as the Internet. Of those who can read, many know only a local language, while the Internet is dominated by English-language content. The Intercultural Collaboration

Experiment 2002 (Nomura *et al.*, 2003) is an experiment headed by the Department of Social Informatics, Kyoto University, Japan, as an initiative to conduct an experiment on open-source software development by multinational teams in Asia. The participants of the experiment are the Faculty of Computer Science and IT (University of Malaya, Malaysia), the Department of Computer Science and Engineering (Shanghai Jiaotong University, China) and the Department of Management and Computer Science (Handong University, Korea). Because one of the unique characteristics of this experimental project is the pursuit of collaboration among heterogeneous groups across country borders, English was not used as a standard language. One of the objectives of the experiment was to see whether the participants were able to break the language barrier through the use of machine translation tools.

Two tools were provided by Kyoto University at the beginning of the experiment, TransBBS and TransWeb. TransWeb is a tool that allows participants to read all web pages in their native language by specifying a URL and selecting a language. All web pages are then translated to the participant's native language. TransBBS is a bulletin board system that incorporates multilingual translation. It provides support for Malay, Chinese, English, Japanese and Korean languages, thus allowing participants in different countries to communicate with each other in their native languages. Each participant can choose a language when viewing messages. Participants are required to use their own native languages to discuss software design during the experiment. During the first track of the experiment, the four teams worked together to add new functions to these tools.

The Chinese team improvised the functionalities of TransBBS to create the TransSearch engine, which translates the query term into one of the five languages. The search result is translated into the selected language. The Japanese team developed TransMail, a web-based mail program, to send messages among team members instead of using the bulletin board provided by TransBBS. The Korean team devised a chat program called TransChat, which functions like a normal chat program with the ability to converse in multiple languages simultaneously. The Malaysian team developed TransSMS, a multilingual SMS solution that enables users from the four nations to send and receive messages in different languages.

TransSMS is a multilingual SMS solution developed under the Intercultural Collaboration Experiment 2002. It provides features that enable users to send and receive text messages in different languages. At the moment, the languages supported are Malay, Japanese, Korean, Chinese and English. TransSMS provides two main features: (i) translating a text message from one language to another and sending the translated text as SMS; and (ii) 'reading' the translated text to the user. The second feature is useful for tourists who do not speak the language of the country that they are visiting. TransSMS can be accessed via the Internet or a Java-enabled phone (Othman and Lakhmichand, 2004).

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17 From Integrated Pest Management to Ecosystem Management: the Case of Urban Lawn

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17.1 Introduction

Integrated pest management (IPM) has provided an excellent framework for the development and application of sound pest-management practices. The basic tenet of this approach relies on frequent monitoring of pest populations to determine thresholds at which action, such as the application of a pesticide, must be taken to prevent the pest from reaching an economic injury level. While this method prevents unnecessary applications of pesticides, it also aims to reduce pest pressure through the deployment of other tactics such as pest-resistant cultivars and a range of other cultural practices. Although originally developed for the management of insect pests, IPM has also been applied to plant disease and weed management where it should more appropriately be called integrated pathogen or weed management.

Although attempts have been made to define IPM broadly, it fails to address some key issues in sustainable production of agricultural and horticultural commodities. For example, apart from its name, IPM does not apply to nutrient management except strictly in the context of pest and disease development. Even today, there are a few soil fertility recommendations that comply with the basic tenets of IPM. Although the need for phosphorus, potassium and micronutrients could be determined based on a pre-planting soil test, there are no acceptable ways to determine the need for nitrogen application based on a soil test, mainly due to its high lability in the soil. Thus, scores of fact sheets and crop management bulletins from all major US universities recommend pre-determined amounts of nitrogen per unit of land area for each crop.

Furthermore, the human desire to achieve absolute control over nature such as completely weed-free rows of crops or weed-free turfgrass lawns, which are often heavily fertilized and excessively mowed to achieve a 'perfect look', defies IPM principles. There is yet another hurdle to IPM presented by the recent development of preventive-use insecticides, such as imidacloprid and thiamethoxam, which are applied long before the population injury threshold of a pest can be determined. The preventive application of such insecticides completely disengages natural enemies (predators, parasites and pathogens) by eliminating their hosts and by being directly toxic to some. Similarly, preventive use of pre-emergence herbicides does not fall within the purview of IPM. This chapter elucidates some specific challenges encountered in the implementation of IPM, using urban lawn as a case study, and

 Integrated Pest Management (eds D.P. Abrol and U. Shankar)

describes a more comprehensive ecosystem-based approach to urban lawn management.

17.2 The Case of Urban Lawn

Turfgrass lawns have emerged as a central part of urban and suburban landscapes throughout North America, and are rapidly becoming a dominant land use in suburban areas (Robbins and Birkenholtz, 2003). Milesi *et al.* (2005) estimated total turfgrass area in the USA at 163,800 km^2 (±35,850 km^2). Turfgrass lawns are also becoming part of emerging commercial urban landscapes in China and India where Western companies have opened offices and manufacturing facilities. Although lawns were cultivated in ancient China and Persia, historians agree that the precursor to the modern lawn was the 'grassy meads' that surrounded British estates (Jenkins, 1994). Originally kept short by browsing sheep, these formal lawns were later maintained by gardeners using scythes (Schultz, 1999). American aristocrats copied this 'manor house' aesthetic. However, it was the invention of the push mower in the 1830s that placed lawn maintenance within the reach of the average homeowner, leading to the rapid spread of lawns in the North American landscape (Schultz, 1999). As early as 1880, state agricultural colleges began breeding improved grass cultivars for turfgrass (Beard and Green, 1994). As a result, the preferred modern American lawn is composed of one, or at the most two to three, grass species compared with the original lawns, which had a mixture of native grasses and non-grass species, including nitrogen-fixing legumes, commonly referred to as companion plants. However, amenity turfgrasses in home lawns are under constant scrutiny from the public, and their high cosmetic value has resulted in all non-turfgrass plants being declared as weeds (Schultz, 1999). The discovery of selective chemical herbicides during the Second World War, the development of synthetic fertilizers and the rise of the suburban American dream among the growing middle classes during the 20th century led to the rapid expansion in lawn area and growth of the modern lawn-care industry (Jenkins, 1994). Wear-tolerant amenity grasses now occupy over 50 million home lawns, 14,500 golf courses, and many parks, athletic fields, cemeteries and sod farms. Between golf courses and professional and homeowner lawn care, turf maintenance has become an annual industry of over US$45 billion (Potter, 1998).

Besides recreational uses, turfgrass lawns sequester carbon, reduce dust and air pollution, control soil erosion, capture and clean water runoff, provide soil improvement and restoration, moderate temperature, reduce glare and noise, reduce pollen and human disease exposure, and improve the physical and mental health of urban populations (Beard and Green, 1994; Grewal, 2007). Unfortunately, increased lawn coverage has coincided with increased chemical inputs, especially over the last three decades, when cosmetic standards for home and commercial lawn management have increased dramatically (Bormann *et al.*, 1993): 70 million out of 95 million households in the USA (74%) use chemical pesticides and fertilizers (NGA, 2000). Approximately 15 million kg of active pesticide ingredients are sold for home and garden use annually, a figure that has been rising (Aspelin, 1997). The desire of homeowners to achieve a 'perfect' lawn look has resulted in the establishment of a system of lawn care that relies heavily on routine and often calendar-based applications of chemical fertilizers and pesticides. The adoption of best-management practices including IPM and biological control has been extremely slow in both private and commercial lawn-care systems. Homeowners lack training to accurately monitor pest and agronomic problems to make sound decisions about the need for pesticide and fertilizer applications, but their unwillingness to pay if a treatment is not made discourages the implementation of IPM even by professional lawn-care companies. Thus, the application of a fertilizer or pesticide on each visit to the customer's lawn has become the foundation of commercial lawn-care practice.

Lawn inputs are expensive for the homeowner, and pose significant health and environmental risks. While the use of lawnmowers contributes to noise pollution, emissions from lawnmowers contribute to global warming (Priest *et al.*, 2000). It is ironic that regulation of lawnmower emissions has substantially lagged behind compared with other vehicle emissions (Grewal and Grewal, 2005). Research and testimony by the Environmental Protection Agency (EPA) point to the risks of lawn chemical exposure in and around the household (Guerreo, 1990; Ohio EPA, 1999). Human exposure assessment research has demonstrated that many lawn chemicals are far more persistent than previously thought in indoor environments (Nishioka *et al.*, 1999). While many of these same chemicals are also commonly used in production agriculture, the proximity of the site of application to the home poses unique problems. Lawn pesticides and herbicides accumulate in house dust and on carpets, where small children are placed at disproportionate risk (Lewis *et al.*, 1991, 1994; Nishioka *et al.*, 1999). Lawn chemical applications have also been shown to lead to contamination through deposition of the product on clothing (Leonas and Yu, 1992). Experimental measurement of the chemical transport vectors demonstrates the ease with which they track into homes and present ongoing exposure risks (Nishioka *et al.*, 1996, 1999). The effects of these toxins on children are still not well understood, especially their chronic exposure. Modelled impacts of the pesticide chlorpyrifos, however, suggest serious risks from continuous exposure (Zartarian *et al.*, 2000).

A water-quality assessment report indicated the detection of one or more pesticides in 99% of urban stream samples, with insecticides detected more frequently and at higher concentrations in urban watersheds than any other watershed land use, including agriculture (United States Geological Survey, 1999). As many fertilizer compounds contain a range of non-micronutrient substances that remain unused in plant metabolism, overuse can lead to an increase in toxic elements in the underground water supply (Iskander, 1994). These chemicals are also transported above ground or through soils to watercourses where they impact on ambient water quality and biodiversity (Watson and Baker, 1990).

Irrigation of lawns is a major contributor to urban water overuse, especially in semi-arid and drought-prone areas (Anderson *et al.*, 1980; Nelson, 1992; Lant, 1993). This issue is so acute that many municipalities have even considered their legal rights to the large quantities of return flow to aquifers from homeowner watering (Oad and DiSpigno, 1997). Waste production including the impact of grass clipping disposal on shrinking landfill space and the expense and risks involved in disposing of hazardous waste such as partially used household yard and garden chemicals are also enormous problems associated with conventional lawn management. In fact, excess grass clippings are also a problem at municipal composting facilities, where the large amounts of clippings arriving in spring and summer can overload the system with material containing high levels of nitrogen and lead to the process becoming anaerobic, resulting in air pollution violations from the odours of methane, ammonia and other gasses released (McDonald, 1999).

Lawn chemicals can also disrupt ecological processes, such as predator/parasite complexes and organic matter breakdown that are important to the natural functioning of an ecosystem. In low-maintenance or unmanaged grassland ecosystems, the layer of live and dead plant material that accumulates on the soil surface is a key resource that is rapidly decomposed by a diverse array of detritivores. However, in lawns, these detritivore communities are disrupted by pesticide and fertilizer applications, resulting in the build-up of 'thatch', due to a low rate of decomposition (Potter, 1994). Excessive thatch favours major diseases and insect pests (Halisky *et al.*, 1981; Davis and Smitley, 1990a,b), reduces pesticide efficacy due to adsorption and presents an impenetrable barrier to the movement of biological, microbial and chemical pesticides into the

root zone. Pest-management systems that either use no chemical pesticides and fertilizers or rely on using less could make greater use of natural ecological processes such as thatch decomposition and biological controls to enhance system stability. Indiscriminate use of lawn pesticides has also resulted in the development of resistance of the target pests and pathogens. For example, several insect species have developed resistance against several classes of insecticides (Reinert and Portier, 1983; Cherry and Nagata, 2007; Ramoutar *et al.*, 2009). Therefore, the very concept of the lawn is now being questioned by the emerging urban environmentalist movement and there is a growing anti-lawn sentiment in parts of the USA and Canada (Robbins and Sharp, 2003a; Sandberg and Foster, 2005; Robbins, 2007). In fact, several municipalities in Canada have banned the 'cosmetic use' of pesticides on lawns because of potential health effects and environmental contamination.

