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Preface

Strictly speaking, biological control, or biocontrol, is defined as the use of living organisms to reduce the abundance or impact of pests. These living organisms, known as biocontrol agents, are natural enemies or antagonists of the target pests and include arthropods, nematodes, fungi, oomycetes, bacteria and viruses. They are deployed according to three general strategies: importation, augmentation (which includes inundation and inoculation) and conservation. In this book, we put forward a broader definition of biocontrol that also embraces the products of living organisms as well as sterile or incompatible insect pests. To avoid any ambiguity, we have used the term "extended biocontrol" in the title and wherever needed to account for these inclusions. Extended biocontrol encompasses biological control sensu stricto, autocidal control, and the use of semiochemicals and plant-derived biopesticides.

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Introduction

The field of biological control is currently undergoing an expansion that is closely tied to our growing awareness of the problems associated with massive synthetic pesticide use in agriculture and efforts to identify alternatives. And yet, the idea of biological control itself – using the tools provided by nature to manage crop parasites and pests – is not new. The main reasons why "biological" approaches are not more widely used in agriculture in industrialized countries are that synthetic pesticides have long been cheap, easy to access and effective, and users were largely unaware of their negative impacts on the environment, biodiversity and even human health. But things have changed, especially in Europe, and many countries have begun enacting more proactive policies. This has helped bring sustainable farming systems and environmental protection back to the fore while pushing agricultural research to explore new paths. Areas that had previously been overlooked are now being reconsidered and will surely become the cornerstones of crop protection in the future.

This book rounds up the latest research in biological control and crop protection methods based on natural pest control, which we will refer to here as "extended biocontrol". The authors take a critical look at the various solutions that are, or will one day be, available to agriculture. The book also explores the underlying concepts that are key to understanding and making use of biological interactions in cropping systems as well as the possible applications under consideration or already being used in the field. Readers should note that there are other approaches to crop protection, such as the use of resistant plants or preventive measures, that are not covered in this book.

Crops Need to Be Protected – But Differently

Humans and other organisms, including pathogens and insects, have likely been competing for crops since the advent of agriculture (Stukenbrock et al. 2007). In the West, the oldest references to plant protection methods date back to the Romans

(Cato the Elder ca. 160 BC). In Europe, the modern history of crop protection emerged in the nineteenth century following the appearance of major pests and diseases: the introduction of grapevine powdery mildew from 1845 and the demonstration – and then broad use – of sulphur to control it, and the introduction of grapevine downy mildew in 1878 and the development of Bordeaux mixture in the early 1880s.

However, the European agricultural world experienced a sea change just after the Second World War with a newfound political drive to intensify production to feed the continent's population. Agricultural research efforts steadily increased yields; France, for example, achieved food self-sufficiency in the 1970s and was able to begin generating trade surpluses. This extraordinary progress was made possible by radical shifts in farm structure, greater mechanization, access to abundant fertilization and intensive genetic selection efforts to obtain more productive crop varieties.

In terms of food security, this agricultural intensification was a success. However, it also made crops more vulnerable to pests. Merely growing dense crops of the same species opened the door to epidemics and the proliferation of pests. Organizing farms into large fields of genetically homogeneous crops and applying generous quantities of nitrogen fertilizers only worsened things. The solution to the problem, initially solved by systematically applying chemical pesticides, created new problems that are now up to us to tackle.

The use of synthetic pesticides in agriculture – and more specifically, herbicides and insecticides – rose following the Second World War. Synthetic fungicides appeared in the 1960s, and became more widespread in the 1970s with formulas containing systemic and curative active ingredients. These increasingly efficient products successfully managed pests and diseases while allowing the new varieties to reach their full yield potential. They made intensive production systems possible while limiting health risks. But for farmers, pesticide use also become something of a crutch, and some analysts would even say an addiction (Bonnefoy 2012; Valo 2012). Their heavy use led to two major problems: first, the selection of resistance in target pests, and second, major environmental and health impacts.

