

BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Biopesticides for sustainable agriculture

Edited by Professor Nick Birch, formerly The James Hutton Institute, UK

Professor Travis Glare, Lincoln University, New Zealand



Contents

Series list	xii
Introduction	xvii

Part 1 General

1	Improving methods for developing new microbial biopesticides <i>Susan M. Boyetchko, Agriculture and Agri-Food Canada, Canada</i>	3
1	1 Introduction	3
1	2 The role of biopesticides	3
1	3 The economics of biopesticides	5
1	4 Strategic framework for biopesticide development	6
1	5 Microbial exploration and discovery	10
1	6 Fermentation and formulation	13
1	7 Conclusion and future trends	15
1	8 References	17
2	Implementing biopesticides as part of an integrated pest management (IPM) programme <i>József Kiss, Szent István University, Hungary; and Marc Delos, Académie d'Agriculture de France, France</i>	21
2	1 Introduction	21
2	2 Biocontrol products and biopesticides: definitions	23
2	3 Regulatory assessment of the use of biopesticides in IPM programmes	25
2	4 The use of biopesticides in different crop production systems	27
2	5 Integrating biopesticides into IPM programmes	30
2	6 Using biopesticides in IPM programmes in practice: a case study	35
2	7 Where to look for further information	38
2	8 References	40

3	Improving regulatory approval processes for biopesticides and other new biological technologies in agriculture <i>Wyn Grant, University of Warwick, UK; and Roma Gwynn, Biorationale, UK</i>	45
1	Introduction	45
2	Establishing a regulatory framework for biopesticides	46
3	Pesticide regulation in the European Union (EU)	47
4	The development of the biopesticide sector and new regulatory requirements	49
5	Challenges in improving the regulatory framework for biopesticides	50
6	Current EU regulation of biopesticides	52
7	Global national initiatives in biopesticide regulation	54
8	Developing good regulatory practice	56
9	Conclusions	58
10	Where to look for further information	59
11	References	59

Part 2 Microbial biopesticides, entomopathogenic nematodes and mites

4	Advances in the use of entomopathogenic fungi as biopesticides in suppressing crop pests <i>Enrique Quesada-Moraga, Meelad Yousef-Naef and Inmaculada Garrido-Jurado, University of Córdoba, Spain</i>	63
1	Introduction	63
2	Natural occurrence and biodiversity	64
3	Mode of action	66
4	Delivery methods	66
5	Use of entomopathogenic fungi as biopesticides	69
6	Advantages and limitations of entomopathogenic fungi as biopesticides	84
7	Market overview	85
8	Conclusion and future trends	87
9	Where to look for further information	87
10	References	89
5	Advances in the use of entomopathogenic bacteria/microbial control agents (MCAs) as biopesticides in suppressing crop insect pests <i>Tejas Rao and Juan Luis Jurat-Fuentes, University of Tennessee, USA</i>	99
1	Introduction	99
2	Overview of commercialized entomopathogenic bacteria	102

3 Commercialized spore-forming bacterial entomopathogens	105
4 Spore-forming bacteria with potential for future commercialization	113
5 Commercialized non-spore-forming entomopathogenic bacteria	114
6 Non-spore-forming entomopathogenic bacteria with potential for commercialization	116
7 Microbial control agents (MCAs)	117
8 Advantages and drawbacks on the use of bacterial biopesticides	118
9 Future trends in research	119
10 Where to look or further information	121
11 References	122
6 Advances in the use of Bt genes in insect-resistant crops <i>Salvatore Arpaia, ENEA Research Centre Trisaia-Rotondella (MT), Italy</i>	135
1 Introduction	135
2 <i>Bacillus thuringiensis</i> (Bt) insecticidal toxins	136
3 Incorporating Bt-expressing GM plants in integrated pest management (IPM)	138
4 Insecticide resistance	141
5 Conclusion and future trends	143
6 Acknowledgements	145
7 Where to look for further information	145
8 References	146
7 Plant growth-promoting bacteria (PGPBs) as biocontrol agents against invertebrate pests <i>Luca Ruiu, Università degli Studi di Sassari, Italy</i>	151
1 Introduction	151
2 Plant growth promotion	152
3 Action against plant pathogens	153
4 Potential against invertebrate pests	154
5 Applications in agriculture and forestry	156
6 Case study: <i>Pseudomonas protegens</i>	156
7 Conclusion and future trends	160
8 Where to look for further information	160
9 References	161
8 Advances in the use of entomopathogenic viruses as biopesticides in suppressing crop insect pests <i>Martin Erlandson, Agriculture and Agri-Food Canada, Canada</i>	167
1 Introduction	167
2 RNA viruses	171
3 DNA viruses	173

4 Conclusion	188
5 Where to look for further information	188
6 References	189
9 Advances in the use of entomopathogenic nematodes (EPNs) as biopesticides in suppressing crop insect pests	195
<i>Albrecht M. Koppenhöfer, Rutgers University, USA; David I. Shapiro-Ilan, USDA-ARS, USA; and Ivan Hiltbold, University of Delaware, USA</i>	
1 Introduction	195
2 Entomopathogenic nematode (EPN) biology and ecology	196
3 Entomopathogenic nematode (EPN) use in different cropping systems and climatic zones	203
4 New developments to improve efficacy	218
5 Conclusion and future trends	221
6 Where to look for further information	222
7 References	223
10 Advances in the use of entomopathogenic oomycetes as biopesticides in suppressing crop insect pests	233
<i>Aurélien Tartar, Nova Southeastern University, USA</i>	
1 Introduction	233
2 Oomycetes as invertebrate pathogens	235
3 Case study: <i>Lagenidium giganteum</i> as a biocontrol agent against insects	237
4 Mode of infection by entomopathogenic oomycetes	239
5 Oomycetes as sources of novel biopesticidal metabolites	240
6 Conclusion and future trends	243
7 Where to look for further information	244
8 References	244
Part 3 Natural substance-based biopesticides	
11 Advances in the use of semiochemicals in integrated pest management: pheromones	251
<i>Gadi V. P. Reddy, USDA-ARS, Southern Insect Management Research Unit, USA; Anamika Sharma, Montana State University, USA; and Angel Guerrero, Institute of Advanced Chemistry of Catalonia-CSIC, Spain</i>	
1 Introduction	251
2 Structural diversity and specificity of pheromones	254
3 Established uses of pheromones	256
4 Pheromone inhibitors	263

5 Advancement and future perspectives	266
6 Conclusion and future trends	271
7 Acknowledgement	271
8 Where to look for further information section	271
9 References	272
12 Possible use of allelochemicals in integrated pest management (IPM)	283
<i>Toby Bruce, Keele University, UK</i>	
1 Introduction	283
2 Allelochemicals for pest management	284
3 Botanical pesticides	285
4 Repellents	286
5 Attractants	287
6 Defence activators	288
7 Overcoming barriers to improve use of allelochemicals in integrated pest management (IPM)	289
8 Conclusion	291
9 Where to look for further information	292
10 References	292
13 Peptides as novel biopesticides	297
<i>Lin Bao, Robert M. Kennedy, Kyle Schneider, Alvar Carlson and Andy Renz, Vestaron Corporation, USA</i>	
1 Introduction	297
2 Venom peptides as biopesticides	298
3 The discovery of novel venom peptides	299
4 The first commercialized venom peptide-based biopesticide: SPEAR	301
5 Other commercialized peptides: harpin and trypsin modulating oostatic factor (TMOF)	302
6 Plant expression of venom peptides	303
7 Plant defensin and other antifungal peptides	306
8 Summary and future trends	307
9 References	308
14 Development of plant-derived compounds as biopesticides	315
<i>Barbara Thuerig and Lucius Tamm, Research Institute of Organic Agriculture (FiBL), Switzerland</i>	
1 Introduction	315
2 Legal framework and authorization of plant-derived plant protection products: an overview	317

3 Development of plant-derived plant protection products	320
4 Conclusion	329
5 Where to look for further information	329
6 References	330
Index	335

Introduction

With increasing concern about the environmental impact of synthetic pesticide use, including their impact on beneficial insects, the problem of insect resistance and the lack of new products, there has been an increasing interest in developing alternative biopesticides to control insect and other pests. This collection reviews the wealth of research on identifying, developing, assessing and improving the growing range of biopesticides. Part 1 reviews research on developing new biopesticides in such areas as screening new compounds, ways of assessing effectiveness in the field and improving regulatory approval processes. Part 2 summarises advances in different types of entomopathogenic biopesticide including entomopathogenic fungi and nematodes and the use of Bt genes in insect-resistant crops. The final part, Part 3, assesses the use of semiochemicals such as pheromones and allelochemicals, peptide-based and other natural substance-based biopesticides.

Part 1 General

The first chapter of the book begins with a discussion of improving methods for developing new microbial biopesticides. Chapter 1 highlights the role and economics of biopesticides, analyses the strategic framework for biopesticides development and provides an overview of the biopesticide development process. The chapter then focuses on microbial exploration and discovery and draws attention to the biological characterisation and development of biopesticides. A section on fermentation and formulation is also provided, focusing on various fermentation methods and a selection of formulations useful to biopesticide production. The chapter concludes by providing an overview of the future of biopesticides as an emerging industry as well as giving recommendations for future research.