17.3 IPM in Urban Lawn Care

The IPM concept is an effective tool in reducing the overuse of chemical pesticides in agricultural and horticultural production systems. The IPM concept promotes the integration of multiple tactics including cultural practices and biological control, but action (e.g. the application of a pesticide) is taken only when justified through adequate sampling. Although urban lawns are not cultivated for direct sale as a crop, they are consumed indirectly as aesthetic, personal and property values. As such, a typical high-maintenance home lawn in the midwestern USA receives: (i) two to three herbicide applications annually, two for broadleaf weeds such as dandelion and clover and the third for annual grasses such as large or smooth crabgrass; (ii) two insecticide treatments, one in the summer for either bill bug or chinch bugs and the other in autumn for white grub control; and (iii) a fungicide treatment. In addition, home lawns receive two to four applications of fertilizers annually delivering 75–195 kg N/ha/year (Danneberger, 1993; Cockerham and Minner, 1999). Recent research shows that homeowners are still unable to achieve acceptable lawn quality and weed and pest suppression, despite this reliance on four-, five- or six-step lawn-care programmes that recommend calendar-based application of fertilizers, herbicides and insecticides (Cheng *et al.*, 2008b). While lawn-care company technicians are often able to produce the desired quality (extremely green, weed- and pest-free) lawns, do-it-yourself homeowners often fail to accomplish this goal, most probably due to a lack of knowledge and training and the ineffectiveness of many consumer lawn-care products.

Alumai *et al.* (2009a) compared aesthetic (lawn quality), biological (weeds and insect pests) and economic (management costs) effectiveness of a *commercial* (managed by a professional company), *consumer* (managed using consumer lawn-care products following the label instructions), *IPM* (pesticide applications based on monitoring and thresholds), *organic* (monitoring and need-based organic and natural product applications) and an *untreated* programme. The percentage weed cover was the lowest in the commercial followed by the IPM, organic and consumer programmes, respectively. The commercial programme had a lower white grub density than all the other programmes, and the organic programme had a lower white grub density than the untreated programme. The commercial programme had the highest lawn quality, while the untreated programme had the lowest. The IPM and organic programmes did not differ significantly in lawn quality, but both rated significantly higher than the consumer programme. Annual costs (which included monitoring costs in the case of the IPM and organic programmes) were highest in the commercial (US$382/0.05 ha) followed by the organic (US$305/0.05 ha), IPM (US$252/0.05 ha), and consumer programme (US$127/0.05 ha), respectively. They concluded that the commercial programme produced the highest lawn quality and best weed and insect control, and was also the

most expensive. The IPM and organic programmes were cheaper than the commercial programme and produced acceptable lawn quality. Although the consumer programme was the cheapest, it did not produce acceptable weed control or lawn quality compared with the untreated programme.

Encouraged by the above findings, Alumai *et al.* (2009b) conducted a 2-year study in collaboration with a professional lawn-care company to compare biological (weed, insect and disease), aesthetic (lawn quality) and economic (lawn-care programme cost) effectiveness of an IPM programme, in which pesticides were applied on the basis of treatment thresholds, with a standard programme in which pesticides were applied on a calendar basis without pest monitoring. Although the weed incidence was low, the IPM programme had significantly more lawns with the presence of weeds than the standard programme during 2005 and 2006. However, only 21% of the IPM lawns required herbicide applications in 2005, and none exceeded the treatment threshold (5% weed cover) in 2006 compared with 100% of the standard-programme lawns being treated for weeds in both years. The IPM programme also had significantly more lawns with insect damage than the standard programme during June 2005 and August 2005, but not in September 2005 and throughout 2006. Only 28% of the IPM lawns required insecticide applications in 2005 and none exceeded the threshold (5% insect damage) in 2006, whereas all of the lawns in the standard programme received insecticide treatments in both years. Although lawn quality was high for both programmes (>8, on a scale of 1–9), it was significantly higher for standard than for IPM-programme lawns during 2005 and in June 2006 and September 2006 but not in August 2006. The annual lawn-management costs were lower for the IPM programme (US$281.50), which included the cost of monitoring, than for the standard programme ($458.06). In the IPM programme, 31% of customers who continued with the study in 2006 did so because they were satisfied with the programme. Among those who did not continue with the programme, 33% cited weed or insect problems, while 33% expected better results. On the conclusion of the study in 2007, the lawn-care company decided not to continue with the IPM programme as they did not want to lose any customers.

While these studies clearly demonstrate the effectiveness of IPM in reducing overall pesticide inputs (i.e. the number of lawns receiving pesticide applications) and lawn-care costs, they illustrate difficulties in convincing homeowners and lawn-care providers to voluntarily adopt IPM. A comprehensive survey of homeowners in Ohio also revealed that concern for the environment did not motivate homeowners to adopt IPM practices or accept alternatives to turfgrass lawn (Blaine *et al.*, 2012). In another study, Robbins *et al.* (2001) found that the users of high-input lawn chemical systems were more likely to be wealthy, well-educated and knowledgeable about the negative environmental impacts of their actions than non-users. While easy-to-use diagnostic tools, particularly for use by homeowners, to determine thresholds at which pesticides should be applied may increase IPM adoption, especially by those who have the time and who care enough about the environment, these findings raise doubts about whether additional IPM education will increase IPM adoption more broadly.

The concept of turfgrass lawn as a dominant component of the urban landscape is now well entrenched in North American cultural psychology (Bormann *et al.*, 1993; Jenkins, 1994). Regardless of cost, time or labour for frequent maintenance, awareness about the negative impact of lawn chemicals on human health and the environment, and the near-impossibility of achieving a 'perfect look', lawns are considered 'part of nature', essential to the value of the property, and are perceived as important in residents' sense of achievement of social status and acceptance in the neighbourhood (Blaine *et al.*, 2012). While there are challenges to the implementation of IPM, the proximity and widespread familiarity of the urban lawn ecosystem to

North Americans presents a huge opportunity to raise awareness about local and global environmental and sustainability issues. More than 80% of the US population and more than 50% of the world population now live in cities where all future population growth is projected to occur (Cohen, 2003). Indeed, urban ecosystems form a broad and growing front as the world population is becoming more urbanized and human impacts on the environment are becoming more severe, resulting in both local and global feedback loops that place humans and the ecosystems on which they depend at risk. Unfortunately, urban areas – where most people live – have been the least studied ecosystems on the planet and have long been neglected, particularly by ecologists (Pickett *et al.*, 2001). As homeowner education about management practices and their ecological underpinnings is a fundamental prerequisite for the adoption of best-management approaches to lawn care, a basic understanding of urban lawn ecology is critical. Thus, we need a new paradigm and a transformative approach to tackle the dilemma of the urban lawn.

17.4 Ecosystem Management – a Novel Approach

I propose a new approach 'ecosystem management' that builds on certain tenets of IPM but is much broader and focuses on the maintenance of ecological integrity and sustainability of the ecosystem while maximizing the desirable services it produces for both current and future generations. The ecosystem management approach was originally developed for the sustainable management of forests, and there are several different definitions. For example, Grumbine (1994) reviewed the then evolving concept of ecosystem management, identified ten dominant themes and coined a new definition of ecosystem management as a strategy that 'integrates scientific knowledge of ecological relationships within a complex sociological and values framework toward the general goal of protecting native ecosystem integrity over the long-term'. The Ecological Society of America's committee on the scientific basis for ecosystem management defined ecosystem management as a 'management driven by explicit goals, executed by policies, protocols, and practices, and made adaptable by monitoring and research based on our best understanding of the ecological interactions and processes necessary to sustain ecosystem structure and function' (Christensen *et al.*, 1996). This committee explicitly stated that ecosystem management must include the following: (i) long-term sustainability as a fundamental value; (ii) clear operational goals; (iii) sound ecological models and understanding; (iv) an understanding of the complexity and interconnectedness; (v) recognition of the dynamic character of ecosystems; (vi) attention to context and scale; (vii) acknowledgement of humans as ecosystem components; and (viii) commitment to adaptability. In the planning literature, ecosystem management is defined as 'a process that aims to conserve major ecological services and restore natural resources while meeting the socio-economic, political, and cultural needs of current and future generations' (Brussard *et al.*, 1998). Here, the principal objective of ecosystem management is considered to be the efficient maintenance and ethical use of natural resources (Szaro *et al.*, 1998), and the interrelations of socio-cultural, economic and ecological systems are considered paramount to understanding the circumstances that affect environmental goals and outcomes (Lackey, 1998).

Although the current definitions differ somewhat in their emphasis on specific aspects, the overarching goal of the ecosystem management approach is clear, which is to obtain *human-desired* goods and services from ecosystems *sustainably*. In this sense, ecosystem management is the ecological analogue to the economic stewardship of a trust or endowment dedicated to benefit all generations (Christensen *et al.*, 1996). It is also evident that ecosystem management requires the application of ecological science to obtain or generate ecosystem goods and services for human

well-being. What is unclear, however, is how to implement such an approach. Christensen *et al.* (1996) acknowledged that moving from concepts to practice is a daunting challenge and stated that it certainly requires the following: (i) defining sustainable goals and objectives; (ii) reconciling spatial scales; (iii) reconciling temporal scales; (iv) making the system adaptable and accountable; and (v) a role of scientists in the ecosystem management. Perhaps due to its genesis in the sustainable management of forests (natural resources), as currently defined the implementation of the ecosystem management concept in privately owned parcels of land such as individual farms and home landscapes presents unique challenges. For instance, although the goal of a farmer can be to maximize farm productivity in both the short and the long term (as in any business), the required long-term monitoring, research and ecological modelling for individual farms may be both intellectually and cost prohibitive, especially when it is not clear exactly what to monitor as the models of natural systems are not very precise and are subject to change. As acknowledged by Christensen *et al.* (1996), it is unlikely that society will accept 'science as a model for ecosystem management' in the absence of a clearer understanding of the importance of uncertainty to both science and management.

In the context of managed agricultural and horticultural ecosystems, *ecosystem management* can be defined as a holistic approach in which ecological interactions and processes are recognized and carefully and minimally engineered to obtain desirable goods and services, while minimizing disservices and building and sustaining ecosystem composition, structure and function. Here an explicit objective is to minimize the need for external inputs, particularly inorganic fertilizers, pesticides, tillage and so on, by maintaining desirable biological diversity and functionality of the food webs through careful engineering of the ecological interactions. It is acknowledged that most current agricultural and horticultural ecosystems are dysfunctional, as characterized by the 'ecosystem distress syndrome', showing significant changes in nutrient cycling, community diversity, successional retrogression, primary productivity, organism size distribution, pest and disease incidence, and amplitude of population fluctuations (Rapport *et al.*, 1985). Thus, restoring and maintaining ecological integrity is an explicit goal. It is also acknowledged that ecosystems are complex and have emergent properties; therefore, adaptive management is the most appropriate. Ecosystems are also open systems with respect to exchanges of organisms, matter and energy; thus, minimizing the production and export of disservices is another goal. In this approach, humans are considered an integral part of the ecosystem and thus, as a component species, their needs, actions, knowledge, beliefs and socio-political institutions are considered fundamental to the development and practice of the approach, as opposed to the strictly biocentric or ecocentric values approach (Noss and Cooperrider, 1994) in which humanism or anthropocentrism is rejected.

To practice the proposed ecosystem management approach, the common fundamental ecological principles are explicitly identified, and are then used as tools to obtain the desired ecosystem services sustainably. The most common fundamental ecological principles may include: (i) human intervention; (ii) resource competition; (iii) herbivory; (iv) predation and parasitism; and (v) biogeochemical cycling. The underlying premise of this *ecological principle tools-based ecosystem management approach* is the inherent property of 'trade-offs'; that is, any manipulation of these ecological principles may lead to both desirable and undesirable outcomes in the short or long term. For example, the principle of human intervention in the ecosystem involves 'feedback' loops, which can positively or negatively influence the ecosystem and human well-being in the short or long term. The five most common ecological principles are elaborated below and their use in building a comprehensive ecosystem

management approach for urban lawns is illustrated. It is expected that this *ecological principle tools-based ecosystem management approach* will be refined over time and used in diverse ecosystems with appropriate modifications.

17.5 Application of the Ecosystem Management Approach to Urban Lawn

The first step in the application of the ecosystem management approach is to identify the major biotic and abiotic components of the target ecosystem and potential ecological interactions among them. It is acknowledged that current knowledge of ecological interactions and ecosystem functioning is provisional and subject to change as science progresses. Therefore, management approaches must be viewed as hypotheses to be tested by continued research and monitoring. Fig. 17.1 shows the various biotic and abiotic components of the urban lawn ecosystem and identifies the potential ecological interactions among them. Major biotic components of the lawn ecosystem include the lawnowner (human), grass (turfgrass), weeds (undesirable grassy and non-grassy plant competitors), herbivores (pests and pathogens), natural enemies (predators and parasites) and detritivores (bacteria, fungi, nematodes and other micro- and macro-arthropods), while the abiotic components include soil, water and climate. In case of the urban lawn, five fundamental ecological principles are those described in the previous section. Below is a brief description of each of these principles followed by a brief discussion on their manipulations to obtain desirable ecosystem services sustainably from the urban lawn while minimizing the generation of internal and external disservices.