Although the environmental and health impacts related to pesticides have attracted considerable media attention in recent years, we have known about these issues for some time. Already in 1962, American biologist Rachel Carson shone a spotlight on the problem with her book *Silent Spring*. Little by little, the public authorities started banning the most dangerous substances (e.g. organochlorine insecticides like DDT). Even more recently, the discovery of the sublethal effect of neonicotinoids on bees (Henry et al. 2012) led the authorities to take steps to ban the use of some of these products in Europe.

Public attitudes have also changed markedly as of late with regard to pesticide use, and producers can no longer turn a blind eye. The many press articles on conflicts between farmers and local residents are a telling example. Consumers are quite vocal in their desire for fresh produce that is free of pesticide residues, although general attitudes remain paradoxical: while shoppers are asking explicitly for untreated food, there is an implicit demand for cheap, perfect-looking fruit on shop shelves.

Extended Biocontrol: A Fresh Look at an Age-Old Approach

The term “extended biocontrol” used in this book refers to a set of crop pest-control methods based on natural mechanisms that go beyond those included in the narrow definition of biological control. Extended biocontrol includes the use of natural enemies, microbial control, semiochemicals (such as pheromones) and biopesticides.

Many of these approaches have been around a long time. The first examples of biological control supported by a scientific approach date back to the late nineteenth and early twentieth centuries (see Chaps. 3 and 4). Additionally, farmers have always used biological pest control methods on an empirical basis in subsistence and small-scale farming. The Creole garden, mentioned in Chap. 20, is an excellent example of this. By capitalizing on natural mechanisms to protect plants, the goal is to give modern agriculture new ways to use methods that had fallen by the wayside when we tried to ignore the earth’s natural checks and balances through fertilization and chemical protection. Whether inspiration comes from observing nature (you can see ladybirds eating aphids right in your own garden) or from analysing farmers’ first-hand knowledge (e.g. planting mixtures of mutually beneficial species), the challenge is to develop targeted academic research on biocontrol approaches with practical applications for productive agriculture to feed the world and provide a livelihood for farmers’ families. Although this is chiefly a biological and ecological engineering issue, there are also essential sociological and economic dimensions (presented in Chaps. 18, 19 and 20). We cannot simply substitute biological solutions for chemical solutions and keep our current cropping systems (see, especially, Chap. 20). Shifting from chemical to biological solutions will require a total overhaul of our farming systems: they must be redesigned and rebuilt, with proper support measures for producers.

The Biocontrol Arsenal

To get back to the main topic, what exactly are the “natural mechanisms” we are talking about? Chemical protection is based on a very straightforward idea: you buy a product that is compatible with a standard sprayer, put on protective clothing, and apply the product at the recommended dose (while trying not to think too much about the long-term impacts on the environment or the people living near your field). By comparison, extended biocontrol offers an array of options, which are all partially effective and must be used in certain ways. Sometimes, the application methods may still not be fully worked out.

And yet, these approaches boast some very interesting advantages. One major problem with chemical pesticides is that the pest populations they are meant to control can quickly develop resistance (just like how overuse of antibiotics can lead to bacterial resistance). Although some questions remain about the sustainability of extended biocontrol solutions (see Chap. 23), insects rarely develop resistance to their predators. Resistance in pests is related to their genetic variability and the long-term arms race coevolution with their enemies. Thus, what is problematic for chemical pesticides can be turned into an advantage here: biological control agents also have an ability to adapt that can be exploited for sustainable pest control (see Chap. 4).

Moreover, the range of solutions that are or may one day be available means that we can build crop protection systems around different constraints, with certain beneficial effects materializing once the systems are fully redesigned and the pest control methods are well mastered. One example is the evolution of greenhouse farming in the Almeria region of Spain. Following a health scandal linked to the presence of unauthorized pesticide residues in peppers, which led European buyers to switch suppliers, a radical conversion to biological control was carried out in this farming area, leading to the nearly total elimination of chemical control of thrips. Around 70% of local farmers adopted this approach, versus just 4% in 2006. This conversion was accompanied by improved technical expertise through training and financial support from the state (Global G.A.P. 2016). However, environmental and labour problems also arose along with this new form of intensification, serving as a reminder that while a biological approach to crop protection has undeniable advantages, it is not intrinsically virtuous (Mandard 2019). Biological control in greenhouses has also been remarkably successful. For example, in the Netherlands, more than 90% of tomatoes, cucumbers and peppers are grown using integrated pest management strategies that include biological control methods, resistant varieties and climate control.