Chapter 2 reviews the implementation of biopesticides as part of an integrated pest management (IPM) programme. It begins by highlighting the way biopesticides and other biocontrol agents can be integrated into IPM programmes. The chapter then discusses regulatory assessment of when it is safe and appropriate to use biopesticides and the use of biopesticides in different crop production systems. The chapter also provides a case study of the French government's programme to move its agricultural sector from reliance on synthetic pesticides to the use of more sustainable biocontrol techniques.

Moving on to the final chapter of Part 1, Chapter 3, the subject is improving regulatory approval processes for biopesticides and other new biological technologies in agriculture. The chapter begins by discussing how a regulatory

framework for biopesticides can be established. It moves on to review the regulation of pesticides in the European Union (EU) and how the biopesticide sector and new regulatory requirements have been developed. The chapter also highlights the challenges faced in improving the regulatory framework for biopesticides and discusses current EU regulation and global national initiatives in biopesticides. The chapter concludes by reviewing how good regulatory practice can be developed.

Part 2 Microbial biopesticides, entomopathogenic nematodes and mites

Part 2 begins with Chapter 4, a discussion of the advances in the use of entomopathogenic fungi as biopesticides in suppressing crop pests. Hypocrealean ascomycetes have a set of properties such as their unique mode of action by direct penetration through the cuticle, potential for mass production, and their newly discovered ecological roles as endophytes and/or plant growth promoters which put them at the forefront of the global development of alternative control strategies. The chapter first addresses general aspects related to the natural occurrence and biodiversity, life cycle and delivery methods of the entomopathogenic fungi. It uses case studies to review the use of entomopathogenic fungi as biopesticides for the control of a wide range of insect and mite pests including locust and grasshoppers, soil dwelling insect pests, piercing and sucking insect and mite pests, stored-grain pests, forestry pests, invasive pests, and medical and veterinary pests. Finally, the advantages and limitations of entomopathogenic fungi as biopesticides together with a market overview are provided, with a concluding section indicating possible future directions in research on these fungi.

The next chapter, Chapter 5, addresses the advances in the use of entomopathogenic bacteria/microbial control agents (MCAs) as biopesticides in suppressing crop insect pests. The chapter first provides an overview of commercialised entomopathogenic bacteria and then discusses commercialised spore-forming bacterial entomopathogens. It also draws attention to non-spore forming entomopathogenic bacteria with potential for commercialisation and provides a section on microbial control agents (MCAs). A section on the advantages and drawbacks on the use of bacterial biopesticides is included before the chapter concludes by discussing research needs and emerging initiatives and providing future research trends in the subject.

Chapter 6 focuses on the advances in the use of Bt genes in insect-resistant crops. Insecticidal toxins derived from the soil bacterium *Bacillus thuringiensis* (Bt) have been widely used as microbial pesticides to selectively control juveniles of Lepidoptera, Coleoptera, Diptera and soil-dwelling nematodes. Synthetic genes derived from different strains of Bt have been used to produce

genetically-modified (GM) plants resistant to insects. The chapter begins with an introduction to *Bacillus thuringiensis* (Bt) insecticidal toxins and moves on to discuss how Bt genes can be incorporated into integrated pest management (IPM). It also reviews the importance of effective insecticide resistance programs before providing a section on potential areas for future research and sources for further information.

The subject of Chapter 7 is on plant growth-promoting bacteria (PGPBs) and how they can be used as biocontrol agents against invertebrate pests. Plant growth promoting bacteria (PGPBs) represent an important resource for agricultural crops, providing several benefits to the plant such as optimizing the use of environmental resources, improving plant health and resistance to biotic and abiotic factors, and directly acting against plant pathogens and invertebrate pests. The chapter begins by reviewing plant growth promotion and how plant growth-promoting bacteria (PGPBs) can be used against plant pathogens. It also addresses their potential against invertebrate pests and applications in agriculture and forestry. The chapter provides a case study on the soil-dwelling bacterium *Pseudomonas protegens* as a PGPB with dual action of biocontrol against both plant pathogens and invertebrate pests. It concludes by providing potential areas for future research.

Chapter 8 reviews advances in the use of entomopathogenic viruses as biopesticides in suppressing crop insect pests. It begins by discussing insect pests and their impact on the environment and how insect pest control has developed over time. It also highlights how viruses have been used as potential insect control agents. The chapter first analyses RNA viruses, specifically focusing on tetraviruses and reoviruses. It then looks at DNA viruses, focusing on Parvoviridae, Poxviridae, Nudiviridae and Baculoviridae. As baculoviridae is the most studied group of insect-specific viruses, the chapter also provides information on attributes of baculoviruses, an analysis of baculovirus genomics and molecular biology, a discussion on biopesticide development and reviews baculovirus production and formulation. It also provides a paragraph on the development of host-resistance to baculoviruses. The chapter concludes by providing an overview of how insect-specific viruses have potential for development as biological control agents of insect pests in integrated pest management (IPM) systems.

The next chapter, Chapter 9, discusses advances in the use of entomopathogenic nematodes (EPNs) as biopesticides in suppressing crop insect pests. Entomopathogenic nematodes have been commercialized as biopesticides since the 1980s. Since the 1990s, research on the application, biology, and ecology of these biocontrol agents has seen exponential growth, and since the mid-2000s basic research on EPNs has further expanded due to the use of the nematodes and their symbiotic bacteria as tractable model systems for many basic biological and ecological questions. The

emphasis of the chapter is on the use of EPNs as biopesticides in a wide range of agricultural and other commodities. However, the chapter also provides overviews on EPN biology and ecology and mass production and application technology and interactions with other management tools as they are necessary for a better understanding of the strengths and limitations of EPNs as biopesticides. The chapter also provides a section on potential areas for future research.

Chapter 10, the final chapter of Part 2, reviews advances in the use of entomopathogenic oomycetes as biopesticides in suppressing crop insect pests. It begins by discussing how oomycetes have evolved from invertebrate pathogens to plant pathogens, what they are commonly known as today. The chapter also provides a case study on *Lagenidium giganteum* as a biocontrol agent against insects. The chapter moves on to review how the mode of infection can be established by entomopathogenic oomycetes, and how oomycetes can be used as sources of novel biopesticidal metabolites. It concludes by providing potential sources for further information and directs researchers to key conferences in the field.

Part 3 Natural substance-based biopesticides

The final part of the book begins by addressing the advances in the use of semiochemicals in integrated pest management, specifically focussing on pheromones. Semiochemicals enable intra- and interspecific chemical communication in insects. Among them, pheromones are species-specific and have been used in the management tactics for control of many insect pests for the last 50 years. During this time, important advancements have been made in understanding pheromones and their uses, particularly pheromone receptor neurons and pheromone inhibitors. Chapter 11 presents the latest developments of pheromones and their applications as key elements to specifically control insect pests within IPM programs. It also includes a section which provides researchers with potential sources for further information on the subject.

The next chapter, Chapter 12, discusses the possible use of allelochemicals in integrated pest management (IPM). Allelochemicals are produced and released into the environment by one species and have an effect on another species. They include attractants, repellents and toxic defence compounds which could provide valuable tools for use in IPM. The chapter begins by reviewing allelochemicals for pest management and also discusses botanical pesticides. Sections on repellents, attractants and defence activators are also provided. The chapter concludes by discussing the importance of overcoming barriers to improve the use of allelochemicals in pest management (IPM).

Chapter 13 reviews the use of peptides as novel biopesticides. Venom peptides are small toxic proteins originated from animal venoms. They have attracted great interest as a route to develop the next generation of novel, effective and safe biopesticides. The chapter begins by reviewing the characteristics and discovery of a range of venom peptides. It shows how inhibitory cysteine knot (ICK) type peptides, characterized by strong selectivity/specificity, stability and capacity for production at high volume, were used to develop the first commercialised peptide-based insecticide: SPEAR®. It also discusses other commercialised peptides and techniques to incorporate venom into plants to protect against insect and fungal attack.

The final chapter of the book, Chapter 14, reviews the development of plant-derived compounds as biopesticides. It begins by providing an overview of the legal framework and authorization of plant-derived plant protection products, then goes onto discuss how these products are developed. The chapter focuses on how plant-derived substances with activity against plant pathogens can be identified, as well as the efficacy of botanicals and novelty of invention and IP protection. It also highlights the importance using raw material of a high quality for plant-derived plant protection products. The chapter also provides a section on the extraction process that is used to produce extracts suitable for plant protection products. The chapter concludes by highlighting the growing interest in environmentally-friendly produced agricultural products, including plant-derived plant protection products, and also provides potential sources of further information on the subject.

Part 1

General

Chapter 1

Improving methods for developing new microbial biopesticides

Susan M. Boyetchko, Agriculture and Agri-Food Canada, Canada

- 1 Introduction
- 2 The role of biopesticides
- 3 The economics of biopesticides
- 4 Strategic framework for biopesticide development
- 5 Microbial exploration and discovery
- 6 Fermentation and formulation
- 7 Conclusion and future trends
- 8 References

1 Introduction

Biopesticides are attracting global attention as pest management tools that have a role in sustainable pest management. They are broadly defined as the deliberate use of living organisms, such as bacteria, fungi, viruses and nematodes, and their natural products (bioactive compounds produced as secondary metabolites) that directly or indirectly harm weeds, reduce the impact of plant diseases, or control the insect pests. Although the scientific community has been involved in biopesticide technology development for decades, it is only in recent years that it has been recognized as a viable emerging technology that is acceptable for mainstream use, rather than niche market products (Vincent et al., 2007; Bailey et al., 2009; Glare et al., 2012, 2016; Morán-Diez and Glare, 2016). They have generally been accepted as environmentally friendly technology because they are naturally occurring and thus considered attractive alternatives to synthetic pesticides. This is not to say that all naturally derived microbes are safer than chemicals because some of the mycotoxin-producing fungi can be considered very toxic to humans and animals.