17.5.1 The principle of human intervention

The principle of human intervention is the most important ecological principle guiding the development of the ecosystem management approach in urban lawn ecosystems. Humans are the dominant biotic component of urban lawn ecosystems and their actions directly or indirectly

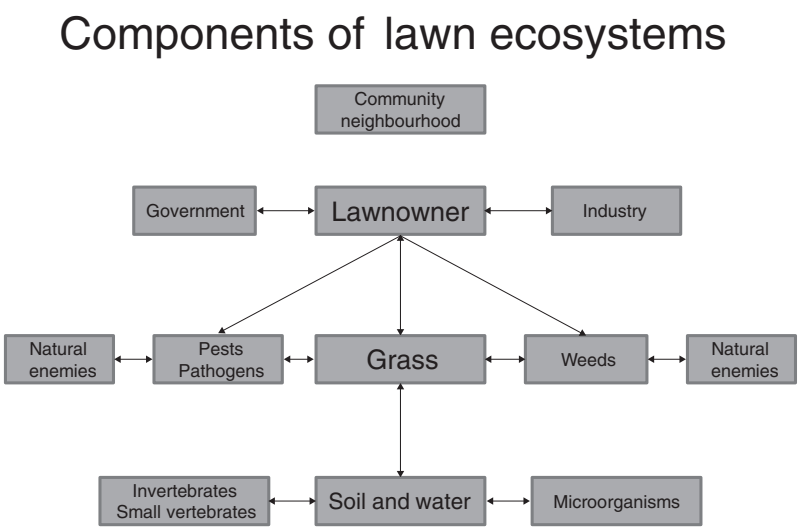

Fig. 17.1. Biotic and abiotic components of the urban lawn ecosystem and their interactions represented by the arrows. Note humans (lawnowners) are considered a component species and an integral part of the ecosystem.

influence all biotic and abiotic components and their interactions. In fact, lawns are artificial ecosystems that exist solely because of human action prompted mainly by aesthetics and recreational needs. Humans eliminate existing vegetation, disturb the soil profile and establish turfgrass lawns. The lawnowner's choice of grass species and cultivar to be planted defines the ensuing interactions between the grass and weed species (resource competition), between the grass and pest or pathogen species (herbivory), between the herbivores and natural enemies (predation and parasitism), and between biomass production and the detritus food web (biogeochemical cycling). Human action directed against weeds, pests and pathogens (e.g. pesticide applications) directly or indirectly influences above- and below-ground food chains (e.g. predation and parasitism) and affects the functions of the soil food web (e.g. nutrient release, antagonism). Human actions such as the application of fertilizers or pesticides, mowing and watering can also influence human and environmental health, biodiversity and biogeochemical processes including carbon sequestration, nutrient cycling, and energy and material flows, both locally and globally.

In order to use the human intervention principle most appropriately as a tool in lawn ecosystem management, we must understand the needs, actions, knowledge, beliefs and socio-political institutions of humans as a component ecosystem species. Although the need for recreational space (an area to play and socialize) may have been the original motivation for the establishment and maintenance of lawns, aesthetics of orderliness has now become the primary driver for most human intervention in the modern lawn ecosystem. A survey of middle-class, midwestern home-owning families (n = 325) living in suburban and rural communities around Chicago revealed an almost unanimous (98%) consensus on the value of lawns as the primary component of the residential landscape (M. Quigley, personal communication). Regardless of recognized cost, endless maintenance and the impossibility of accomplishing a 'perfect look', lawns are considered both 'part of nature' and essential to the value of a property. The respondents recognized that their lawns did not look 'good' for more than a fraction of the year (4.7 months on average), and that amount of time spent maintaining the lawn was often more than the time they spent using or enjoying the lawn. These results suggested that, while the species compositions of lawns could be changed, the overwhelming majority of Americans would not replace their turf with anything that did not look like a lawn. Lawnowners striving for a 'perfect lawn look' routinely use external inputs including mowing, watering, chemical fertilizers and pesticides. Well-tended, weed-free, monoculture turfgrass lawns have become a status symbol in suburban neighbourhoods and are considered essential to the maintenance of property values of homes and a desirable norm to be accepted in the neighbourhood (Blaine *et al.*, 2012). In fact, homeowners' passion for weed-free lawns, and acceptance of herbicidal methods to achieve it, seems to contradict society's general antipathy toward pesticides (Robbins, 2007). Although, humans receive economic (e.g. an increase in property values and employment in the landscape industry), environmental (dust reduction, soil erosion control, water runoff reduction and climate amelioration) and social (recreation and exercise) benefits from lawns, Robbins (2007) has built a convincing case for the influence of urban lawns on human psychology in North America. He explains the way lawnowners become turfgrass subjects, through a coercive economy, fraught with uncertainty and anxiety, mediated by a moral dedication to community and family.

It is intuitive to think that lawnowners' attitudes and behaviours are influenced by their knowledge, beliefs and values, and therefore that their lawn and landscape management practices (actions) may reflect their environmental ethics. However, social science reveals that lawnowners' decisions are strongly influenced by external factors such as the community, neighbourhood and

industry. Robbins and Sharp (2003b) found that well-educated homeowners who profess to know the negative effects of lawn chemicals on the environment tend to use more lawn chemical inputs than less educated homeowners who are unaware of the effect of lawn chemicals on the environment. Homeowners in rural areas use lower lawn inputs and are less likely to employ a lawn-care company than those in urban and suburban areas (Blaine *et al.*, 2012). Most homeowners believe that their or their neighbours' own application of fertilizers and pesticides are less harmful to the environment than the applications by the lawn-care companies (Blaine *et al.*, 2012). Furthermore, most homeowners adopt lawn-care practices to 'fit in' with their neighbourhood norms (Blaine *et al.*, 2012), and some homeowners even feel pressured to adopt lawn-care practices that they admit are not environmentally friendly. Homeowner associations often have by-laws, also referred to as 'weed laws', which dictate that lawns must be 'weed free' and kept mowed at a certain height, which also discourages the use of alternative ground covers (Robbins *et al.*, 2001). It has also been noted that angry homeowners may mow their neighbour's unmanaged lawns to send them a message to comply with the neighbourhood norms (Robbins, 2007).

Homeowners' attitudes and behaviours are strongly influenced by the powerful marketing strategies used by the lawn-care industry. Beautiful pictures of lawns used in product advertisements on television and in gardening magazines promote the aesthetics of orderliness, which is neither realistic nor practical under most 'real world' urban situations (Robbins, 2007). Companies often include frightening pictures of insects, such as magnified white grubs showing frowning mandibles and extensive lawn damage, to sell additional insecticide treatments. Schultz (1999) suggested that, after the launch of the first broadleaf lawn herbicide, which also eliminated the then-admired clover, the herbicide manufacturer Scotts launched a relentless marketing programme to discredit clover in the lawn. This programme started with articles questioning the value of clover in a lawn and ended with clover classified as a weed, with recommendations for its eradication. These powerful marketing strategies coupled with pressure from formal and informal neighbourhood organizations promote a system of high-input lawn management. Robbins and Sharp (2003b) argued that the peculiar growth and expansion of the moral economy of the lawn is the product of a threefold process in which (i) the lawn chemical industry has implemented new and innovative styles of marketing that (ii) help to produce an association of community, family and environmental health with intensive turfgrass aesthetics and (iii) reflect an increasing local demand by consumers for authentic experiences of community, family and connection to a non-human biological world through meaningful work. Although the use of a lawn-care company is certainly a luxury (Blaine *et al.*, 2012), it is considered a status symbol in affluent suburban neighbourhoods. In the USA, about 25% of high-income homeowners hire a commercial lawn-care company and about 40% engage in 'do-it-yourself' lawn-care practices (Blaine *et al.*, 2012). While lawn-care company technicians are often able to produce an acceptable lawn look (extremely green, weed- and pest-free), do-it-yourself homeowners often fail to accomplish this goal even when using industry recommended four- or five-step lawn-care programmes (Cheng *et al.*, 2008b). Although this is partly the result of a lack of knowledge and training in accurate product application, research has confirmed that some consumer products fail to provide acceptable weed and insect control, even when label recommendations are followed (Alumai *et al.*, 2009a). Most lawn-care companies employ trained technicians (or will train them on the job) but also use liquid formulations of products, compared with the granular formulations available to consumers. The inability of the lawnowner to achieve the expected results promotes repeated overuse and often misuse of products. On the other hand, lawn-care

companies may also overuse water-soluble fertilizers to ensure greener lawns compared with their competitors and will apply pesticides preventively to avoid call-backs. Interestingly, most lawn-care company professionals believe that their competitors overuse fertilizers, herbicides and insecticides compared with their own companies (Blaine *et al.*, 2005). Using both campus and home lawn experiments in collaboration with lawn-care companies, recent research has documented the effectiveness of the IPM approach in reducing herbicide and insecticide applications (Alumai *et al.*, 2009a,b), yet the acceptance of the IPM approach by lawn-care companies and homeowners remains low.

Obviously, the use of the human intervention principle as a tool in the lawn ecosystem has trade-offs that can produce desirable or undesirable outcomes. For example, while the application of a chemical fertilizer may increase aesthetic appeal (greening) of a turfgrass lawn, it may also result in an increased need for mowing and clippings removal and water pollution through nutrient runoff. Similarly, the application of a chemical pesticide may reduce pest pressure, but it may also disrupt natural enemy complexes, which may result in resurgence of the pest or the emergence of a new pest problem. Application of a broad-spectrum pesticide may also harm the detritivore community, thus halting decomposition (biogeochemical cycling), resulting in the accumulation of dead plant material commonly referred to as 'thatch'. Furthermore, the chemical pesticide may even become the source of water pollution through runoff and groundwater percolation (export of disservices). However, if used with a goal to build and sustain ecosystem structural and functional integrity, the principle of human intervention can produce desirable (more appropriately acceptable) lawn quality in both the short and long term. Thus, a thorough education of society (including the industry, government and policy makers) in ecological science is *essential* for practice of the ecosystem approach. The positive and negative influence of human intervention on other ecological interactions in the lawn ecosystem is explored further below.

17.5.2 The principle of resource competition

The second most important ecological principle in building the ecosystem management approach to the urban lawn is resource competition. In nature, organisms compete for resources including space, nutrients and sunlight, and studies on the mechanisms of interspecific competition for resources (Tilman, 1990, 1997, 1999) suggest several potential mechanisms for competitive suppression of unwanted species. In urban lawns, turfgrass plants compete with grassy and non-grassy weedy species for space, light, nutrients and water. Most lawn weeds are opportunistic species that become established in response to disturbances that create a gap, exposing the soil to sunlight. The source of weed seeds is predominantly the soil seed bank, although some seeds are blown in by wind or moved by animals or humans. The soil seed bank in suburban allotments contains thousands of viable weed seeds representing 30 or more species (Cardina *et al.*, 2002). Seeds of some weed species can live for many years in the soil, germinating in response to environmental cues associated with soil disturbance. In north-central and north-eastern USA, dandelion (*Taraxacum officinale*) and crabgrass (*Digitaria sanguinalis*) are considered the most objectionable weed species in urban lawns.

The ability of turfgrass to compete with weedy species depends on the species involved, soil pH, nutrient availability, biotic and abiotic stresses, and cultural practices (human intervention) such as mowing and fertilizer applications. van Dersal (1932) reported that common turfgrass species such as bent and bluegrass require acidic soil conditions, and a pH of 5.5 can exclude clover. Grass species also differ in their requirements for water and fertility. For example, Frank *et al.* (2004) reported that buffalograss (*Buchloe*

dactyloides) requires less irrigation and fertilization than other turfgrasses. It is a C4 grass native to the Great Plains of North America, and possesses exceptional drought tolerance and good heat and cold tolerance. The authors recommended 98 kg N/ha and a mowing height of 5.0–7.5 cm for sustained quality, colour and density. Richie *et al.* (2002) demonstrated that deeper and less frequent irrigation of established tall fescue grass on sandy loam soils in southern California improved visual quality. In north-eastern and north-central USA, Kentucky bluegrass, perennial ryegrass and tall fescue are most commonly planted as lawn grasses. Kentucky bluegrass is used most widely, mainly due to its fine texture, followed by perennial ryegrass and tall fescue, respectively. However, Kentucky bluegrass requires higher maintenance than the other recommended species and hence is less competitive against weedy species under low maintenance. It turns brown and goes dormant if not irrigated in the summer, is prone to thatch formation and does not do well on poor soils (Danneberger, 1993). In contrast, 'turf-type' tall fescue requires low maintenance, develops a deep root system, tolerates low fertility and compacted soils, and survives drought conditions better. It also germinates fast, tolerates both shade and full sun and holds up well under heavy traffic situations, and is therefore more competitive against weedy species. Although perennial ryegrass is heat and cold tolerant, establishes quickly and forms a dense canopy that can crowd out weeds, it also requires higher maintenance than tall fescue and hence is less competitive against weedy species under low maintenance.