The reasons that biological control in greenhouses is successful have nothing to do with idealistic intentions on the part of professionals, who are unwilling to take risks on high-value produce when pesticides cost so little. Instead, producers cite enhanced technical efficiency given the emergence of pesticide resistance, the possibility of using pollinators in greenhouses, no need for turnaround time or harvest delays, a lack of phytotoxicity, reduced monitoring and fewer necessary interventions, lack of risk for workers applying treatments, and consumer expectations. Biological control is perceived as a profitable, efficient system with multiple benefits. When combined with climate control in greenhouses and resistant varieties, biological control can support a coherent crop protection system in a confined environment, which can be difficult to extrapolate to the open field.

This book takes a comprehensive look at the different techniques available for extended biocontrol, which includes classical, augmentative and conservation biological control. Extended biocontrol also encompasses the sterile insect technique; the use of microorganisms that promote plant health along with those for microbial pest control; semiochemicals that can trick insects' senses; and various natural substances with a direct action on pests (biopesticides).

The Different Types of Biological Control

The first part of this book deals with the use of natural enemies. After a review of the general concepts and underlying scientific principles (Chaps. 1 and 2), the different practical approaches are then explained (Chaps. 3, 4 and 5). The second part of the book delves into conservation biological control, which relies on natural trophic interactions at the landscape scale (Chaps. 6, 7 and 8): here, the aim is not to deploy one organism (natural enemy) against another (pest), but rather to support and encourage natural predator and parasite relations that limit the proliferation of crop pests.

Classical biological control involves identifying a natural enemy, usually from the pest's native range, and then releasing it into the area to be protected. This approach is mainly used against invasive pests that have been accidentally introduced. The process (Chap. 3) entails:

- conducting an inventory of the natural enemies of the pest in its native range,
- characterizing the species (which may lead to a thorough review of a complex of morphologically similar species, but with different host ranges; see Chap. 1),
- characterizing the natural enemy's effectiveness and host range,
- assessing the possibilities of producing the natural enemy and setting up rearing facilities,
- releasing the natural enemy in the field and monitoring its dynamics.

Classical biological control has several major advantages. A successful introduction will have a sustained effect and not lead to significant additional costs, and the natural enemy can develop in hard-to-access areas. When everything comes together, it can be remarkably effective. However, this approach does not generate commercial profits and it must be implemented by public organizations with the support of relevant partners and sectors. One good example, as explained in Chap. 3, is the introduction of the parasitoid wasp *Torymus sinensis* to control the oriental chestnut gall wasp.

Augmentative biological control, described in Chap. 4, is based on the mass production of natural enemies that are then released in large numbers in a specific area, such as a greenhouse, to achieve immediate results. It works well on endemic pests and can replace classic chemical pesticides. This approach and the market for it are growing considerably, but efforts are mainly focused on high valued-added crops grown under cover. The number of new commercialized natural enemies increased sharply through the 1990s and then declined after 2000, partly due to more restrictive regulations on importing and introducing exotic species (based on the Nagoya Protocol on Access and Benefit-sharing, which followed on from the Convention on Biological Diversity and entered into force on 12 October 2014). Research efforts have been refocused on natural enemies that are endemic to the area in question, rather than relying so heavily on exotic species.

At first glance, these biological control approaches may seem relatively conventional, but the various chapters devoted to them, as well as Chaps. 18, 19, 20 and 21, show that scientific and technological research is essential to their current development and enhancing their effectiveness. The overarching aim is to use the most recent conceptual advances and cutting-edge technologies to develop solutions based on the understanding and mastery of biological systems. One of the most striking examples of this is the sterile insect technique, described in Chap. 5.