2 The role of biopesticides

Several factors have precipitated the necessity for developing biopesticides as pest control strategies to synthetic pesticides. These include the deregistration or

phasing out of older chemistries for agricultural and horticultural crop protection by the regulators and phasing out of numerous synthetic pesticides in lawns and gardens (Bailey et al., 2009; Boyetchko, 2017). Government legislation was enacted to ban synthetic pesticides for cosmetic use in urban municipalities in Canada through the Pest Management Regulatory Agency (PMRA). For example, Halifax, Nova Scotia, became the first Canadian city to ban chemicals within city limits in 2000 (Bailey et al., 2009). More than 100 urban municipalities in Canada followed suit, while the provinces of Ontario, Quebec, and Manitoba have ratified similar bans for cosmetic use of pesticides within urban municipalities. These regulations have thus generated a demand for biopesticides. However there are not sufficient biopesticide products to replace those that have been forbidden for use by the public and/or city employees. Therefore there is an even more urgent need to conduct future research for the discovery, development, and registration of new biopesticide products. The legislation accordingly supported policy changes within the Canadian regulatory body to register lower-risk pest control products. Biopesticides were thus classified under the Reduced-Risk Products Initiative (<http://www.hc-sc.gc.ca/cps-spc/pest/index-eng.php>; Bailey et al., 2009). This program helped expedite the registration of currently registered biopesticides in the United States which led to the harmonization of regulations with the US EPA and Canadian PMRA. During this time, there was a significant increase in PMRA registrations because registrants were allowed to undergo a pre-submission consultation process and the length of time it took to register biopesticides was reduced. In addition to the changes in federal legislation, the federal government created the Pest Management Centre (PMC) in 2003, an entity that helped in the delivery of new biological control products by jointly funding research to aid in their registration. PMC also provided support to encourage the adoption of these new pest management tools.

The general public has a greater awareness for environmentally friendly and safe products in agriculture and forestry, and for use in home gardens.(Ott et al., 1991; Magnusson and Cranfield, 2005; Bailey et al., 2009; Glare et al., 2012) . Therefore there is a greater expectation for organic and pesticide-free products. Agricultural practices such as the continued use of chemicals that pollute soil, water, and food supplies are putting more pressure on industry to provide suitable products for pest management and this practice has forced the industry to evaluate current pest management practices and how chemical pesticide loads have affected our environmental performance. Changes in consumer acceptance of biopesticide technology are thus driving the need for such products and are leading the initiatives into the development of 'green' technologies for food production in grocery stores and farmer's markets.

Another major issue of concern related to traditional chemical pest control products is the development of pesticide-resistance to chemicals making them ineffective to the pests they were intended to control (Boyetchko and Rosskopf,

2006; Lazarovits et al., 2007; Bailey, 2010; Bailey et al., 2009; Glare et al., 2016). Repeated use of synthetic pesticides has created this problem, but researchers and the industry involved in their manufacture were skeptical to believe that pesticide-resistance could occur until the scientific community reported that such problems were occurring. For example, there are numerous examples of (i) insecticide-resistance to pyrethroids, organophosphates, and carbamates with insects; (ii) herbicide-resistance to acetyl-CoA carboxylase (ACCase), acetolactate synthase (ALS), and 5-enolpyruvylshikimate-3-phosphate (EPSPS or glyphosate) with weeds; and (iii) fungicide-resistance to Qo1 and azole fungicides with plant pathogens (Hawkins et al., 2019).

3 The economics of biopesticides

The discovery of new synthetic chemicals with novel modes of action is becoming more expensive and difficult. It is estimated that their discovery is decreasing by 1-2% per annum, while the biopesticide industry is expected to grow by over \$1 billion by 2010, and projections indicate the biopesticide market to exceed \$4 billion by 2023 (Oerke, 2006; Thakore, 2006; Bailey et al., 2009; Glare et al., 2012). The cost to develop a new synthetic pesticide is more than \$250 million and takes at least 10 years for a product launch (Oerke, 2006; Kelly and Allen, 2011; Glare et al., 2012). Conversely, it takes fewer microbial strains to discover a new biopesticide and can cost approximately \$3-5 million to commercialize in the United States and take 3 years to get to market. In the EU, it typically takes 5-7 years to get a biopesticide to market and there are fewer biopesticide-active substances registered in the EU versus the United States, mainly due to greater complexity of EU-based biopesticide regulations (Balog et al., 2017).

In the past, the multinational companies did not invest in many biopesticide products. The commercialization of the majority of biopesticides was engaged by small to medium-sized companies (Bailey, 2010; Bailey et al., 2009; Boyetchko, 2017). Investment in infrastructure for the discovery of synthetic chemicals is expensive and facilities and equipment can be specialized for their discovery. Large firms invested in discovery and development of synthetically derived compounds whereas a change in infrastructure investment is required for microbial-based technology, which requires access to fermentation capabilities. Moreover, formulation of chemical pesticides may or may not be compatible with biopesticide technology. Therefore, small- to medium-sized enterprises (SMEs) were often responsible for biopesticide development. It was anticipated that multinational companies would tackle the screening of microorganisms and their secondary metabolites once they acquired small to medium-sized biopesticide companies (Glare et al., 2016). For example, Becker Underwood was acquired by BASF, and Bayer CropScience purchased

Agraquest and Prophita, in addition, Syngenta purchased Valent BioSciences and Pasteuria BioScience. This investment by multinationals demonstrated their recognition of the growing value of the biopesticide market. Nonetheless, multinationals have the resources to devote to R&D however, their focus has been on major crops such as corn, soybean, rice, potato, and cotton being the main emphasis. Other markets that remain overlooked are pulse crops, small and large fruit crops and high value field and greenhouse vegetable crops and other horticultural crops. This therefore creates an opportunity for biopesticides to fill this niche.

4 Strategic framework for biopesticide development

One of the major challenges to the development and commercialization of biopesticide products is how to manufacture them at an affordable cost and in a consistent manner. Fermentation systems, formulation technology, and spray application or delivery of biopesticides has often been ignored in the R&D phase by researchers. It should be borne in mind that the initial screening of microbial organisms for biopesticides represents the first step in the discovery phase. The perception of finding a suitable organism that effectively controls the target pest by senior managers and the general public unfamiliar with the complexities of biopesticide development often assumes that once the organism has been discovered, that is the end of the process. However, the microbe must be mass-produced through fermentation systems, subsequently formulated into a stable form and applied using spray application systems to facilitate their delivery to the soil, seed, and/or foliage (Boyetchko and Peng, 2004; Hynes and Boyetchko, 2006). Developing a suitable fermentation system requires an understanding of the microbial physiology of the organism and a suitable formulation often involves the knowledge of the microbe's mode of action in order to facilitate delivery of the biopesticide. Contrary to the popular belief that once a microbial organism has been discovered with biopesticidal properties in the laboratory, the final biopesticide product is imminent, a biopesticide product is one where all the processes related to its manufacture are considered, including platform technologies related to fermentation, formulation, and spray application (Fig. 1; Boyetchko and Peng, 2004). The taxonomy, biological characterization, mode of action, and efficacy are among the factors that are core to the selection of the biopesticide organism. The platform technologies are interrelated and closely linked and their complexities have been underestimated (Boyetchko and Peng, 2004; Boyetchko et al., 2007; Bailey, 2014). They are integral factors to consider with the biopesticide product development. The goal is to find 'nature's best' with respect to the microbe selected for biological control. However, fermentation or formulation may not necessarily improve a mediocre microbe, but a superior microbial agent may

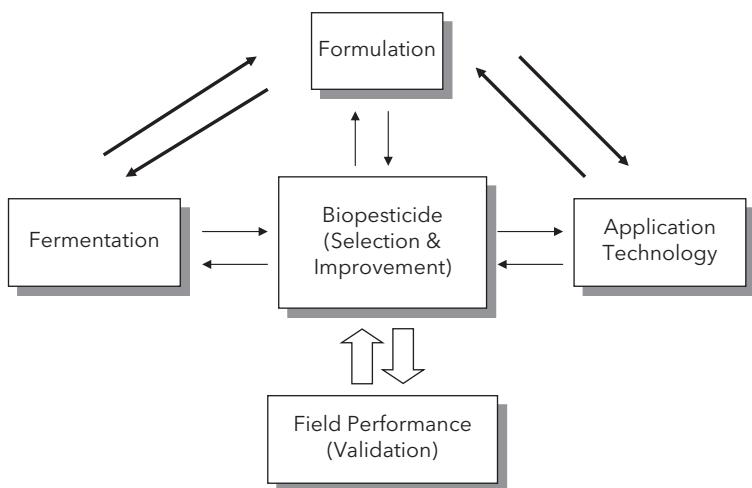


Figure 1 Strategic framework for evaluation and development of biopesticides. Source: adapted from Boyetchko and Peng (2004).

have to be sacrificed if it cannot be readily grown in suitable fermentation media or it cannot be readily formulated. Compromises are sometimes made in the selection process.