Studies show that turfgrass species can influence the type and severity of weeds and patterns of weed occurrence over time. Richmond *et al.* (2006) reported that some weed species, such as white clover (*Trifolium repens*), corn speedwell (*Veronica arvensis*) and dandelion were more prevalent in plots seeded with tall fescue compared with perennial ryegrass during the lawn-establishment phase. However, over time the prevalence of these species decreased in tall fescue plots to levels similar to or less than those in perennial ryegrass plots when no fertilizer or irrigation was provided. After 5 years of subsequent low maintenance without any fertilizer or herbicide applications, total weed cover and cover by dandelion, buckhorn plantain (*Plantago lanceolata*) and white clover (*T. repens*) were significantly higher in perennial ryegrass than in tall fescue, whereas cover by heal-all (*Prunella vulgaris*) was higher in tall fescue than in perennial ryegrass (A. Alumai, S.O. Salminen, D.S. Richmond, J. Cardina and P.S. Grewal, unpublished data).

Turfgrass grass and weedy species can differ in their resource requirements, and adjustments in resource supply rates (human intervention) may determine the outcome of interspecific competition, allowing desired species to competitively exclude or suppress weedy species (Tilman *et al.*, 1999). In the 140-year-old Park Grass Experiment at Rothamsted in the UK, Tilman *et al.* (1999) found that dandelion abundance was highly dependent on potassium fertilization and on liming but not on the addition of other nutrients. In this experiment, potassium fertilization led to a 17–20-fold increase, while liming led to a two- to threefold increase in dandelion abundance. In a greenhouse study in Minnesota, it was shown that dandelions had a higher requirement for potassium and had the biomass more limited by potassium than any of the five grass species tested. They also reported that in Minnesota lawns that had not received fertilizer or herbicide, dandelion density and abundance were significantly positively correlated with its tissue potassium levels.

High rates of nitrogen fertilization (100–300 kg N/ha/year) can reduce populations of crabgrass, dandelion and other broadleaf weeds in cool-season tufgrassses, although annual bluegrass may be favoured (Busey, 2003). Turner *et al.* (1979) reported that crabgrass encroachment was significantly affected by phosphorus application, with a greater amount of crabgrass in no-phosphorus plots than in fertilized turf. They also reported that

dandelion encroachment was greatest in the turf receiving limestone applications and tended to decrease with increasing phosphorus rates. They suggested that encroachment was related to competition rather than the nutritional requirements of the weeds, as encroachment tended to decrease as the turfgrass clipping yield increased. Sincik and Acikgoz (2007) reported that in grass/white clover mixes, nitrogen fertilization significantly reduced the white clover percentage in harvested clippings in the second and third years after planting.

Other cultural practices (human invention) such as mowing can also influence competitive interactions between turfgrass and weedy species. Lush and Rogers (1992) reported that ryegrass biomass was directly related to turf cutting height, whereas tiller density was inversely related to cutting height. Both height and tiller density can influence the competitive ability of turfgrasses. Lower cutting heights and increased mowing frequencies result in reduced root production, decreased shoot growth and increased dark respiration (Krans, 1975), which may weaken turfgrass plants. Proper mowing frequency (semi-weekly) and cutting height (6.25 cm) tend to alleviate mowing stress and improve turfgrass quality in Kentucky bluegrass. Dernoeden *et al.* (1993) reported that a high mowing height (8.8 cm) was the best cultural practice for reducing smooth crabgrass encroachment and maintaining tall fescue cover. In the absence of irrigation and fertilizer inputs, Dernoeden *et al.* (1994) noted that Bighorn blue fescue and Aurora hard fescue maintained higher quality and better resisted weed invasion compared with the Silverado and Rebel II cultivars of tall fescue. In field-lot studies, it was found that weed cover varied significantly with mowing height and mowing frequency in tall fescue and perennial ryegrass plots (A. Alumai, S.O. Salminen, D.S. Richmond, J. Cardina and P.S. Grewal, unpublished data). For both grass species, plots mowed at 5 cm had significantly higher total weed cover than plots mowed at 8.9 cm on each sampling date. For tall fescue, total weed cover and cover of thymeleaf speedwell and buckhorn plantain were significantly higher in plots mowed at 5 cm than in plots mowed at 8.9 cm. Tall fescue plots mowed biweekly (2-week intervals) at 8.9 cm had significantly lower dandelion, heal-all and buckhorn plantain cover than plots mowed weekly or biweekly at 5 cm height. For perennial ryegrass, total weed cover and white clover cover were significantly higher in plots mowed weekly at 5 cm than in plots mowed biweekly at 5 cm, and weekly or biweekly at 8.9 cm. Dandelion cover was significantly higher only in perennial ryegrass plots mowed biweekly at 5 cm compared with plots mowed weekly at 5 or 8.9 cm. Heal-all cover was significantly higher in perennial ryegrass plots mowed at 5 cm than in those mowed at 8.9 cm, but no significant differences were observed with mowing frequency. Perennial ryegrass plots mowed weekly at 5 cm had significantly lower thymeleaf speedwell than plots mowed biweekly at 5 cm. These results illustrate that cultural practices such as fertilization and mowing can influence weed patterns in urban lawns through direct or indirect affects on resource competition between turfgrass and weedy species, and that careful manipulation of the resource supply can produce desirable lawns with fewer weeds.

Fungal endophyte (*Neotyphodium* spp.) infection in cool-season grasses can also influence competitive interactions between turfgrass and weedy species. By modifying the resource allocation patterns in the turfgrass plant, the endophytes can alter host phenotypic plasticity (Hill *et al.*, 1990, 1991; Belesky and Fedders, 1995; Cheplick, 1997; Malinowski and Belesky, 1999; Malinowski *et al.*, 2000; Rahman and Saiga, 2005) and competitive ability (Peters and Zam, 1981; Marks *et al.*, 1991; Clay *et al.*, 1993; Mathews and Clay, 2001). Endophyte-infected perennial ryegrass and tall fescue produce more tillers and biomass than uninfected plants, suggesting that the endophyte provides a competitive advantage to host plants (Latch *et al.*, 1985; Clay, 1987). In greenhouse experiments, Marks *et al.* (1991) studied inter- and intra-specific

competition between endophyte-infected and uninfected tall fescue and perennial ryegrass. Endophyte-infected tall fescue plants were heavier and more competitive than non-infected plants in intra- and interspecific competition. In this study, the endophyte had a negative effect (trade-off) on competition in the perennial ryegrass cultivar used. Clay and Holah (1999) showed that plant species diversity declined and tall fescue dominance increased in unmanaged field plots containing endophyte-infected tall fescue relative to uninfected plots without a difference in total productivity. They showed that 40% of plant biomass was composed of weedy grasses in 4-year-old plots of non-infected tall fescue compared with only 15% in plots with endophyte-infected tall fescue. During the course of their study, weedy forbs biomass declined by 50% without the endophyte and by 90% with the endophyte-infected grass. In mowed turfgrass lawns, it was found that endophytes had a significant effect on weed cover in both tall fescue and perennial ryegrass plots (A. Alumai, S.O. Salminen, D.S. Richmond, J. Cardina and P.S. Grewal, unpublished data).

Endophytes may also enhance biotic (Clay, 1988; Kimmons *et al.*, 1990; Dahlman *et al.*, 1991; Latch, 1993; Breen, 1994) and abiotic (West *et al.*, 1993; Elmi and West, 1995) stress tolerance in turfgrass plants, although Lewis (2004) found no consistent effect of the endophyte on perennial ryegrass stress tolerance. Other studies also indicate that endophyte infection can have negative ecological consequences (trade-offs) for perennial ryegrass when competing with a fast-growing, aggressive weed species such as large crabgrass (Richmond *et al.*, 2003). Endophyte infection reduced the ability of perennial ryegrass to compete with large crabgrass under establishment conditions, and crabgrass reproductive potential increased as a result. Although endophyte infection had little influence on competitive interactions between tall fescue and dandelion, dandelion was a significantly stronger competitor against endophyte-infected perennial ryegrass compared with uninfected grass (Richmond *et al.*, 2003). These findings underscore the ecological contingency of the endophyte–host relationship and illustrate trade-offs.

Biotic stresses such as insect pests (herbivory) can weaken turfgrass plants, reducing their competitive ability against weeds and facilitating weed invasion and establishment in turfgrass lawns. Hardy *et al.* (1985) reported that among the reasons for enhanced competitive ability of endophyte-infected tall fescue was resistance to insect herbivory. Funk *et al.* (1983) found that infected ryegrass maintained greater cover under severe insect stress, whereas uninfected stands persisted poorly and were more heavily invaded by weed species. In studies on plant competition among grasses with and without fungal endophytes and herbivores (fall armyworm, *Spodoptera frugiperda*), Clay *et al.* (1993) found that herbivory reduced plant growth whereas endophyte infection increased it. Endophyte-infected perennial ryegrass and tall fescue plants had nearly twice the biomass of non-infected plants when herbivores and competing (non-endophytic) grasses were both present. In monocultures, Richmond *et al.* (2004a) found that root herbivory by Japanese beetle (*Popillia japonica*) grubs significantly reduced the number of tillers per plant and the above- and below-ground biomass of perennial ryegrass and tall fescue but had no effect on dandelion. In mixtures of dandelion and either grass species, herbivory significantly reduced the number of grass tillers and above- and below-ground biomass, whereas endophyte infection significantly reduced the number of perennial ryegrass tillers but had little effect on tall fescue. Herbivory significantly increased the number of dandelion leaves and above- and below-ground biomass in mixtures with perennial ryegrass or tall fescue. In field-plot experiments, the prevalence of weedy species in perennial ryegrass and Kentucky bluegrass mixtures treated annually with insecticides was lower compared with untreated plots (P.S. Grewal, D.S. Richmond and S. Salminen, unpublished data). Weed invasion into stands of turfgrass suffering from different

levels of billbug damage also differed significantly while very few weed seedlings were present at the undamaged site (D.S. Richmond and P.S. Grewal, unpublished data). However, at the other two sites where moderate to severe billbug damage was present, weeds (large crabgrass, dandelion and black medic) established rapidly when overseeded compared with the undamaged sites. Although it is known that insects can reduce the vigour of turfgrass plants, only rarely is the connection made between insect damage and other ensuing problems such as disease or weed invasion. In fact, we tend to think of weeds, insects and diseases as separate and independent concerns in turfgrass management and, to a large extent, the conventional approach towards research and extension reflects this way of thinking. However, there is a great deal of interdependence among these components, even if the connections are not always obvious. This research highlights the connection and importance of insects in weed management through the combined interaction of the principles of resource competition and herbivory.

17.5.3 The principal of herbivory

While turfgrass supports a wide variety of living organisms, less than 1% of these organisms acquire pest or pathogen status requiring control (Klein *et al.*, 2007). Root-feeding white grubs, stem- and crown-feeding weevils and foliage- and stem-feeding caterpillars are pests on turfgrasses worldwide, but other groups have a more limited geographical distribution (Potter, 1998; Vittum *et al.*, 1999). Plant-parasitic nematodes and fungal pathogens attacking turfgrasses are also found worldwide, with specific species predominating in different regions. Herbivory by insects, nematodes and plant pathogens can cause significant damage to turfgrass lawns and the use of chemical pesticides is currently the most commonly used method of control (Waltschke *et al.*, 1995; Niemczyk and Shetlar, 2000). While populations of most pests and pathogens are kept under check for the most part by their predators, parasites, pathogens and antagonists, pest outbreaks and disease epidemics may occur occasionally.