Finally, the idea of permanent biological regulation at the cultivated landscape scale can be generalized using the principles of community ecology and conservation biological control, which consists of maintaining and developing beneficial populations by managing the vegetation in field margins, modifying certain farming practices, introducing companion plants or planting refuges. Chapters 6, 7, and 8 explain why it is important for recommendations to be founded on science-based knowledge of community ecology. They also reveal how, in turn, the issues raised by the practical application of recommendations can inform this discipline and its theoretical expectations, such as regarding the link between food web functioning and structure at the landscape level.

Microbial Control

Scientists now recognize that plants have their own microbiota, and that this microbiota influences plant health. Modern tools used in genomics and functional biology have produced considerable insights into the assembly rules of these microbiota and their interactions with plants, their physiology, and especially their immunity. Such approaches have also shed new light on microbial antagonism, which can limit pathogen development, for example, this phenomenon has long been known to occur in suppressive soils.

Chapters 9 and 10 lay out the approaches available to microbial community ecology to tackle these issues and develop solutions for crop protection and agro-ecological management of farming areas. Once again, there is strong application potential, and efforts should go well beyond the few commercially available strains sold as natural protective or beneficial organisms to allow for integrative management of the microbial environment of crops. The scientific challenge to be addressed here has largely been set, but current developments are very promising.

Although practical applications for microbiota management in agriculture will not be immediately available, some microbial strains and compounds produced by microbes are already being used. Chapters 11 and 12 provide a summary of such organisms and products along with the conditions and limits of their use.

As with insects, examples of microorganisms being used for pest management can be found as early as the end of the nineteenth century, and research on the subject was produced throughout the twentieth century. There has been renewed interest in this type of solution in recent years, and more than 200 strains of microorganisms are currently authorized for crop protection worldwide. However, developing effective operational solutions requires more research to understand the modes of action involved (antibiosis, hyperparasitism, stimulation of plant immunity, competition, etc.) and the conditions for good efficacy in the field.

Botanical Biopesticides

Plant extracts have been used for millennia as a means of pest control in agriculture, and the gradual banning of entire classes of chemical pesticides following new European regulations has intensified interest in these natural substances. Chapters 13 and 14 explain what these substances are and the challenges to be addressed in order to develop their use. While some, such as pyrethrins, are well known and have even inspired synthetic products, there is still major development potential for new botanical biopesticides. These products are popular with the general public, but they pose many challenges in terms of research and development, from diversifying effective products to facilitating their production and adapting their formulation, as well as identifying non-target effects.

Semiochemicals

Other types of chemical compounds known as semiochemicals (pheromones, kairomones, etc.) that regulate the behaviour of many organisms already play a key role in crop protection, but they will become increasingly important in the future. Chapters 15, 16 and 17 take a closer look at these compounds and their role in insect biology, and then explain how they can be used for crop protection. Their purpose is to attract insects into a trap or throw them off track as they seek out a mate or host plant. The research involved in such processes runs the gamut from genomics to chemical ecology and behavioural analysis. New genomics approaches can now be used in reverse chemical ecology: researchers start by analysing the genome and identifying coded proteins to then trace their way back to the odorant molecules that activate the olfactory receptors. It is easy to see how pest control using semiochemicals might gradually replace chemical insecticides in field crops. But it is also a very complementary approach to other methods, such as the use of companion plants to attract or repel certain pests.

From the Lab to the Field

Even as laboratories continuously learn more about biological systems, turning a proof of concept into an operational solution requires in-depth knowledge of the conditions of effectiveness, adoption and extended biocontrol use in cropping systems. Chapters 18, 19, 20 and 21 explore these aspects in detail, covering the technical, regulatory, economic and organizational considerations. One key idea here is that extended biocontrol will not fully replace chemical control. Instead, it must fit into a comprehensive redesign of cropping systems in order to make the agroecological transition a reality and reduce the world's dependence on synthetic pesticides.

Finally, it is important to note that biological approaches are not always flawless and could potentially pose problems for non-target organisms, cause uncontrolled proliferation or prove to be unsustainable if a pest evolves. "Natural" is not synonymous with "safe", and an active substance used on a large scale may have undesirable effects on non-target organisms (including people). Although we might agree that the potential risks associated with the use of extended biocontrol methods are much lower than the proven and major risks posed by synthetic pesticides, we must take a cautious approach. The two final Chaps. 22 and 23 address these issues.