The earlier prototype models of biopesticides were manufactured using simple formulations but the intricacies between the platform technologies are much more complicated (Auld and Morin, 1995; Fravel, 1999; Auld et al., 2003). Lack of appropriate formulation may have led to ineffectual or inconsistent field performance and fermentation of the microbial organism may be too expensive or scale-up unacceptable that the product commercialization was abandoned which led to an 'orphaned' biopesticide product (Butt et al., 2001; Hallett, 2005; Hynes and Boyetchko, 2006).

4.1 The process of biopesticide development

Biopesticide discovery and development follows a process of incremental steps that are unique for each target pest-biopesticide system. Different research or industry groups follow different stepwise protocols to facilitate in the selection of promising biopesticide candidates (Boyetchko and Svircev, 2009; Bailey et al., 2009; Bailey, 2010; Köhl et al., 2011; Ravensberg, 2011; Glare et al., 2012). The 'Biopesticide Innovation Chain' (Fig. 2) was developed in response to a need for several researchers in the disciplines of weed science, entomology, and plant pathology to follow a similar blueprint to develop microbial biopesticides. The innovation chain depicts nine critical stages for developing a biopesticide product using a series of 'Go vs. No-Go' criteria in order to make decisions on

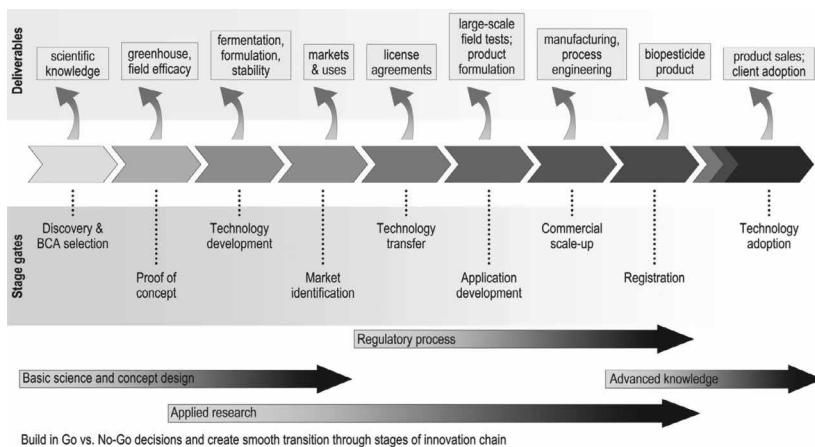


Figure 2 Solution for delivery of biopesticides: AAFC biopesticide science innovation chain. Source: adapted from Boyetchko and Svircev (2009).

the feasibility of the organisms and the target pest. This concept is applicable to any type of biopesticide (i.e. bacteria, fungi, viruses, natural products) and is appropriate for any type of crop pest (i.e. weed, invertebrate/insect pest, plant pathogen). The stages of the biopesticide innovation chain include (i) discovery and selection; (ii) proof of concept; (iii) technology development; (iv) market identification; (v) technology transfer; (vi) application development; (vii) commercial scale-up; (viii) registration; and (ix) technology adoption. The innovation chain describes the process for developing a biopesticide without considering the scientific terminology specific to each crop protection discipline; everyone will be describing the process using similar terminology. This road-map forces the researcher to proceed to the subsequent steps while not being fixated on one or two stages, thus preventing him/her from advancing toward the end goal: developing a biopesticide.

The early stages of discovery involve characterization of the biopesticide agent, assessing growth characteristics, basic understanding of the biology, genomics, and initial *in vitro* and *in vivo* screening bioassays that measure efficacy on the target pest (Bailey et al., 2009; Bailey, 2010). Proof of concept entails evaluation of biopesticide performance on live plants under controlled-environmental conditions where light, temperature, and relative humidity/moisture conditions can be monitored. It is then essential to bring the evaluation to the field, where possible, in order to demonstrate the adequacy of the biopesticide performance. Technology development involves the bioprocesses of fermentation/mass-production, formulation, and application technology for delivery of an efficacious, stable biopesticide with qualities that contribute to an 'adequate' shelf life for a microbe that is easy to grow and

deliver to the target pest. As previously indicated, these platform technologies that are critical to biopesticide development are often linked. Recent advances have been publicly available, but, unfortunately, industry has often kept fermentation and formulation as proprietary or trade secret information. Several fermentation and formulation may be tested before a final process is selected (Bailey, 2014).

The importance of market considerations cannot be underestimated since they will dictate the success or failure for commercialization. Although this area is not necessarily a research consideration to scientists, it is important because it defines the field of use and the market the biopesticide is being developed for. It will pre-determine the experimentation and scientific data required to register the product. For example, will the biopesticide be developed for use in the horticultural or greenhouse market, in large acreage field crops, or for use in forestry or for the home gardener? Another key feature of the innovation chain is that it encourages additional applications and crop pests into other potential use patterns, thus broadening to other target pests and production systems (Boyetchko and Rosskopf, 2006; Hynes and Boyetchko, 2006). A great deal of understanding of the microbial physiology and biochemistry is also required, but the business side of the research is an essential factor.

The later stages in the innovation chain (i.e. technology development, technology transfer) test the robustness of the earlier decisions by actively working with various stakeholders involved in the development of biopesticides. These include the industry partner, other collaborators, and regulators necessary to develop the data required for the registration package. Moreover, involvement of additional stakeholders generates 'buy-in' of the new technology which will help with the adoption of the biopesticide into the field of use. For example, the innovation chain can be used as a tool for the training of farmers and crop protection consultants in the safe and effective use of biopesticides. Moving into commercial scale-up can still affect the final stages of product development and is more often led by industry, often requiring significant investment back into technology development to registration, and technology adoption, which is usually directed by the industry partner and extension agrologists. The champion of the technology is usually the lead scientist or inventor who should work collaboratively with industry until the technology adoption has occurred. The corporate memory or 'know-how' and intuition of the lead scientist, along with his/her scientific expertise, can ease in the transition phases toward commercialization. Do not assume that the technology will be successful once it has been handed off to industry, without input from the scientist who originally discovered the biopesticide. The lead scientist can provide guidance into the overall idea and direction of the scientific discovery, along with a number of unsuccessful trials that had been attempted but failed during the process of developing the biopesticide.

5 Microbial exploration and discovery

If we are to identify the ‘nature’s best’, a systematic approach is essential during the exploration and discovery phase to thoroughly evaluate the microbial biodiversity. This biodiversity provides excellent opportunities for finding microbial strains with suitable traits for biocontrol potential (Boyetchko and Peng, 2004).

Numerous discoveries of biopesticides have taken place in university and government institutions resulting in the licensing of the technology to industry. Many of these institutions may be in possession of undervalued but extensive microbial culture collections which can be exploited for the discovery of future biopesticide candidates. To add value to these collections, it is essential to conduct additional mass-screening of these same microbes against additional target pests, whether they are for insecticidal, fungicidal, or herbicidal activity using established *in vitro* and *in vivo* screening protocols. Over the years, a researcher can accumulate numerous future biopesticide candidates which can be stored under –80°C conditions until required. Future collections can thus access potential microbes based on the taxonomy of related pests they are known to be inhibitory to. Different strains to the same genus species may perform differently from one another and should not be readily discarded. Glare et al. (2012) described how Trichobank™, a culture collection of over 2000 isolates of 21 *Trichoderma* spp. has been effectively utilized to select for promising biopesticide candidates with various growth characteristics for control of several soil-borne diseases. Similarly, Bailey et al. (2009) explained their philosophy behind screening for microorganisms as bioherbicides. Once the target weed is selected, several hundreds to thousands of fungi and bacteria that have been collected through surveys were isolated from a broad range of environmental conditions and evaluated to select those microbes that possess a list of desirable characteristics. Therefore, a systematic approach for selection of appropriate biopesticide candidates is conducted. For example, the fungal pathogen, *Phoma macrostoma*, was initially discovered to be a foliar pathogen, but its bioherbicidal potential to control Canada thistle and chickweed was only discovered when it was found to be effective when applied to the soil (Bailey, 2014). The potential for *P. macrostoma* as a bioherbicide was only discovered when the researchers understood what was the best method to apply the fungal pathogen and what was its mode of action. No longer are potential biopesticide candidates developed by a fortuitous discovery, as was the case with *Colletotrichum gloeosporioides* f.sp. *malvae* to control round-leaved mallow (Boyetchko et al., 2007; Bailey, 2010). Experienced researchers are developing their own road-map and selection process for screening biopesticide organisms.