Cultural practices (human intervention) such as mowing, fertilization and grass cultivars can strongly influence the type and abundance of pests and diseases in turfgrass. For example, Williamson and Potter (1997) reported that mowing could remove 75–90% of the eggs of black cutworm (*Agrotis ipsilon*) on creeping bentgrass (*Agrostis palustris*) putting greens. Seastedt (1985) reported that the total biomass of scarabaeid grubs more than doubled when tallgrass prairie was mowed. Similarly, maximum densities of white grubs occurred in pastures under intermediate levels of sheep grazing (Hutchinson and King, 1980). Mowing increases the energy allocation to foliage at the expense of roots, crowns and flowers (Ruess *et al.*, 1983), which typically results in reduced root carbohydrate reserves, a lower carbon:nitrogen ratio (Caldwell *et al.*, 1981; Ruess *et al.*, 1983) and an increased concentration of nitrogen in the roots, even though the total amount of nitrogen remains unchanged (Seastedt, 1985). Roots of grasses have an abundant food supply but one that is low in nitrogen compared with above-ground plant tissues (Brown and Gange, 1990). Because nitrogen availability is limiting to most herbivores (Mattson, 1980), elevated levels in roots promote increased growth rates and survival of below-ground herbivores (Seastedt, 1985; Seastedt *et al.*, 1985; Brown and Gange, 1990). Crutchfield *et al.* (1995) reported that grub feeding resulted in greater foliar yield reduction in turfgrass that received both nitrogen and high irrigation compared with one without. Potter *et al.* (1996) reported that masked chafer (*Cyclocephala lurida*) grubs were consistently smaller and less abundant in high-mown turf and turf that had been treated with aluminium sulfate to reduce soil pH. High mowing and the application of aluminium sulfate prior to beetle flights reduced the total biomass of white grubs in tall fescue by as much as 55 and 77%, respectively. Elevated cutting heights of turf

are typically associated with deeper rooting and an increased growth rate, diameter and total production of roots and rhizomes (Beard, 1973). Therefore, Potter *et al.* (1996) speculated that these changes might reduce the food quality for grubs by producing tougher, more fibrous root systems.

Davidson and Potter (1995) found that fall armyworms developed faster when reared on foliage from plants treated with medium or high rates of urea than on unfertilized tall fescue, but the development rates were not affected by the presence of endophytes. They also reported that greenbugs (*Schizaphis graminum*) preferred fertilized, endophyte-free fescue and that bird cherry-oat aphids (*Rhopalosiphum padi*) developed fastest on fertilized and endophyte-free tall fescue. The densities of leaf hoppers, flea beetles and Staphylinidae were generally higher in fertilized than in non-fertilized turf. Flea beetles and two of the five most abundant species of leaf hoppers were less numerous in endophyte-infected plots. Crutchfield and Potter (1995) found that dandelion and white clover were particularly poor hosts for Japanese beetle and masked chafer grubs, respectively. Both fed and grew on many other weed species.

Although insect resistance is rarely the deciding factor when establishing a new turfgrass lawn, genetic resistance among grass species and cultivars to particular leaf- and stem-feeding pests, especially within warm-season turfgrasses, has been identified (Reinert *et al.*, 2004). For example, Jackson *et al.* (1981) reported that Kentucky bluegrass (*Poa pratensis*), tall fescue (*Festuca arundinacea*) and chewings fescue (*Festuca rubra*) were susceptible to greenbugs, but creeping bentgrass (*Agrostis* spp.), Bermuda grass (*Cynodon dactylon*), perennial ryegrass and zoysia grass (*Zoysia* spp.) were not. Crutchfield and Potter (1994) reported that Japanese beetle grubs consistently preferred perennial ryegrass over other turfgrasses, while masked chafers showed no preference. Potter *et al.* (1992) reported that hard fescue (*Festuca ovina*), endophyte-free tall fescue and perennial ryegrass were more favourable than creeping bentgrass for the growth of masked chafers, whereas Kentucky bluegrass was a poor host for both Japanese beetles and masked chafers. Bughrara *et al.* (2003) found that tall fescue cultivars were more tolerant of European chafer (*Rhizotrogus majalis*) grubs than other grass species.

Presence of fungal endophytes in stems and leaf sheaths of cool-season grasses including tall fescue and perennial ryegrass can impart resistance against many herbivores. These endophytes produce an array of alkaloids that act as feeding deterrents (Johnson *et al.*, 1985) or are toxic to herbivorous insects and nematodes (Clay, 1988; Kimmons *et al.*, 1990; Dahlman *et al.*, 1991; Breen, 1994). Three classes of fungal metabolites, the indole diterpenes, the ergot alkaloids and peramine, have been implicated in insect resistance of endophyte-infected perennial ryegrass. Indole diterpenes identified in endophyte-infected perennial ryegrass are lolitrems A to E, lolitriol and paxilline. The lolitrems and paxilline are toxic to Argentine stem weevil (*Listronotus bonariensis*) (Gallagher and Hawkes, 1986). The ergot alkaloids can be classified into three structural classes: (i) the ergopeptine alkaloids; (ii) the clavine alkaloids such as argoclavine; and (iii) the lysergic acid derivatives. Ergopeptine alkaloids occur widely in endophyte-infected grasses, with some six ergopeptine alkaloids having been identified in perennial ryegrass. Ergopeptine alkaloids have been shown to affect the fall armyworm, Argentine stem weevil and Japanese beetle. Peramine, a cyclic dipeptide derived from proline and arginine, occurs widely in endophyte-infected grasses and seems to play an important role in the resistance of ryegrass to the Argentine stem weevil, sod webworm (*Fissicrambus mutabilis*) and greenbug aphid (Siegel *et al.*, 1990). Peramine functions solely as a feeding deterrent to insects. These endophytes also reduce the reproduction of plant-parasitic nematodes (West *et al.*, 1988; Kimmons *et al.*, 1990).

Insect, nematode and mite pests of lawns that can potentially be managed with use of endophytic grass cultivars are listed in Table 17.1. However, endophyte-based

Table 17.1. Major insect, mite and nematode pests of lawns in cool-season turf and promising biological control options (compiled from the literature and the author's unpublished data).

	Biological control options			
Species	Endophytes	Nematodes	Fungi	Bacteria
Insects				
Annual bluegrass weevils (*Hyperodes* spp.)		+[a,b]	+[c]	
Armyworm (*Pseudaletia unipuncta*)	+	+[a]		+[d]
Bluegrass billbug (*Sphenophorus parvulus*)	+	+[a,b]	+[c,e]	
Chinch bug (*Blissus leucopterus leucopterus*)	+		+[c]	
Hairy chinch bug (*Blissus leucopterus hirtus*)	+		+[c]	
Black cutworm (*Agrotis ipsilon*)	–	+[a]		–
Bronzed cutworm (*Nephelodes minians*)	–	+[a]		–
Fall armyworm (*Spodoptera frugiperda*)	+	+[a]		+[d]
Greenbug (*Schizaphis graminum* Rondani)	+			
Bluegrass webworm (*Parapediasia teterrellus*)	+	+[a]		+[d]
Cranberry girdler (*Chrysoteuchia topiaria*)	+	+[a]		+[d]
Larger sod webworm (*Pediasia trisecta*)	+	+[a]		+[d]
Vagabond crambus (*Agriphila vulgivagellus*)	+	+[a]		+[d]
Asiatic garden beetle (*Maladera castanea*)		+[b]		
Black turfgrass ataenius (*Ataenius spretulus*)		+[b]		
European chafer (*Rhizotrogus majalis*)		+[b]		
Green June beetle (*Cotinus nitida*)		+[b]		
Japanese beetle (*Popillia japonica*)		+[b]		+[f]
Phylophaga spp.		+[b]		
Northern masked chafer (*Cyclocephala borealis*)		+[b]		
Oriental beetle (*Anomala orientalis*)		+[b]		
Southern masked chafer (*Cyclocephala lurida*)		+[b]		
Mites				
Clover mite (*Bryobia praetiosa*)	+			
Winter grain mite (*Penthaleus major*)	+			
Plant nematodes				
Hoplolaimus galeatus		+[a]	+[g]	
Criconemoides spp.		+[a]	+[g]	
Tylenchorhynchus spp.	+		+[g]	
Helicotylenchus spp.	+		+[g]	

+, Control; –, no control; [a], *Steinernema carpocapsae*; [b], *Heterorhabdidis bacteriophora*; [c], *Beauvaria bassiana*; [d], *Bacillus thuringiensis kurstaki*; [e], affects only adults; [f], *Bacillus popillae*; [g], *Myrothecium verrucaria*.

pest resistance is not a panacea (Potter, 2005). Endophytes have not been found in Kentucky bluegrass, creeping bentgrass or warm-season turfgrasses, and attempts to transfer them into these grasses have been unsuccessful. Endophyte-based resistance can vary in response to seasonal and environmental factors (Kennedy and Bush, 1983; Breen, 1992, 1994; Salminen *et al.*, 2005) as well as to cultural practices including fertilization and mowing. Salminen *et al.* (2005) reported that the dry weight of fall armyworm feeding on endophyte-infected tall fescue or perennial ryegrass was significantly lower than for those feeding on uninfected grasses, but the presence of the endophyte had no effect on survival. They identified a four-alkaloid model consisting of a plant alkaloid, perloline and three fungal alkaloids, ergonovine, chanoclavine and ergocryptine, explaining 47% of the variation in fall armyworm weight when feeding on tall fescue. For perennial ryegrass, a three-alkaloid model consisting of a plant alkaloid, perloline methyl ether and two fungal alkaloids, ergonovine and ergocryptine, explained 70% of the variation in fall armyworm dry weight. The levels of these alkaloids most closely linked to armyworm

performance increased linearly or curvilinearly with increasing temperature.

Alkaloid concentrations in endophytic grasses can be manipulated by the application of nitrogen and phosphorus fertilizers (Marten *et al.*, 1974; Fribourg and Loveland, 1978; Lyons *et al.*, 1986, 1990; Arechavaleta *et al.*, 1992; Malinowski *et al.*, 1998). Arechavaleta *et al.* (1992) reported that the application of nitrogen increased the concentration of major ergopeptide alkaloids in tall fescue cultivar Kentucky 31. They demonstrated that the source of nitrogen was also important, as all concentrations of NO_3 increased the ergopeptide alkaloid content as opposed to NH_4^+, which was effective only at high concentrations (34 mg N per pot). Ergopeptide concentrations were the highest in drought-stressed plants grown at –0.5 MPa and fertilized at a moderate or high nitrogen rate. Malinowski *et al.* (1998) reported that ergot alkaloid accumulation in roots of tall fescue increased linearly with phosphorus availability in the soil; however, in the shoots, the alkaloid concentration increased with increasing phosphorus availability in the soil from 17 to 50 mg P/kg but declined at 96 mg P/kg. In a greenhouse study, Cheng and Grewal (2009) showed that organic fertilizers such as Cockadoodle Doo, Vigoro, Corn Gluten and Nature's Touch with enzymes increased the concentrations of most ergot alkaloids and perloline (a plant alkaloid) in shoots of endophyte-infected tall fescue compared with the most commonly used chemical fertilizer, Scotts Turf Builder.

The levels of ergot alkaloids in endophytic turfgrasses can also be manipulated by mowing height and mowing frequency. In a greenhouse study, Salminen and Grewal (2002) found that the major ergot alkaloids including ergonovine, ergocristine and ergocriptine significantly increased in tall fescue with increased mowing height from 2.5 to 7.5 cm. In another greenhouse experiment, Salminen *et al.* (2003) tested the effect of two mowing frequencies, once a week (weekly) or once every 2 weeks (biweekly) on ergot alkaloids. They found that decreased mowing frequency caused a fivefold increase in the amount of ergovaline and a 2.6-fold increase in ergonovine. They also found that as the concentrations of ergonovine in the shoots increased due to less frequent mowing, the dry weight of the fall armyworm decreased. A 2-year field study was conducted to assess the influence of endophyte infection and mowing regime (mowing height and frequency) on weed cover and ergot alkaloid levels in tall fescue and perennial ryegrass (A. Alumai, S.O. Salminen, D.S. Richmond, J. Cardina and P.S. Grewal, unpublished data). In both grass species, endophyte-infected grass plots had significantly lower weed cover than endophyte-free grass plots, and plots mowed at 8.9 cm height had lower weed cover than plots mowed at 5.0 cm. Mowing frequency (weekly or biweekly) had no significant influence on weed cover in plots of either grass species. Five ergot alkaloids – chanoclavine, ergocristine, ergocryptine, ergonovine and ergovaline – were extracted in both endophyte-infected grass species, the levels of which varied significantly with mowing regime. In tall fescue, plots mowed at 5.0 cm had significantly lower chanoclavine and ergonovine levels than plots mowed at 8.9 cm, and more frequent mowing resulted in lower levels of chanoclavine, ergocristine, ergocryptine and ergonovine regardless of mowing height. In perennial ryegrass, chanoclavine, ergocryptine, ergonovine and ergovaline levels were significantly lower at 5.0 cm than at 8.9 cm, and more frequent mowing resulted in significantly lower levels of chanoclavine and ergonovine.