Challenges to Be Tackled

Generally speaking – and this will be apparent throughout the book – the development of extended biocontrol requires research in systematics, ecology, demogenetics of small populations, the major biological functions of the relevant organisms (olfaction, symbiosis, immunity, etc.), and the interaction mechanisms among plants, microbes, pests and natural enemies. It also requires methodological developments to enable operational solutions and a redesign of cropping systems to integrate them.

The organisms that are suitable for extended biocontrol are potentially numerous, but the production, packaging and application problems related to their use remain largely unresolved. Such issues are engineering related and require cooperation between research and industry on subjects that deal not only with biology but also chemistry and even robotics.

The performance of biological control agents varies from one individual to another within a given species. Choosing the most effective strains or lines for pest control requires the development of phenotyping methods to assess the behaviours and traits to be selected. Science is still just scratching the surface of this issue, but further research could significantly increase the potential of extended biocontrol.

If we push the idea further, we might imagine eventually breeding these organisms. This raises issues of professional conduct and ethical acceptability, but conventional breeding is a possibility here, as is genetic transformation. Without going quite so far, one main advantage of biological organisms is their potential for natural diversity, which could help cope with the issue of resistance in target pests. The case has already been made for one particular entomopathogenic virus, with the selection of a second-generation virus capable of infecting codling moth lines that developed resistance to the original strain (see Chap. 23).

Extended biocontrol methods are generally very specific. While this is an important advantage that helps protect non-target species, it is also a disadvantage, because crops are attacked by multiple pests. Rather than focusing on a particular crop or pest, we must adopt a holistic vision of the farming system, integrating all beneficial ecological processes contributing to pest control, whatever their scale (from the landscape to the plant). This will involve combining landscape planning that supports natural enemies along with the use of companion plants, pheromone traps, natural insecticides, etc. Agricultural production would then benefit from a full range of ecosystem services associated with natural pest control as well as specific extended biocontrol actions.

Transitioning to biological-based crop protection will involve more than merely replacing one technology with another. The change will be more extensive and will require considering the production system as a whole and doing away with methods that are partially or totally incompatible with biological control. Extended biocontrol specialists and agricultural scientists must work together to redesign cropping systems accordingly.

Biological control is now widely used in high-tech greenhouses and climate-controlled environments. These systems have the benefit of being partly separated from the outside environment, and must deal with some pests that would not fare well or survive outdoors in winter. Meanwhile, biological control in the open field, particularly in vineyards, orchards and large fields of cash crops, remains a major challenge to tackle.

Creating landscapes that support natural pest control is crucial for crop protection. Pest outbreaks are largely the result of modern farming practices, with large, very homogeneous fields and reduced interactions with biodiversity reservoirs. Acting at the landscape scale should make it possible to reduce the overall risk, and in doing so benefit all practitioners. This raises fundamental questions in terms of community ecology, as well as for public policy and how stakeholders are organized.

Indeed, the challenge goes beyond the technical dimension of the production system and must involve the various players, right down to consumers. The economic, social and political dimensions of the expected innovations and changes remain largely unexplored.

Introductions of exotic pests are becoming increasingly frequent due to climate change and intense global trade. Official eradication strategies often revolve around the use of pesticides, and chemical products may indeed seem more effective. However, they pose a clear danger to the environment, and success is not guaranteed. One example is the case of the western corn rootworm, which was introduced into France in 2002; attempts to eradicate it failed. Biological control has an important role to play here, and there are several examples of notable successes (Cock et al. 2016), but effective ways of dealing with most introduced pests are still out of reach. Anticipating the arrival of exotic pests and planning ahead for possible biological control methods is a strategic issue for agriculture.