Endophytic bacteria and fungi found in vascular plants are an underexplored bioresource as promising biological control agents (Vega,

2008; Glare et al., 2012; Eljounaidi et al., 2016; Rybakova et al., 2016; De Silva et al., 2019). Endophytes grow inside the plant for part or all of its life cycle but do not cause visible symptoms. Almost all vascular plants examined to date have been found to harbor bacterial and/or fungal endophytes. It is believed that many endophytic microbes may have originated in the rhizosphere and phylloplane and they gained entry through natural openings or plant wounds. Due to the diversity of species discovered to be endophytic, it is postulated that they may prove to be very suitable biopesticides and plant-growth promoters. Endophytes can be introduced as biocontrol agents through seeds and propagated plant material such as those introduced via tissue culture (Eljounaidi et al., 2016). Once endophytes enter the plant, they will not be subjected to environmental stresses that many other nonendophytic biocontrol agents can succumb to. This may mitigate the need to develop formulations to protect them from unfavorable environmental conditions. Some endophytes may demonstrate their biocontrol potential by secreting antifungal and antibacterial metabolites, thereby inhibiting the competition of pathogens, or they may exhibit mycoparasitic activity (i.e. parasitism of one microorganism by another microorganism) (de Silva et al., 2019). Those endophytic microbes that are entomopathogenic are surmised to act as a feeding deterrent or by antibiosis. Commonly isolated bacteria that can become endophytic belong to the genera *Bacillus*, *Pseudomonas*, *Agrobacterium*, *Paenibacillus* and *Enterobacter*. Common endophytic fungal microbes belong to *Fusarium oxysporum*, *Trichoderma* spp., *Colletotrichum gloeosporioides*, and *Phomopsis* spp.

Many biopesticide products were developed because they targeted a single major pest (Glare et al., 2012; Bailey, 2014; Boyetchko, 2017). Narrow host-range possessed by these biopesticides was initially considered an asset, if not essential. A narrow host-range contributes to environmental safety and limits the risk of a biopesticide affecting nontarget crops and other beneficial microbiota and microfauna. This trait has also restricted its success to a niche market product. However, it was later considered a hurdle to commercialization because industry was hesitant to invest in technology with such a small market potential. It was recognized that it is important to select a product that has activity against multiple pests in order for industry to get a return on their investment. Moreover, it is not practical or economical to develop a crop protection product for use by farmers on a single target pest when agriculture and forestry ecosystems are comprised of multiple crop pests. For example, bioinsecticide products containing *Bacillus thuringiensis* have had remarkable success in the marketplace because they kill several invertebrate pest species (Côté, 2007). Similarly, the fungus *Chondrostereum purpureum* was developed as a broad-spectrum bioherbicide to control a number of woody tree species and shrubs for vegetative management in forests and riparian lands and

utility-rights-of-way (Hintz, 2007). Control of a broad spectrum of plant diseases is also possible with the commercial product Serenade®, hence adding to its commercial value (Marrone, 1999, 2002).

5.1 Biological characterization and development

The discovery of new and promising microbial candidates as active ingredients for biopesticides has far out-paced the knowledge and related technology required to bring them to commercialization. Although there are numerous biopesticides registered for use globally (Vincent et al., 2007), including over 100 biopesticide-active ingredients in the United States and more than four different microbial-based active ingredients registered in Canada since 1972 (Fravel, 1999; Hynes and Boyetchko, 2006; Bailey et al., 2009; Kabaluk and Gazdik, 2011), the public still wonders why more biopesticides are not commercially available. It has been pointed out that scientific efforts in biological control have not contributed to the lack of commercial success of biopesticides, but that researchers have failed to focus on resolving critical technical elements such as formulation and methods for stabilization of living organisms (Zorner et al., 1993). It is true that a great deal of research effort has focused on the search for microbial agents with pest control potential, but technological challenges that include economical methods for mass production and scale-up, viable formulation and application strategies, and shelf life have been encountered and have contributed to the lack of commercial success (Auld and Morin, 1995; Boyetchko and Peng, 2004). However, the long-term commitment toward biological control has been predominantly supported by public research institutions such as government and universities (Boyetchko, 2017), while investment by industry to commercialize this technology has been sorely lacking. Access to public funding has been one constraint to this area; biopesticide research requires long-term commitment to funding, while many funding agencies provide financial support for projects that are 3–4 years in duration. In addition, the teams of researchers dedicated in understanding the fundamentals of pest biology and ecology and the agronomists necessary to implement pest management practices, have not been active participants in the application and implementation of biopesticide technology. It should be recognized that the success of biopesticides will depend on the involvement of multidisciplinary research teams consisting of crop protection specialists in either entomology, weed science, or plant pathology, microbiologists, natural products chemists, agronomists, molecular biologists, and economists with a vested interest in biopesticide adoption (Fig. 3). Biopesticides should not be considered a stand-alone pest management tool. A holistic approach using various tools for controlling pests can often lead to an additive or

Index

- AA10 proteins 175
AAFC. *see* Agriculture and Agri-Food Canada (AAFC)
ACCase. *see* Acetyl-CoA carboxylase (ACCase)
ACC-deaminase 153, 154
Acetolactate synthase (ALS) 5
Acetyl-CoA carboxylase (ACCase) 4
ACL. *see Allium cepa* lectin (ACL)
AcMNPV. *see* *Autographa californica* MNPV (AcMNPV)
ACP. *see* Asian citrus psyllid (ACP)
Adoxophyes honmai 261
Aedes aegypti 109, 138
AgMNPV. *see* *Anticarsia gemmatalis* MNPV (AgMNPV)
Agraquest 6
Agriculture and Agri-Food Canada (AAFC) 270
'Agriculture at a Crossroads' 138
Agriotes spp. 270
 A. obscurus 73
Agrochemical industry 46
Agrochemical insecticides 297
Agro-ecological approach 22
Agroecology Project 35
Agro-ecosystems 22-23
Agrotis ipsilon 206
Agrotis segetum 206
Alarm pheromones 253
Aleurodicus rugioperculatus 80
Alfalfa 289
Allelochemicals 252
 attractants 287-288
 botanical pesticides 285-286
 defence activators 288-289
 in integrated pest management 289-291
 overview 283-284
for pest management 284-285
repellents 286-287
Allium cepa lectin (ACL) 305
Allomones 284
Alphaentomopoxviruses 174, 175
Alphanudivirus 176
ALS. *see* Acetolactate synthase (ALS)
Amber disease 114
Ambushers 199
AMPs. *see* Antimicrobial peptides (AMPs)
Amyelois transitella 209
Androctonus australis scorpion insecticidal toxin (AAIT) 305
Annual arable crops 29
Anopheles gambiae 109, 117
Anoplophora glabripennis 81
Anthonomus grandis 254
Anticarsia gemmatalis MNPV (AgMNPV) 186
Antimicrobial peptides (AMPs) 306
Arabidopsis thaliana 302
Arvalis 37
Asian citrus psyllid (ACP) 80
Asian longhorned beetle. *see* *Anoplophora glabripennis*
Aspidiotus nerii 254
Attract-and-infect system 82
Attract-and-kill strategy 73, 75
Attract and kill technology 269, 271
Attractants 284
Autographa californica MNPV (AcMNPV) 179, 181, 182
Auxiliary macro-organisms 24
Azadirachta indica 286, 326
Azadirachtin 286
Bacillus spp.
 B. anthracis 105
 B. cereus 105

- B. firmus* 154
B. pumilus 155
B. subtilis 154
B. thuringiensis (Bt) genes 11, 102, 143-145, 298
 incorporating GM plants in IPM 138-141
 insecticidal toxins 136-138
 insecticide resistance 141-142
 overview 135-136
- Bactrocera* spp.
B. invadens 78-79
B. oleae 78
- Baculoviruses 178, 299
 attributes of 178-180
 biopesticide development 182-186
 genomics and molecular biology 180-182
 host resistance development to 187
 production and formulation 187
- Banana weevil. see *Cosmopolites sordidus*
- Barricade gel 219
- BASF 5
- Bayer CropScience 5
- BCAs. see Biocontrol agents (BCAs)
- Beauveria bassiana* 36, 75, 76, 80-82
- Becker Underwood 5
- Bemisia tabaci* 80
- Betaentomopoxvirues 174, 175
- Betanudivirus* 176
- Billbug species 212
- Binary (Bin) proteins 108-109
- Biochemical pesticides 24
- Biocontrol agents (BCAs) 23, 25, 27-29, 38
- Biopesticide innovation chain 7-8
- Biopesticides and Pollution Prevention Division (BPPD) 54, 301
- Bio Pesticides Steering Group (BPSG) 318
- Black cutworm. see *Agrotis ipsilon*
- Black vine weevil. see *Otiorhynchus sulcatus*
- Blattella germanica* 176
- Bombyx mori* 117, 252
- Botanical pesticides 285
- BPPD. see Biopesticides and Pollution Prevention Division (BPPD)
- BPSG. see Bio Pesticides Steering Group (BPSG)
- Bracon hebetor* 300
- Brevibacillus laterosporus* 102, 113, 155
- Brown-banded cockroach. see *Supella longipalpa*
- Budded virions (BV) 178
- Burkholderia* spp. 155
B. rinojensis 102
- BV. see Budded virions (BV)
- Cabbage stem flea beetle. see *Psylliodes chrysoccephalus*
- Calisoga* spp. 300
- Candidatus Liberibacter asiaticus* 80
- Capnodis tenebrionis* 75
- Carbamates 4
- Cellar spider. see *Segestria florentina*
- Cerambycid beetle. see *Prionus californicus*
- Ceratitis capitata* 68, 78, 79
- Ceutorhynchus picturans* 36
- Chemical mediators 24
- Chilo suppressalis* 254, 264
- Chitinases 158, 159
- Chondrostereum purpureum* 11
- Chromobacterium subtsugae* 102
- Chrysodeixis includens NPV 187
- Citrus greening disease. see Huanglongbing (HLB)
- Citrus leafminer. see *Phyllocoptis citrella* S.
- Clearwing moth 208
- Coat protein (CP) 305
- Codling moth 208. see *Cydia pomonella*
- Coleoptera 204, 266
- Colletotrichum gloeosporioides* 10
- Commercialized entomopathogenic bacteria 120-122
 advantages and drawbacks 118-119
 general pathogenicity and resistance 103-105
 microbial control agents (MCAs) 117-118
- non-spore-forming
Burkholderia rinojensis 115-116
Chromobacterium subtsugae 115
Pseudomonas spp. 116-117
Serratia entomophila 114-115
Yersinia entomophaga 116
- overview 99-103
- spore-forming
Bacillus thuringiensis (Bt) 105-108
Brevibacillus laterosporus 113
Clostridium bifermentans 113-114
Lysinibacillus sphaericus 108-111
Paenibacillus spp. 111-113
- Coniothyrium minitans* 31, 37
- Conotrachelus* spp.
C. nenuphar 208
C. psidii 209