The above studies illustrate that cultural decisions including endophyte infection levels and mowing regimes may play an important role in the management of weed and insect species in turfgrass lawns. In the north-eastern USA and regions with similar climates, the use of endophyte-infected turfgrasses mowed at 8.9 cm once a week should provide a sustainable, low-maintenance turfgrass lawn. As endophytes are present in the seed (Neil, 1940, 1941), new lawns containing endophytic grass species can easily be established, but the total renovation of existing lawns can be expensive and sometimes impractical.

Richmond *et al.* (2000) demonstrated that once overseeded into existing stands of bluegrass, endophyte-infected ryegrass can establish and its proportion of the stand increases over time, providing effective control of the bluegrass billbug (*Sphenophorus parvulus*). They found that the proportion of the endophytic ryegrass plants relative to uninfected plants necessary to achieve acceptable levels of bluegrass billbug and sod webworm control was about 30%. Overseeding, therefore, can provide a cost-effective means of using endophyte-infected cultivars in existing turfgrass lawns.

17.5.4 The principle of predation and parasitism

Predatory beetles, parasitic wasps, entomopathogenic nematodes (EPNs), and fungal and bacterial pathogens such as milky disease (*Bacillus popillae*) contribute to natural insect mortality and can be carefully manipulated to play a greater role in suppressing herbivore populations. Cockfield and Potter (1984a) found nine species of Lycosidae and over 40 species of staphylinids on Kentucky bluegrass and tall fescue lawns, but more species of Carabidae were collected from bluegrass than tall fescue. Lopez and Potter (2000) showed that the ant *Lasius niger* preyed heavily on black cutworm eggs and first instars and on Japanese beetle eggs, and the predation was lower in golfcourse fairways than in roughs. Fewer grubs were found in areas where ants were abundant than where ants had been controlled by insecticides. It has been reported that natural below-ground biocontrol activity in urban vacant lots and gardens in Ohio varied between 51 and 98%, with major contributions by ants and microbial pathogens and to a lesser extent by EPNs (Yadav *et al.*, 2012). Ants showed higher predatory activity in vacant lots (60 ± 33.4%) than in urban gardens (33.3 ± 22.2%) whereas microbial pathogens exhibited higher activity in urban gardens (27.8 ± 15%) than vacant lots (8.3 ± 16.7%). Thus, predation by indigenous ants, microbial pathogens and EPNs could provide an important buffer against pest outbreaks in urban lawns.

Cultural practices (human intervention) can either enhance or decrease predation and parasitism on herbivores in turfgrass lawns. Black turfgrass ataenius (*Ataenius spretulus*) grubs have been found to be three- to tenfold more abundant in fairways than in adjacent roughs (Smitley *et al.*, 1998; Rothwell and Smitley, 1999), and Smitley and Rothwell (2003) found a higher incidence of *Paennibacillus* sp. infection of black turfgass ataenius larvae in the rough (47.4%) compared with fairway (26.4%). Rogers and Potter (2004) showed that plantings of the nectar-producing *Paeonia lactifolia* established in a stand of turf significantly increased Japanese beetle parasitism by the predatory wasp *Tiphia vernalis*. Most carabids and styphalinids consumed immature black turfgrass ataenius. *Harpalus affinis* adult beetles and *Philonthus* sp. larvae consumed 100% of the available ataenius grubs (Jo and Smitley, 2003). Cockfield and Potter (1985) reported that tall fescue supported fewer predators than Kentucky bluegrass, specifically the females of Erigonidae, Linyphiidae and Carabidae, but populations of Erigonidae, Linyphiidae and Carabidae were lower in high-maintenance bluegrass lawns under commercial lawn care. Arnold and Potter (1987) found that trap catches of predacious arthropods, specifically Araneae, Staphylinidae and Carabidae, were significantly reduced by insecticides, particularly by late summer soil treatments with diazinon. Cockfield and Potter (1983) reported that chlorpyrifos, bendicarb, trichlorfon and isofenphos had the greatest negative effect on predacious arthropods with some taxa reduced for up to 6 weeks, and Cockfield and Potter (1984b) found that chlorpyrifos application reduced predation on sod webworm eggs. Terry *et al.* (1993) reported that a single surface application of isazofos in mid-June caused significant short-term reductions in the abundance of spiders, ants, staphylinids, carabid larvae, histerids and other predominantly predatory arthropods. More significantly, they noted that the pupae of

fall armyworm and the eggs of Japanese beetle implanted into treated or control plots 1–2 weeks after treatment sustained predation losses as high as 60 and 74%, respectively, within 48 h. Predation on Japanese beetle eggs was reduced by as much as 70% in plots that had been treated previously with isozofos or carbaryl. Kunkel *et al.* (1999) found that pitfall trap capture of predatory coleopteran larvae and hister beetles was reduced by imidacloprid and bendiocarb, and Kunkel *et al.* (2001) demonstrated that topical ordietary exposure of the carabid *Harpalus pennsylvanicus* to bendiocarb was lethal. Exposure to imidacloprid caused a high incidence of sublethal neurotoxic effects including paralysis, impaired walking and excessive grooming. Intoxicated beetles were highly vulnerable to intraguild predation by ants. The natural rates of Japanese beetle parasitism by *T. vernalis* on a golf course rough were significantly lower in plots treated with full or half label rates of imidacloprid in early May compared with untreated turf (Rogers and Potter, 2003).

EPNs of the species *Heterorhabditis* and *Steinernema*, the entomopathogenic bacteria *Bacillus thuringiensis* and *B. popillae* (milky disease), and the fungal pathogens *Beauveria bassiana* and *Myrothecium verrucaria* can also be applied innundatively to control lawn insect pests (Warren and Potter, 1983; Grewal and Georgis, 1998; Grewal, 1999; Grewal *et al.*, 2005b; Potter, 2005) (Table 17.1). *B. thuringiensis* serovar *japonensis* strain Buibui has shown particular promise for the control of white grubs (Bixby *et al.*, 2007; Mashtoly *et al.*, 2009). EPNs are viable alternatives to chemical insecticides and are well suited for IPM programmes, particularly for soil pests (Grewal *et al.*, 2005a). While most insect pests are susceptible to EPNs, non-targets are unaffected (Georgis *et al.*, 1991). Although initial studies provided mixed results, control of white grubs with EPNs has improved in the last 20 years due to the improved matching of nematode host-finding behaviour with the insect biology (Gaugler *et al.*, 1997) and the discovery of more effective nematode strains (Grewal *et al.*, 2002, 2004; Koppenhöfer and Fuzy, 2003). New nematode strains such as *Heterorhabditis zealandica* X1 and *Heterorhabditis bacteriophora* GPS11 applied as rescue treatments are significantly more effective than previously studied species and strains, and provide control of both the Japanese beetle and the northern masked chafer (*Cyclocephala borealis*) that is equivalent to or better than the most widely used curative insecticide, trichlorfon (Grewal *et al.*, 2004; Power *et al.*, 2009).

Although EPNs are most frequently applied as inundative biological control agents with a relatively short-term effect, nematode populations can be established to provide longer-term 'foundational' control. Successful inoculative releases of EPNs have occurred in Florida, Ohio and New Jersey in the USA and in Germany (Barbercheck and Hoy, 2005). For instance, Klein and Georgis (1992) reported 84% control of Japanese beetle larvae in spring in Ohio following single applications of *Steinernema carpocapsae* and *H. bacteriophora* in the previous autumn. In another inoculative release effort, the mole cricket nematode *Steinernema scapterisci* was released into pastures at several sites in Florida during the summer of 1985. Based on evaluation of field-collected mole crickets over a 5-year period, the nematodes were confirmed to have become established at all the release sites (Parkman *et al.*, 1993). EPNs applied in the spring not only provide control of overwintered white grubs in the spring but also provide suppression of the billbug in late spring and summer and of the next-generation white grubs in the autumn due to their recycling in the target hosts (P.S. Grewal and K.T. Power, unpublished data). Therefore, EPNs can be used as a foundational pest control in which they are inoculated to establish in the soil to provide more sustained pest control. Such an approach should be more cost-effective due to less frequent application of nematodes and insecticides. In a survey of golf courses in Ohio, Alumai *et al.* (2006) recovered EPNs from 43% of the fairways

and 57% of the rough areas but none from the greens. They concluded that EPNs are more likely to occur in less intensively managed sites that receive fewer chemical inputs and have relatively high sand content and moderate silt, organic matter, phosphorus and magnesium content. Such studies can facilitate the development of conservation approaches for the use of EPNs.

An intriguing interaction between EPNs and plant-parasitic nematodes suggests that EPN applications can suppress plant-parasitic nematode populations (Smitley *et al.*, 1992; Grewal *et al.*, 1997). Single applications of *S. carpocapsae* or *Steinernema riobrave* at a rate of 1 billion infective juveniles/acre reduced the soil populations of the sting (*Belonolaimus longicaudatus*), ring (*Criconemella* sp.) and root-knot nematode (*Meloidogyne* sp.) by 90–95% in turfgrass (Grewal *et al.*, 1997). This suppression also lasted more than 8 weeks after treatment. Further research in this area has revealed that EPNs and their symbiotic bacteria produce several effects on plant-parasitic nematodes including repellency, mortality and reduction in egg hatch and fecundity (Grewal *et al.*, 1999). A recent study suggested that EPN applications to the soil can induce general plant defences, which may contribute to resistance against plant-parasitic nematodes (Jagdale *et al.*, 2009). Harnessed properly, such unexpected ecological interactions (ecological 'surprises') may provide sustainable solutions to the most difficult pest problem, the plant-parasitic nematodes.

There is potential for both synergistic and antagonistic interactions between EPNs and grass endophytes as mortality factors of herbivorous insects. Studies show that Japanese beetle grubs fed on the roots of endophyte-infected tall fescue, fine fescue and perennial ryegrass are more susceptible to *H. bacteriophora* than those fed on the roots of non-infected grasses (Grewal *et al.*, 1995). Although the fungus and its alkaloids are concentrated in above-ground plant tissues, as much as 15% of the lolines and smaller amounts of ergot alkaloids may occur in tall fescue roots (Siegal *et al.*, 1987; Potter *et al.*, 1992; Justus *et al.*, 1997). Loline alkaloids extracted from *Neotyphodium*-infected tall fescue deterred feeding by third-instar Japanese beetle grubs on agar-based medium (Patterson *et al.*, 1991; Grewal *et al.*, 1995). Reduced survival and weight gain of white grubs in infected grasses in some field trials has also been observed (Murphy *et al.*, 1993; Davidson & Potter, 1995). Reductions in the populations of plant nematodes in the soil surrounding the infected plants (Pederson *et al.*, 1988; West *et al.*, 1988; Kimmons *et al.*, 1990) suggest that deterrents may either be exuded from the roots (Latch, 1993) or reach the soil through the fallen leaf litter. Kunkel and Grewal (2003) found that fall armyworm larvae feeding on endophyte-infected plants were significantly less susceptible to EPNs and that this reduction in susceptibility stemmed from the deleterious effects of endophyte-mediated alkaloids on the nematode's symbiotic bacteria released inside the insect haemocoel (Kunkel *et al.*, 2004; Richmond *et al.*, 2004b). Thus, these cascading lethal and sublethal effects of endophytes on below-ground herbivores and predatory and parasitic organisms can have both negative and positive outcomes. However, if carefully manipulated, endophyte-mediated interactions may lead to enhanced sustainability of biological control services provided by the natural enemies in urban lawns.

Bottom-up (endophyte-mediated) and top-down (EPN-mediated) control of insect herbivore populations can also influence competitive interactions between turf grass and weeds. Selective below-ground herbivory by white grubs on grass roots can provide competitive advantage to dandelions (D.S. Richmond and P.S. Grewal, unpublished data) against turfgrass. However, increased susceptibility of white grubs feeding on the roots of endophytic turfgrasses to EPNs can restore the competitive ability of tufgrass plants against dandelions. Although there is a significant body of literature dealing with the issue of endophyte-mediated herbivore defence and plant community dynamics, the ability of natural enemies to regulate populations of

herbivorous insects has not been considered simultaneously. As a result, the relative strength of bottom-up (endophyte-mediated changes in plant quality) versus top-down (natural enemies of phytophagous insects) forces has not been appreciated with respect to their impact on plant competition. These studies document the combined impact of these forces on herbivorous insects with respect to their indirect effects on plant community ecology.