- Contarinia nasturtii* 261
 Cooperation in Science and Technology (COST) 88
 Corn earworm. *see Helicoverpa zea*
 Corn rootworms (CRW) 203
Cosmopolites sordidus 209
 COST. *see* Cooperation in Science and Technology (COST)
 Cotton boll weevil. *see Anthonomus grandis*
 Cotton bollworm. *see Helicoverpa armigera*
 CP. *see* Coat protein (CP)
CpGV. *see* *Cydia pomonella granulovirus (CpGV)*
 CPV. *see* Cypoviruses (CPV)
 Crinkler (CRN) 241, 242
 CRISPR/Cas9 system 264
 CRN. *see* Crinkler (CRN)
 Crop Protection Programme 55
 CRPs. *see* Cysteine-rich proteins (CRPs)
 Cruisers 199
 CRW. *see* Corn rootworms (CRW)
 Crystal (Cry) proteins 106–108
 Cucumber beetle. *see Diabrotica balteata*
Culex spp.
C. pipiens 109
C. pipiens fatigans L. 255
C. quinquefasciatus 109, 110
Curculio caryae 208
 Cutworms 75–76
Cydia pomonella granulovirus (CpGV) 185–186
Cydia spp.
C. nigricana F. 260
C. pomonella 261
 Cypoviruses (CPV) 172–173
 Cysteine-rich proteins (CRPs) 306
 Cytokinins 153
 DAPG. *see* 2,4-diacetylphloroglucinol (DAPG)
 Daring jumping spider. *see Phidippus audax*
 Data Requirement on Pesticide Registration 55
 Death valley of innovation 325
Delia radicum 76–77
 'Demonstration Farms Integrated Plant Protection' 27
Dendroctonus rufipennis 81
Dendrolimus punctatus 1 (DpCPV-1) 173
Dendrolimus spectabilis CPV 1 (DsCPV-1) 173
Densoviruses 173–174
 Department for International Development (DFID) 55
Deroceras reticulatum 216
 Desert bush spider. *see* *Diguetia canities*
Desmodium 288
 DEXiPM tool 27
 DFID. *see* Department for International Development (DFID)
Diabrotica spp. 203
D. balteata 255
D. barberi 203
D. virgifera 30–31, 74–75, 136
D. virgifera virgifera 203
 2,4-diacetylphloroglucinol (DAPG) 158
 Diamondback moth. *see* *Plutella xylostella* (L.)
Diaphorina citri 80
Diaprepes abbreviatus 209, 210, 217
Diguetia canities 300
 Diptera order 204
Dociostaurus maroccanus 70, 71
DpCPV-1. *see* *Dendrolimus punctatus* 1 (DpCPV-1)
 Draft Assessment Report 53
Drosophila spp.
D. melanogaster 117, 155
D. suzukii 68
DsCPV-1. *see* *Dendrolimus spectabilis* CPV 1 (DsCPV-1)
 EC₅₀ 323
 EC/1007/2009 48
 Economic injury level 267
 Ecophyto plans 35–37
 Eden Bioscience 303
 EDNA. *see* Extracellular DNAs (eDNA)
 EF-1 α gene 85
 EFSA. *see* European Food Safety Authority (EFSA)
 egt gene 182
 Endophytes 10–11, 68–69
Enhancin gene 182
 5-enolpyruvylshkimate-3-phosphate (EPSPS) 5
 Entomopathogenic eukaryotes 239–240
 Entomopathogenic fungi (EPF) 87–89
 advantages and limitations 84–85
 as biopesticides 69–83
 delivery methods 66, 68–69
 market overview 85–86
 mode of action 66
 natural occurrence and biodiversity 64–66
 overview 63–64

- Entomopathogenic nematodes 195–196
 biology 196–198
 chemical ecology 200–201
 combinations, with control agents 220–221
 dispersal and foraging strategies in 199–200
 environmental manipulation 220
 factors affecting survival and efficacy 201–203
 formulation and application technology 219–220
 future trends 221–222
 host range, host immune response, and EPN virulence 198–199
 mass production 218–219
 persistent native strain for long-term pest suppression 215–216
 slug-parasitic nematodes 216
 strain improvement and stabilization 218
 use in forestry 214–215
 use in greenhouse production 214
 use in landscape plants and nurseries 213–214
 use in maize/corn 203–206
 use in mushroom production 215
 use in orchards 208–210
 use in relation to cost 217
 use in small fruit 207
 use in turfgrass and pastures 210–213
 use in vegetable and tuber crops 206–207
- Entomopathogenic oomycetes, advances in use of
 future trends 243–244
 infection mode 239–240
 as invertebrate pathogens 235–237
Lagenidium giganteum as biocontrol agent 235, 237–239
 main species of 235
 as sources of novel 240–243
- Entomopathogenic viruses, advances in use of 167–171
 DNA viruses 173–187
 RNA viruses 171–173
- Entomopoxviruses 174–176
- Environmental Protection Agency (EPA) 4, 54, 137, 298
- Environmental risk assessment (ERA) 143–145
- EPA. see Environmental Protection Agency (EPA)
- EPF. see Entomopathogenic fungi (EPF)
- EPPO. see European Plant Protection Organization (EPPO)
- EPS. see Extracellular polysaccharide (EPS) matrix
- EPSPS. see 5-enolpyruylshkimate-3-phosphate (EPSPS)
- ERA. see Environmental risk assessment (ERA)
- Erwinia amylovora* 302
- EU Framework Directive 91/414/EEC 22
- EU Framework Directive 2009/128/EC 22, 30, 87
- European Commission 53
- European corn borer (ECB). see *Ostrinia nubilalis*
- European Economic Area Agreement (1994) 48
- European Farming Systems Project 27
- European Food Safety Authority (EFSA) 26, 47, 53, 87, 143
- European Plant Protection Organization (EPPO) 25, 51
- EU Strategic Research Agenda 23
- Extracellular DNAs (eDNA) 158
- Extracellular polysaccharide (EPS) matrix 158
- False codling moth. see *Thaumatotibia leucotreta*
- FAO. see Food and Agriculture Organization (FAO)
- Farmer Field Schools (FFS) 33, 35
- Federal Food, Drug, and Cosmetics Act (FFDCA) 301
- Fenpicoxamide 37
- Fermentation methods 14
 solid-substrate 14
 systems 6
- FFDCA. see Federal Food, Drug, and Cosmetics Act (FFDCA)
- FFS. see Farmer Field Schools (FFS)
- Filistata hibernalis* 299
- FitD protein 159
- Food and Agriculture Organization (FAO) 51, 54–56
- Food Machinery Corporation 299
- Food safety 55
- Frankliniella occidentalis* 79
- Fungus gnats 214