17.5.5 The principle of biogeochemical cycling

All ecological interactions in an ecosystem have two consequences: (i) a one-way flow of energy through autotrophs (usually photosynthetic organisms) to heterotrophs, which eat either autotrophs or other heterotrophs; and (ii) a cycling of materials, which move from the abiotic environment through the bodies of living organisms and back to the abiotic environment. This movement of inorganic substances is referred to as biogeochemical cycling as it involves geological as well as biological components of the ecosystem. The geological components are: (i) the atmosphere, which is made up largely of gasses, including water vapour; (ii) the solid crust of the earth; and (iii) the oceans, lakes and rivers, which cover three-quarters of the Earth's surface. The biological components of the biogeochemical cycles include the producers (e.g. plants), consumers and detritivores. As a result of the metabolic activities of the detritivores, inorganic substances are released from organic compounds and returned to the soil, water or air, where they get taken up again by the primary producers, repeating the cycle. For example, NH_4 is formed from nitrogen gas and water by ammonification, and is converted to NO_2 or NO_3 by bacteria via nitrification and then converted back to nitrogen gas via denitrification. Plants can take up NH_4 or NO_3 to form amino acids and proteins, which are again broken down to nitrogen by microorganisms.

Soil is the foundation of all plant life in terrestrial ecosystems. The top 6–15 cm layer of organically rich soil provides most of the essential nutrients for plants and is a home to detritivores that take part in biogeochemical cycling and other ecological processes such as predation, parasitism and antagonism. Urban soil is, however, highly disturbed. Craul (1992) defined urban soil as 'a soil material having a non-agricultural, manmade surface layer more than 50 cm thick that has been produced by mixing, filling or by contamination of land surfaces in urban and suburban areas'. During the process of urbanization, native vegetation covering the soil is removed and the topsoil is scraped off and mixed, thus destroying its structure and altering its chemical composition. Use of heavy building equipment causes compaction of soil, which results in the loss of pore space, hindering the movement of water, air and organisms. Early in the 20th century, homeowners were advised to grow cover crops for one or more seasons to improve soil tilth and nutrients before seeding turfgrass (Barron, 1923). Current recommendations include amending the soil prior to seeding with organic materials (Pound and Street, 1991; McCoy, 1998). Manures and other organic agricultural wastes are sometimes used for this purpose in field soils (Larney and Janzen, 1996), but transportation costs and the availability of these materials limit their use in urban settings. Urban sources of organic matter such as sewage sludge and yard waste are composted and used as soil conditioners. Incorporation of composted urban waste reduces bulk density and increases the moisture-holding capacity (Darmody *et al.*, 1983; Hornick and Parr, 1987), improves aggregate stability and cation exchange capacity (Aggelides and Londra, 1999) and increases the depth of the topsoil (Darmody *et al.*, 1983). The effects of various composted materials on soil fertility and plant nutrition have been documented in several studies (Darmody *et al.*, 1983; O'Keefe *et al.*, 1986; Sikora and Yakovchenko, 1996; Pascual *et al.*, 1997; Cheng and Grewal, 2009).

Composted materials and humic substances have long been used by the turfgrass

industry as soil conditioners and organic fertilizers (Piper and Oakley, 1917, 1921; Connellan, 1921). Until the 1930s, composted organic amendments served as one of the principle sources of fertilizer used on golf courses (Piper and Oakley, 1921; Welton, 1930). However, the utilization of composts declined dramatically with the advent of synthetic, urea [$(NH_2)_2CO$]-based fertilizers and organic fertilizers such as Milorganite, which offered more consistent and predictable nutrient release characteristics (Westover, 1927). The recent resurgence in the utilization of composted materials has been fuelled by an increase in the diversion of solid wastes from surface waters and landfills and a desire by the public to develop more environmentally sound waste-utilization methods (Sims, 1990; Barkdoll and Norstedt, 1991; He *et al.*, 1992; Schumann *et al.*, 1993).

Much of the research on compost utilization by the turfgrass industry has focused on: (i) the suppression of diseases (Lumsden *et al.*, 1983; Nelson and Craft, 1992; Liu *et al.*, 1995; Craft and Nelson, 1996; Nelson, 1996; Landschoot and McNitt, 1997; Boutler *et al.*, 1999; Garling *et al.*, 1999); (ii) the potential for reducing fungicide use (Skorulski, 1990; Garling, 2000); (iii) the effects of composts on physical and chemical properties of soils (Sims, 1990; He *et al.*, 1992; Giusquiani *et al.*, 1995; Logan and Harrison, 1995; Pagliai and Antisari, 1993); and (iv) improvements in fertility in high-cut turfgrasses (Markland *et al.*, 1969; Sikora *et al.*, 1980; Tester *et al.*, 1982; Landschoot and Waddington, 1987; Tester, 1989; Schumann *et al.*, 1993; Norrie and Gosselin, 1996). Schumann *et al.* (1993) observed enhanced colour and growth of tall fescue and a Kentucky bluegrass and perennial ryegrass mixture for up to 32 days following the application of composted biosolids. In both greenhouse and field studies, applications of composted biosolids at increasing rates resulted in a linear increase in tall fescue clipping yields and foliar nitrogen concentrations (Sikora *et al.*, 1980; Tester *et al.*, 1982; Tester, 1989). Two separate studies evaluating one source of composted biosolids (Landschoot and Waddington, 1987) and two composted paper-mill sludges (Norrie and Gosselin, 1996) applied to establish high-cut turfgrass found the products to be ineffective as fertilizers in the absence of supplemental fertilizer applications. An elevated C:N ratio and low nutrient levels in the composted paper-mill sludges contributed to foliar nitrogen concentrations below recommended levels unless the turf was treated with additional applications of fertilizer (Norrie and Gosselin, 1996).

In recent years, several organic fertilizers have become available in the market for use in turfgrass lawns. Cheng *et al.* (2010) compared the effect of 11 organic fertilizers, each applied at the manufacturer's recommended rate, on quality (an indication of greening effect), shoot and root growth, and shoot nutrient content (an indication of nutrient uptake) and alkaloid content (an indication of insect resistance) in endophytic (infected with the fungus *Neotyphodium coenophialum*) tall fescue in the greenhouse. They measured turfgrass quality weekly on a scale of 1–9 (9 being the best), shoot and root growth monthly, and shoot contents of macro- and micronutrients and of various alkaloids at the end of 4 months. The results indicated that Corn Gluten and Cockadoodle Doo generated the highest turfgrass quality and shoot growth, but Nature's Touch with enzymes enhanced root growth. Compared with the chemical fertilizer, Scotts Turf Builder, the organic fertilizers Cockadoodle Doo, Corn Gluten and Nature's Touch with enzymes generally resulted in better turf quality and higher shoot growth. Although Cockadoodle Doo, Vigoro and Scotts Turf Builder resulted in higher macronutrient contents in turfgrass shoots, there was no correlation between the nutrient contents in the fertilizers and the nutrient contents in the shoot 4 months after application. Significant differences were found for all measured alkaloids among the 13 treatments, but these differences varied with fertilizer. Overall, organic fertilizers produced higher turfgrass quality, growth and insect resistance capacity (alkaloid content) compared with the standard chemical fertilizer, Scotts Turf

Builder. Among the organic fertilizers tested, turf quality, growth, and shoot nutrient and alkaloid contents provide complementary support for the overall superiority of Cockadoodle Doo, Vigoro, Corn Gluten and Nature's Touch with enzymes. Corn Gluten also appears to be an effective pre-emergence herbicide with potential for use in lawn care (Bingaman and Christians, 1995; McDade and Christians, 2000; Alumai *et al.*, 2009a). The application of certain organic fertilizers containing activated stabilized sewage sludge or rendered animal by-products may increase populations of root-feeding black turfgrass ataenius grubs (Potter *et al.*, 2005).

Despite huge initial disturbances, soils under turfgrass lawns support a diverse community of microbes and non-pest invertebrates. The bacterial population in moist litter, grass clippings and thatch of a lawn is commonly in the order of 10^9 organism/cm^2 of litter surface (Clark and Paul, 1970). A New Jersey Kentucky bluegrass/red fescue lawn was found to support 83 invertebrate taxa including insects, mites, nematodes, annelids and gastropods (Streu, 1973). Dozens of beneficial species of rove beetles, ground beetles, ants, spiders, collembolans and earthworms are found in lawn soils (Potter, 1998). The average microbial biomass pool in grasslands has been estimated at 1090 kg C/ha compared with only 700 kg in arable crops and 850 kg in forest systems (Smith and Paul, 1990). In Ohio, microbial biomass in lawn soils exceeded 1140 kg C/ha in late spring (M. Singh and P.S. Grewal, unpublished data). The total number of nematodes per hectare in the top 15 cm of dry soil under turfgrass lawns in Ohio has been estimated at 5.4×10^{10} compared with 4.3×10^{10} in croplands (S.S. Briar and P.S. Grewal, unpublished data). In a study of the energetics of a suburban lawn in Walnut Creek, California, Falk (1976) found the lawn system to be extremely productive with a net productivity of 1020 g/m^2/year compared with maize fields with productivity of 1066 g/m^2/year and exceeding tall grass prairie with an annual productivity of 1000 g/m^2/year. Food utilization per unit area by suburban birds considerably exceeded native grassland bird utilization (46 versus 1.01–2.33 Kcal/m^2/year). Man was the dominant consumer accounting for 10% of the herbivory and nearly 100% of the scavenging. Energy inputs (labour, gasoline, fertilizer, etc.) amounted to 578 Kcal/m^2/year, equalling or exceeding maize production for a comparable net productivity.

Soil food webs in urban soils also appear to be relatively enriched but moderately structured. Briar *et al.* (2007) compared various nematode community indices across croplands, grassy borders along croplands, turfgrass lawns, shrublands and forests, and found all habitats to have moderately enriched food webs (with an enrichment index of 69–78%). Forests, shrublands and turfgrass lawns had high maturity and structure indices, whereas grassy borders had intermediate levels and croplands had the lowest maturity and structure values. Ordination of habitats using nematode indices revealed that grassy borders and turfgrass lawns hold an intermediary position between undisturbed habitats (forests and shrublands) and highly disturbed habitats (croplands). Cheng and Grewal (2009) assessed the structure, dynamics and recovery of the soil nematode food web in tall fescue lawns initiated on the four soil matrices commonly encountered in urbanized areas: topsoil, subsoil, compost-amended topsoil or compost-amended subsoil, and managed under three rates of nitrogen fertilizer application. The total number of nematodes was higher in topsoil than in subsoil, and was also higher in plots with compost amendment than in those without. Overall, nematode food webs in subsoil were poorly enriched and poorly structured but were highly enriched and poorly structured in subsoil with compost. Nematode food webs were highly enriched and poorly to moderately structured in both topsoil and topsoil plus compost treatments. Nematode food webs under all four soil treatments showed a tendency to converge over time. However, no significant effect of nitrogen fertilization was identified on nematode population or community

assembly. Initial levels of calcium, phosphorus, potassium, total nitrogen, NO_3 nitrogen, total carbon and soil organic matter were higher in topsoil than subsoil, and were increased by compost amendment in both substrates. The differences in nutrient pools were maintained throughout the study period of 1 year. Nitrogen fertilization generally increased soil NH_4 and NO_3 nitrogen pools. In addition, during the first 2 months after seeding, topsoil had lower turfgrass cover and higher weed cover than subsoil. After 1 year, turfgrass quality was higher in topsoil than in subsoil and higher following application of high nitrogen levels compared with low and no nitrogen application.

Soil chemical properties can vary with distance to roads and age of urban development within urban areas, and can influence and be influenced by the soil food web (Park *et al.*, 2010a,b). Park *et al.* (2010b) reported that key soil chemical properties can vary in predictable ways with urban age and distance to roads. They identified urban boundaries from the 1920s (old), 1960s (middle) and 2000s (new) for three cities in north-east Ohio (Massillon, Wooster and Canton) and characterized variation in soil chemical properties with length of urbanization period and distance to roads. They observed that two notable spatio-temporal patterns appeared repeatedly in the data set. First, total carbon, total nitrogen and soil organic matter were higher in the soils of old (>50 years) urban sites than in those of newly developed sites. Similar, but not always significant, trends in soil pH and exchangeable calcium were also observed. Secondly, road-side soils had higher pH, calcium and total carbon and nitrogen than interior sites, regardless of urban age. While attempting to determine correlations between the observed soil chemical properties and the soil nematode community, Park *et al.* (2010a) found that most soil nematode variables varied with urban age and season but not with distance from the road. Abundance and genus-level richness of nematodes were greater in old urban sites (50 and 100 years) than those in newly established sites (~10 years). This pattern was particularly significant with the colonizer–persister 2 class nematodes. However, nematode maturity, enrichment and structure indices did not differ with urban age. In October, overall nematode abundance increased, but the structure of the nematode food web degraded, as indicated by a decrease in maturity and structure index values. Correlation analyses revealed that soil nematode abundance variables were positively correlated with soil chemical properties along with urban age but not with distance from the road. The lack of correlation between soil nematode and chemical variables in the road-side soils suggested suppression of soil processes such as decomposition and nutrient cycling in the urban road-side soils. They concluded that the abundance and diversity indices of soil nematodes were particularly useful for detecting differences in soil chemistry and assessing the potential suppression of soil processes in urban areas.