- Galanthus nivalis* agglutinin (GNA) 305
Galleria mellonella 117, 240
 GbNV. *see* *Gryllus bimaculatus nudivirus* (GbNV)
 Genetically modified (GM) crops 135, 139, 142–144
 Genetically modified (GM)-plants 303
 GH5_27 243
 GH20 proteins 242, 243
 Gibberellins 153
Gilpinia hercyniae 168
 Global FFS Network Platform 35
 Glycosylphosphatidylinositol (GPI) anchor 109
 GM. *see* Genetically modified (GM)-plants
 GM crops. *see* Genetically modified (GM) crops
 GNA. *see* *Galanthus nivalis* agglutinin (GNA)
 GPI. *see* Glycosylphosphatidylinositol (GPI) anchor
 'Green' agendas 50
 Green Guard® 69
 Green Muscle® 69
 'Green' technologies 4
Gryllus bimaculatus nudivirus (GbNV) 176
 Guava weevil. *see* *Conotrachelus psidii*
 Gypsy moth. *see* *Lymantria dispar*
Hadronyche versuta 304
 HaSNPV. *see* *Helicoverpa armigera* SNPV (HaSNPV)
 HaSV. *see* *Helicoverpa armigera* stunt virus (HaSV)
 HCN. *see* Hydrogen cyanide (HCN)
Helicoverpa armigera 119, 141, 264
Helicoverpa armigera SNPV (HaSNPV) 182, 186
Helicoverpa armigera stunt virus (HaSV) 172
Helicoverpa zea 206
Heliothis armigera 304
Heliothis virescens 264, 305
Heterorhabditis spp. 197, 198
 axenic 199
H. bacteriophora 202, 203, 206, 209, 214, 221
H. downesi 215
H. indica 209, 210, 214
H. megidis 203
H. zealandica 210
(Z)-9-hexadecenal 254
 ω -hexatoxin-Hv1a (Hv1a) 304
 HLB. *see* Huanglongbing (HLB)
 Hob spider venom. *see* *Tegenaria agrestis*
 Horticulture 55
 Host interactions and baculovirus 178–180
 House fly. *see* *Musca domestica*
 HR. *see* Hypersensitive response (HR)
 Huanglongbing (HLB) 80
Hv1a. *see* ω -hexatoxin-Hv1a (Hv1a)
 Hydrocarbons 254
 Hydrogen cyanide (HCN) 158
Hylobius abietis 214
 Hypersensitive response (HR) 302
Hyphantria cunea 254
 IAA. *see* Indoleacetic acid (IAA)
 IBMA. *see* International Biocontrol Manufacturers Association (IBMA)
 ICK. *see* Inhibitory cysteine knot (ICK)-type peptides
 IJ. *see* Infective juvenile (IJ)
 Indoleacetic acid (IAA) 153
 Induced systemic resistance (ISR) 157–158
 Infective juvenile (IJ) 196–201, 217
 innate longevity of 202
 Infochemical 284
 Inhibitory cysteine knot (ICK)-type peptides 307
 Insect Resistance Action Committee (IRAC) 103
 Integrated pest control (IPC) 22
 Integrated pest management (IPM) and biopesticides 16, 64
 biocontrol products, definitions 23–25
 case study 35–38
 in crop production systems 27–30
 integrating 30–31, 33, 35
 overview 21–23
 regulatory assessment 25–27
 Integrated production 22
 Intellectual property (IP) protection 317
 International Biocontrol Manufacturers Association (IBMA) 38, 49, 52, 53, 56, 59, 320, 329
 International Code of Conduct on the Distribution and Use of Pesticides 22
 International Organization for Biological and Integrated Control (IOBC) 22, 51, 88
 Intracellular pathway models 108
 Invasive fruit fly. *see* *Bactrocera invadens*

- IOBC. *see* International Organization for Biological and Integrated Control (IOBC)
- IP. *see* Intellectual property (IP) protection
- IPC. *see* Integrated pest control (IPC)
- IPM. *see* Integrated pest management (IPM) and biopesticides
- Ips* spp.
- I. avulsus* 81-82
 - I. latidens* 255
 - I. pini* 255
- IRAC. *see* Insect Resistance Action Committee (IRAC)
- Irish potato famine 233
- Isaria javanica* 80
- ISCA 253
- ISR. *see* Induced systemic resistance (ISR)
- Ixodes scapularis* 83
- Japanese beetle. *see* *Popillia japonica*
- Japanese giant funnelweb spider. *see* *Macrothele gigas*
- (Z)-Jasmone 263
- Kairomones 36, 283
- Kisatchie National Forest 81
- Lactones 255
- Lagenidium* spp. 236, 238
- Laginex 238
- Large pine weevil. *see* *Hylobius abietis*
- Lecanicillium muscarium* 80
- Lepidoptera 260
- Lepidopteran pheromones 266
- Lepidoptera order 205
- Leptolegnia chapmanii* 235, 238, 239
- Lobesia botrana* 261
- Locusta migratoria manilensis* 70
- Locust and grasshoppers 69-71
- Lsaria fumosorosea* 80
- Lycoriella ingenua* 79
- Lymantria dispar* 261
- Lysinibacillus sphaericus* 102, 109, 110
- Macrothele gigas* 304
- Magi6 gene 304
- Maize weevil. *see* *Sitophilus zeamais*
- Makes caterpillars floppy (Mcf) toxin 159
- Mamestra brassicae MNPV (MbMNPV) 179
- Matsukemin 173
- Maximum residue levels (MRLs) 55
- MbMNPV. *see* Mamestra brassicae MNPV (MbMNPV)
- MCAs. *see* Microbial control agents (MCAs)
- Mcf toxin. *see* Makes caterpillars floppy (Mcf) toxin
- Mediterranean fruit fly. *see* *Ceratitis capitata*
- Melanoplus sanguinipes* entomopoxvirus (MSEV) 175, 176
- Meso-dispensers 268
- Metarhizium* spp. 65-66, 76
- M. acridum* 70
 - M. anisopliae* 77-80, 220-221
 - M. brunneum* 72-74, 82, 270
 - M. rileyi* 76
- Methyl-branched pheromones 255
- Methyl ketone (MK) 266
- Microbial and Nematode Control of Invertebrate Pests 88
- Microbial biopesticides 16-17
- development 6
 - process 7-9
 - economics 5-6
 - exploration and discovery 10-11
 - biological characterization and development 12-13
 - fermentation and formulation 13-15
 - overview 3
 - role 3-5
- Microbial control agents (MCAs) 117-118
- Microencapsulation 290
- Millennium Ecosystem Assessment 23
- Mole crickets 210-211
- Mosquitocidal (Mtx) activity 109, 110
- Mosquito larvae pathogens 237
- Mosquito-oomycete pathosystems 236
- MRLs. *see* Maximum residue levels (MRLs)
- MSEV. *see* *Melanoplus sanguinipes* entomopoxvirus (MSEV)
- MtDef4 306
- Mtx. *see* Mosquitocidal (Mtx) activity
- Musca domestica* 83
- Mycoinsecticides 66, 68
- Nagoya protocol 322
- Nanoformulation 290
- National Farmers Union (NFU) 51
- Natural substances 24
- Navel orangeworm. *see* *Amyelois transitella*
- Neem. *see* *Azadirachta indica*
- NFU. *see* National Farmers Union (NFU)
- Nonexpressor of pathogenesis-related genes (NPR1) 302
- Non-ionic surfactants 15
- NPR1. *see* Nonexpressor of pathogenesis-related genes (NPR1)

- NPS Pharmaceuticals Inc. 299
 Nudiviruses 176-178
- OBs. *see* Occlusion bodies (OBs)
 Occlusion bodies (OBs) 172, 173, 178, 179
 Occlusion-derived virions (ODV) 178
 (Z)-13-octadecenal 254
 ODV. *see* Occlusion-derived virions (ODV)
Oedaleius senegalensis
 entomopoxvirus 175
Oleander scale. see *Aspidiotus nerii*
 Olfactorily acting 252
 Olive fruit fly. *see* *Bactrocera oleae*
 Oomycete infectious propagules 239
 Oomycetes 233, 243. *see also*
 Entomopathogenic oomycetes,
 advances in use of
 Open field vegetables 29
 Optical brighteners 187
 Orfamide A 158, 159
 Organochlorine pesticides 326
 Organophosphates 4
 Ornate bella moth. *see* *Utetheisa ornatrix* L.
 OrNV. *see* *Oryctes rhinoceros nudivirus*
 (OrNV)
 Orthoptera order 205
Oryctes rhinoceros 177-178
Oryctes rhinoceros nudivirus (OrNV)
 176-178
Oryctes virus 177
Ostrinia nubilalis 135, 140-141
Otiorhynchus spp. 207
O. sulcatus 74, 213
- Paenibacillus* spp.
P. lentimorbus 102
P. popilliae 102, 111, 112
Parvoviridae. see Densoviruses
 Pasteuria BioScience 6
 PCPB. *see* Pest Control Products Board
 (PCPB)
- Pea moth. *see* *Cydia nigricana* F.
 Pecan weevil. *see* *Curculio caryae*
Pectinophora gossypiella 141
 Peptides as novel biopesticides
 future trends 307-308
 harpins 302
 overview 297-298
 plant defensin and antifungal
 peptides 306-307
 plant expression of 303-306
 SPEAR® 301-302
- trypsin modulating oostatic factor
 (TMOF) 302-303
 venom peptides 298-299
 discovery of 299-301
 Perennial production systems 28-29
Periplaneta fuliginosa 174
 Pest Control Products Board (PCPB) 55
 Pesticide Use and Risk Reduction (PURE) 27
 Pest Management Centre (PMC) 4
 Pest Management Regulatory Agency
 (PMRA) 4
 Pest Management Science 292
Phasmorhabditis hermaphrodita 216
 Pheromone receptors (PRs) 256
 Pheromone-responsive neurons (PRNs) 256
 Pheromones 36
 advances in use of
 advancement and future
 perspectives 266-271
 attract and kill 257-259, 261-262
 future trends 271
 mass trapping 257-261
 mating disruption 257-261
 monitoring 256-259
 overview of 251-254
 pheromone inhibitors 263-266
 push-pull strategies 257-259,
 262-263
 structural diversity and specificity
 of 252, 254-256
Phidippus audax 300
Phoma macrostoma 10
 Phospholipase 159
Photorhabdus spp. 199
P. luminescens 115, 159
Phyllocladis citrella S. 261
Phytophthora infestans 233, 234, 240, 242
Pichia pastoris 303
 Pink bollworm. *see* *Pectinophora gossypiella*
Pinus contorta 255
 Plant defensins 306
 Plant-derived plant protection products
 development 320-321
 botanicals efficacy 323-324
 extraction process 327-329
 IP protection 324-325
 novelty of invention 324-325
 plant extracts identification 321-323
 raw material and supply chains
 325-327
 legal framework and authorization
 317-320