In another study, it was found that modification of soil substrate composition could significantly alter the abundance, diversity and development of nitrogen-fixing microbes (S.J. Park, B.B. McSpadden Gardener and P.S. Grewal, unpublished data). The nitrogen-fixing bacteria were monitored using a marker gene, *nifH*. Prior to planting turfgrass, the abundance of *nifH* quantified by quantitative PCR was significantly greater in topsoil treatments than in the subsoil ($P < 0.001$). However, just 2 months after planting, no significant differences in *nifH* abundance were detected between the topsoil and subsoil plots. The results indicated that *nifH* abundance increased over time in exposed subsoil. Over a 1-year period, *nifH* abundance increased in both compost-amended topsoil and subsoil. The compost amendment increased *nifH* abundance most profoundly in the low-carbon subsoil. Analysis of the four *nifH* clone libraries constructed from the soil samples taken 2 months after planting revealed that the composition of *nifH* differed significantly in all the treatments. These data indicated that modification of soil substrate associated

with urbanization significantly altered the abundance and diversity of *nifH*-containing microbes in urban soils.

Common cultural practices (human intervention) can have either positive or negative effects on the structure and function of the soil food web. Potter *et al.* (1985) reported that earthworm density and biomass decline as nitrogen fertilization rates increase, probably due to the increase in soil acidification. They also noted that Collembola were more abundant at intermediate fertilizer rates whereas Acaridae were unaffected by nitrogen fertilization. Potter *et al.* (1990) reported that a single application of the fungicide benomyl or the insectide ethoprop, carbaryl or bendiocarb at label rates could reduce earthworm populations by 60–99% with significant effects lasting for at least 20 weeks. Other insecticides such as diazinon, isofenphos, trichlorfon, chlorpyrifos and isozophos caused less severe but still significant earthworm mortality. They also reported that the abundance of Cryptostigmata, Collembola and ants was drastically reduced in some treatments. Somasekhar *et al.* (2002) reported that a single application of the EPN *H. bacteriophora* GPS11, *H. bacteriophora* HP88 or *Heterorhabditis indica* LN2 significantly reduced the abundance, species richness, diversity and maturity of the soil nematode community by reducing the number of genera and abundance of plant-parasitic nematodes but not that of free-living nematodes. In contrast, trichlorfon reduced the number of genera and abundance and diversity of the nematode community by directly affecting both plant-parasitic and free-living nematodes. Thus, this non-target effect of EPNs can be considered beneficial in urban and agroecosystems where populations of plant-parasitic nematodes can limit crop yields.

Cheng *et al.* (2008b) reported that the use of four- or five-step lawn-care programmes had no significant effect on total soil nematode population and nematode community indices across lawn-care programmes, indicating no differences in net ecosystem productivity. However, professional and 'do-it-yourself' programmes negatively affected microbial biomass and soil organic matter pools in turfgrass soil. They concluded that the soil food web in turfgrass lawns represents a disturbed food web compared with natural grasslands and forest ecosystems, irrespective of the lawn-care programme used. In long-term (15-year) field-plot experiments, Cheng *et al.* (2008a) showed that, although nematode populations and food web indices were not affected by nine different turfgrass management regimes, microbial biomass nitrogen and soil organic matter were affected. Further group analysis revealed that nematode community indices (maturity index and combined maturity index) were significantly lower while the enrichment index was significantly higher under high (223 kg N/ha/year) and medium (171 kg N/ha/year) nitrogen input compared with a low nitrogen input (98 kg N/ha/year) management group, indicating disturbance of the nematode food web. In addition, organic fertilizer-based turf management resulted in significantly higher soil microbial biomass compared with mineral fertilizer management or control conditions, but no differences were found in the nematode community between the two fertilizer types. Herbicide, insecticide or fungicide applications had no significant negative effect on the soil nematode community, microbial biomass or soil organic matter. They concluded that the amount of nitrogen fertilizer influences the soil nematode food web and nutrient pools in turfgrass while pesticides have no effect. They also concluded that, irrespective of the management regime, the soil nematode food webs under turfgrass are more enriched but less structured than those under natural grassland ecosystems, indicating an overall food web disturbance.

Turfgrass lawns have the potential to sequester atmospheric carbon, and their proper management can increase soil organic carbon (SOC) pools. Heckman *et al.* (2000) demonstrated that the return of clippings following mowing compared with clippings removal was associated with fewer weeds and a darker green, more

luxuriant turf. They also reported that the benefits of clippings return were achieved using half (97.6 kg/ha/year) as much nitrogen as is typically applied (195.2 kg/ha/year) for high-maintenance turf. Kopp and Guillard (2002) demonstrated that returning clippings increased clippings dry matter yields by 30–72%, total nitrogen uptake by 48–60%, nitrogen recovery by 62% and nitrogen use efficiency by 52–71%. They also noted that returning clippings did not reduce turfgrass quality, and improved it in some plots. They concluded that nitrogen fertilization rates could be reduced 50% or more without decreasing turfgrass quality when clippings were returned. Qian *et al.* (2003) reported that returning clippings can reduce nitrogen requirements by 25% from 1 to 10 years after turf establishment, by 33% from 11 to 25 years after turf establishment, by 50% from 25 to 50 years after establishment and 60% thereafter. Using the CENTURY ecosystem model, they predicted that, compared with clipping-removal management, returning clippings for 10–50 years would increase soil carbon sequestration by 11–25% and nitrogen sequestration by 12–28% under a high-nitrogen fertilization regime (150 kg N/ha/year), and increase soil carbon sequestration by 11–59% and nitrogen sequestration by 14–78% under a low-nitrogen fertilizer (75 kg N/ha/year) regime. Using the BIOME-BGC ecosystem model, Milesi *et al.* (2005) modelled the growth of warm-season and cool-season turfgrass at a number of sites in the 48 conterminous US states under different management scenarios, simulating potential carbon and water fluxes as if the entire turf surface was managed like a well-maintained lawn. They found that well-watered and fertilized turf grasses act as a carbon sink, storing up to 17 Tg C/year, but at the cost of 695–900 l of water per person/day.) In another study, the effect was determined of long-term (15 years) application of nine treatments varying in the rate, type and amount of fertilizer, and pesticide applications in Kentucky bluegrass lawn experimental plots established at TruGreen Technical Center in Delaware, Ohio, on: (i) SOC and total soil nitrogen concentrations and pools; and (ii) turfgrass quality, biomass and plant cover and their relation with SOC pools (M. Singh and P.S. Grewal, unpublished data). An average of 26.1 Mg C/ha SOC was present in the soil and the SOC pool was not significantly different between treatments for the top 12 cm depth. In the 0–3 cm depth soil, the control and low-nitrogen (98 kg fertilizer/ha/year) treatments had a significantly lower SOC pool at 7.3 Mg C/ha compared with other treatments (nitrogen fertilizer = 171–245 kg/ha/year), which had an average of 8.3 Mg C/ha. In the 3–6 cm depth soil, the SOC pool was lowest in the low-nitrogen fertilizer application at 5.6 Mg C/ha and greater than 6.5 Mg C/ha in all other treatments. These results showed that the SOC pool in the top 12 cm of the soil in turfgrass systems can be influenced by the amount of fertilizer nitrogen and plant cover. Although weeds can reduce turfgrass quality, they increase the total biomass returned to the soil and increase the amount of carbon sequestered in the soil. We quantified the carbon emissions associated with nine different turf management programmes and determined the sustainability of lawns by comparing the data with previously collected SOC data for the same set of programmes. The sustainability index (SI) was calculated as the changes in the gain of carbon sequestered in turfgrass soil for environment benefit compared with the loss of carbon, which results in environmental degradation. The SIs assessed by calculating ratios of gross and net carbon sequestered to carbon emitted were >1 for all programmes, indicating sustainable management of turfgrass lawns for carbon. However, comparing the nine programmes after 15 years, the SI of control and organic treatments was at least fivefold higher than mineral fertilizer treatments. Greater sustainability of urban lawns for carbon can be achieved by reducing or replacing the use of mineral fertilizers with organic fertilizers, returning the clippings and by a progressive decrease in the amount of fertilizer applied with the age of the lawn.

Runoff from urban lawns can carry nutrients and pesticides into storm sewers, which eventually end up in streams and lakes (disservices). A study on nitrogen input from residential lawn-care practices in suburban watersheds in Baltimore County, Maryland, concluded that the annual input of nitrogen from fertilizer is a major component of the urban watershed nitrogen budget and is both spatially and temporally variable (Law *et al.*, 2004). They estimated a mean fertilizer application rate of 97.6 kg N/ha/year with a standard deviation of 88.3 kg N/ha/year. This analysis also indicated that the fertilizer application rate is affected by socio-economic factors and soil characteristics, which include the market value of the house, age of development, soil bulk density and soil nitrogen content. Overmyer *et al.* (2005) reported that the impact of lawn-care pesticides on aquatic systems was directly related to the property values of the homes. Branham *et al.* (2004) reported that runoff occurring at 24–72 h after pesticide application was considerably reduced versus runoff that occurred within 12 h of application. They also found that clippings harvested immediately after pesticide application contained a significant quantity of pesticides. A study by Cheng *et al.* (2008c) indicated that runoff from lawns can differ depending on the soil conditions during lawn establishment. They reported that runoff initiation time was significantly shorter in turfgrass plots established on subsoil (when topsoil is scraped off during urban development exposing subsoil to the surface) compared with plots established on topsoil. The total runoff volume was significantly larger in subsoil plots than in topsoil plots, and the sediment loss was significantly larger in subsoil plots than in topsoil plots. Although the nutrient losses (nitrogen, phosphorus, potassium and sulfur) in runoff from turfgrass were very low, as has been reported previously (Petrovic, 1990), they were significantly different between topsoil and subsoil turfgrass lawns, and among fertilizer treatments. They concluded that the use of organic fertilizers could minimize nutrient losses from lawns established on subsoil compared with inorganic fertilizer.

17.6 Conclusions

Urban lawn is the most familiar ecosystem in many developed countries such as the USA and thus presents a huge opportunity to raise awareness about local and global environmental and sustainability challenges. Urban lawn has become the dominant land use particularly in suburban areas and is valued for its aesthetic and recreational uses. Unfortunately, urban lawn has become an input-intensive system with routine, often calendar-based, applications of fertilizers, pesticides, mowing and irrigation. Although the effectiveness of the IPM approach in reducing fertilizer and pesticide inputs has been demonstrated, the adoption of IPM has been low due to educational and social barriers. As homeowner education of the best-management practices and their ecological underpinnings is a fundamental prerequisite for the adoption of ecological approaches to lawn care, research on urban lawn ecology is needed. Unfortunately, urban lawn is one of the least studied ecosystems on the planet. In this chapter, a novel ecosystem management approach, rooted in basic ecological principles, has been proposed as a framework for understanding and manipulating ecological interactions to build sustainable lawn-care practices. The ecosystem management is defined as a holistic approach in which ecological interactions and processes are recognized and carefully and minimally engineered to obtain desirable goods and services while minimizing disservices and building and sustaining ecosystem composition, structure and function. To practice the proposed ecosystem management approach, five fundamental ecological principles have been explicitly identified that can be used as tools to obtain the desired ecosystem services sustainably. These fundamental ecological principles are: (i) human intervention; (ii) resource competition; (i) herbivory; (i) predation and parasitism; and (i) biogeochemical cycling.

The underlying premise of this new *ecological principle tools-based ecosystem management approach* is the inherent property of 'trade-offs'; that is, any manipulation of these ecological principles may lead to both desirable and undesirable outcomes in the short or long term. The ultimate goal is to minimize the need for external inputs, particularly inorganic fertilizers and pesticides, through building and maintenance of biological diversity and functional food webs. In this approach, humans are considered an integral part of the ecosystem and thus, as a component species, their needs, actions, knowledge, beliefs and socio-political institutions are considered fundamental to the development and practice of the approach as opposed to the strictly biocentric or ecocentric values approach. Manipulations of these five ecological principles to obtain the desired services from the lawn ecosystem have been discussed using examples from recent research. The findings underscore the potential for building a powerful ecosystem management approach to urban lawn and to use the lawn ecosystem to raise public awareness about urban ecology and local and global environmental and sustainability issues.

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