- overview 315–317
- Plant growth-promoting bacteria (PGPBs)** 160–161
- action against plant pathogens 153–154
 - applications in agriculture and forestry 156
 - capability and role 152–153
 - overview 151–152
 - potential against invertebrate pests 154–155
- Pseudomonas protegens* 156–159
- Plant Health Care** 303
- Plant-parasitic nematodes (PPNs)** 154
- Plant pesticides** 24, 36
- Plant protection products (PPPs)** 53, 316
- Plectreurus tristis* 300
- Plum curculio.** *see Conotrachelus nenuphar*
- Plutella xylostella* (L.) 270
- PMC.** *see Pest Management Centre (PMC)*
- PMRA.** *see Pest Management Regulatory Agency (PMRA)*
- Popillia japonica* 72, 111, 255
- Potato virus X (PVX)** 306
- Poxviridae.** *see Entomopoxviruses*
- PPNs.** *see Plant-parasitic nematodes (PPNs)*
- PPPs.** *see Plant protection products (PPPs)*
- Primer pheromones** 253
- Primitive hunting spider.** *see Plectreurus tristis*
- Prionus californicus* 260
- PRNs.** *see Pheromone-responsive neurons (PRNs)*
- Prophyla** 6
- Protected systems** 29
- PRs.** *see Pheromone receptors (PRs)*
- Pseudomonas** spp.
- P. entomophila* 117
 - P. fluorescens* 155
 - P. protegens* 152
- Psylliodes chrysocephalus* 36
- PURE.** *see Pesticide Use and Risk Reduction (PURE)*
- Pure active substance 318
- Push-pull strategy** 36–37
- Push-pull system** 289
- PVX.** *see Potato virus X (PVX)*
- Pyoluteorin** 158
- Pyoverdine** 156
- Pyrethrins** 291
- Pyrethroids** 4
- Pyrrolitetrin** 158
- Pythium** spp.
- P. guiyangense* 235, 238, 239, 241
- P. oligandrum* 238
- Rape winter stem weevil.** *see Ceutorhynchus picitarsis*
- Rapporteur Member State** 53
- REACH.** *see stration, Evaluation, and Authorisation of Chemicals (REACH)*
- Red palm weevil.** *see Rhynchophorus ferrugineus*
- Reduced-Risk Products Initiative** 4
- Registration, Evaluation, and Authorisation of Chemicals (REACH)** 320
- Regulation (EC) 1107/2009** 48, 53, 54
- Regulation (EU) 1107/2009** 316
- Regulation (EC) No 1107/2009** 318
- Regulation (EC) No 2229/2004** 316
- Regulatory approval processes and biological technologies** 59
- biopesticide sector development and requirements** 49–50
- challenges** 50–52
- developing good practice** 56–58
- establishing framework** 46–47
- in European Union (EU)** 47–49
- current 52–54
 - global national initiatives 54–56
 - overview 45–46
- Reoviruses** 172–173
- Repellents** 284
- Rhinoceros beetle.** *see Oryctes rhinoceros*
- Rhizobacterial communities** 151
- Rhynchophorus ferrugineus* 82, 209
- Root weevil.** *see Otiorrhynchus* spp.
- Salvia officinalis* 321
- Saprolegnia parasitica* 237, 240–242
- SAR.** *see Systemic acquired resistance (SAR)*
- Scapteriscus* spp. 210–211
- SCFAH.** *see Standing Committee for Food Chain and Animal Health (SCFAH)*
- Schizocosha ocreata* 83
- Scirtothrips dorsalis* 80
- Sclerotinia** spp.
- S. minor* 37
 - S. sclerotiorum* 31, 37
- Segestria florentina* 300
- Semi-natural habitats (SNHs)** 23, 31, 33
- Semiochemicals.** *see Pheromones, advances in use of*
- Sensory neuron membrane proteins (SNMPs)** 256

- Sep1.see Serine protease (Sep1)
 Serenade® 11
 Serine protease (Sep1) 154
Serratia spp.
 S. entomophila 102, 119, 155
 S. nematodiphila 155
Sesamia nonagrioides 135
 Sex pheromones 253
 Siderophore-producing bacteria (SPB) 153
 Silkworm moth. see *Bombyx mori* L.
Sitona lineatus 269
Sitophilus spp.
 S. oryzae 117
 S. zeamais 81
 S-layer protein (SlpC) 110
 SlpC. see S-layer protein (SlpC)
 Small- to medium-sized enterprises (SMEs) 5
 SMEs. see Small- to medium-sized enterprises (SMEs)
 SNHs. see Semi-natural habitats (SNHs)
 SNMPs. see Sensory neuron membrane proteins (SNMPs)
 Soil-dwelling insect pests 71
 Soil-inhabiting coleopterans
 black vine weevil (see *Otiorhynchus sulcatus*)
 Mediterranean flatheaded peachborer (see *Capnodis tenebrionis*)
 western corn rootworm (see *Diabrotica virgifera*)
 white grub 72
 wireworm 72-74
 Soil-inhabiting dipterans
 cabbage, carrot, and onion root flies 76-77
 forestry pests 81-82
 fungus gnats 79
 hemipteroidea 79-81
 invasive pests 82
 medical and veterinary pests 82-83
 store-grain pests 81
 tephritids 77-79
 tipulids 77
 Soil moisture 202
 Soil temperature 213
 Soil texture 202
Solenopsis invicta 255
 Soricimed Biopharma Inc. 300
 Southern house spider. see *Filistata hibernalis*
 SPB. see Siderophore-producing bacteria (SPB)
 SP-CSPB. see Spore coat and canoe-shaped parasporal body (SP-CSPB)
 Specialized Pheromone and Lure Application Technology (SPLAT®) 253, 268
 SPLAT®. see Specialized Pheromone and Lure Application Technology (SPLAT®)
Spodoptera litura 76
 Spore coat and canoe-shaped parasporal body (SP-CSPB) 113
 Spotted wing drosophila. see *Drosophila suzukii*
 Spray application systems 6
 Stable fly. see *Stomoxys calcitrans*
 Standing Committee for Food Chain and Animal Health (SCFAH) 53
Steinernema spp. 197-199
 S. carpocapsae 199, 202, 206-209, 212, 214, 215
 S. diaprepesi 210
 S. feltiae 203, 206, 214
 S. riobrave 206, 208, 210
Stomoxys calcitrans 83
Streptomyces avermitilis 118, 155
Striga 289
 Sugarbeet weevil. see *Temnorhinus mendicus*
Supella longipalpa 255
 Sustainable Use of Pesticides Directive 318
Synanthedon spp.
 S. exitiosa 208
 S. pictipes 208, 219
Syngenta 6, 300
Synomones 284
 Synthetic agrochemical insecticides 297
 Systemic acquired resistance (SAR) 157-158, 302
 T3SS. see Type 3 secretion system (T3SS)
 Tangler® 269
 Tc proteins. see Toxin complex (Tc) proteins
 Technical grade extract 328
Temnorhinus mendicus 207
 Temperature 202
Tengenaria agrestis 299
 Tetraviruses 171-172
 TFIs. see Treatment frequency indexes (TFIs)
Thaumatomibia leucotreta 209
Thaumetopoea pityocampa 254
 Thrips 214
 Thysanoptera order 205
Tipula paludosa 77, 213

- TMOF. *see* Trypsin modulating oostatic factor (TMOF)
- Tomato leafminer. *see* *Tuta absoluta*
- Toxin complex (Tc) proteins 115, 117
- Toxin oligomeric structures 108
- Toxoflavin 158
- Trap crops 288
- Treatment frequency indexes (TFIs) 26
- Trehalase enzyme 241
- Triatoma infestans* 82-83
- Trichobank™ 10
- Trifluoromethyl ketone (TFMK) 266
- Trypsin modulating oostatic factor (TMOF) 302-303
- Turnip cutworm. *see* *Agrotis segetum*
- Tuta absoluta* 256, 260
- Type 3 secretion system (T3SS) 302
- USDA. *see* US Department of Agriculture (USDA)
- US Department of Agriculture (USDA) 26
- US Environmental Protection Agency in 1995 237
- Uteheisa ornatrix* L. 254
- UV radiation 202
- Valent BioSciences 6
- Vegetative insecticidal proteins (Vip) 106
- Vestaron 301
- Vip. *see* Vegetative insecticidal proteins (Vip)
- Viroden 174
- Virus families, associated with insects 170-171
- Western corn rootworm (WCR). *see* *Diabrotica virgifera*
- Western Integrated Pest Management Center 26
- West Palearctic Regional Section (WPRS) 88
- WGP. *see* Working Group on Pesticides (WGP)
- White grubs 211, 213
- White mould. *see* *Sclerotinia sclerotiorum*
- WHO. *see* World Health Organisation (WHO)
- Working Group on Pesticides (WGP) 318
- World Health Organisation (WHO) 51, 54-56
- WPRS. *see* West Palearctic Regional Section (WPRS)
- Xenorhabdus* spp. 199
X. *nematophilus* 115
- Z11-16:OH 264
- Zeuzera pyrina* 260
- Zymoseptoria tritici* 37