Combining Extended Biocontrol with Other Plant-Health Management Methods

The classic approach to integrated pest management combines different phytosanitary practices and integrates them into the crop management sequence at field level. More specifically, farmers can take into account the agronomic factors likely to reduce the impact of pests and diseases. This type of approach is clearly useful and should be promoted, but one should also note that it has been implemented without solving the fundamental problem of dependence on chemical pesticides. Success with integrated pest management is often local and driven by an innovative farmer or group of farmers; however, when placed within an economic and organizational model, it cannot always be generalized.

From an agroecological perspective, future success will depend on the complete integration of all available crop protection levers. Protection must combine the many possible solutions offered by extended biocontrol, but this alone will not suffice, especially when it comes to controlling pathogens. These methods will have to be used in conjunction with varietal choices based on sustainable and efficient management of resistant varieties, as well as preventive farming practices that can maintain high yields. Solutions will also have to be scaled up from the field level to the cultivated landscape. Pest population dynamics are generally determined at scales larger than the field, and trying to solve a general problem by taking limited local actions without a comprehensive plan can turn into a game of whack-a-mole.

Overhauling crop protection in such a way will require scientists to adopt a multidisciplinary approach (for example, combining population dynamics and economics) and take into account the most relevant biological and time scales. This is where the challenge lies for research, development and end-user networks.

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Part I
Biological Control: Theoretical Foundations and Applications

Xavier Fauvergue

This book provides a broad view of biological control, which we have referred to as extended biocontrol, including the use of natural enemies, sterile male insects, microbiomes, biopesticides and semiochemicals. The rationale behind this more comprehensive perspective is that many natural products that fall beyond the narrow scope of biological control are much more amenable to modern practices and commercialization. Does this mean that biological control sensu stricto is an old-school endeavour – a souvenir from a bygone era when naturalist entomologists with insect nets were at the top of their game in foreign explorations and trial-and-error introductions of exotic natural enemies? In this first section as well as the following one, we will show that exactly the opposite is true.

Biological control, sometimes shortened to biocontrol, is strictly defined as the use of living organisms to reduce the abundance or impact of pests. The three basic strategies – classical, conservation and augmentative (which covers both inoculation and inundation) – involve either introducing small or large numbers of exotic or endemic biological control agents into a target environment, or adapting the target environment to promote the action of the agents already present. A range of organisms, aka natural enemies, can be used: predators (e.g. lady beetles that prey on aphids), parasitoids (e.g. *Trichogramma* wasps that parasitize moth eggs) and pathogens (e.g. bacteria, fungi, viruses, oomycetes), as well as herbivores in the biological control of weeds. The sterile males released in large numbers to manage pests using autocidal control also fit the definition of natural enemies, even if they are rarely considered as such.

This first part of the book will show how these different biological control methods have been modernized through perpetual dialogue between theoretical approaches, methodological developments, naturalist observations, engineering,

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legislation, and finally national and international practices. We will see, for example, how progress in the biology of introduced populations and in modelling underpin a robust conceptual framework for understanding and optimizing biological control agent populations (Chaps. 2 and 4); how the technological advances in molecular biology and high-throughput genomics are ensuring unparalleled relevance in knowledge of beneficial species and populations (Chap. 1); how fresh enthusiasm for the sterile insect technique is generating as many research projects as applications in the field (Chap. 5); or finally, how classical biological control, which involves the use of exotic organisms, has adapted to new legislative rules for the exchange and introduction of living organisms around the world and thus maintained its status as the preferred method for controlling invasive pests (Chap. 3).

The history of modern biological control stretches back several decades for some methods (augmentative biological control and autocidal control), while classical biological control has been around for more than a century. The use of these strategies shows no signs of abating. The following chapters clearly demonstrate a renewed momentum in this field, fuelled by the emergence of new frontiers in science and our desire to make the most of them.

Chapter 1
Integrative Systematics and Adaptations of Natural Enemies to Their Hosts

Nicolas Ris, Éric Pierre, and Jean-Claude Streito

1.1 Introduction

Beneficials, or beneficial organisms, is a broad term used to describe organisms that provide ecosystem services in an agronomic context. They are useful in agriculture because they enhance soil quality, pollinate crops and control other organisms that damage crops: weeds, pathogens and plant-eating pests. When used for this last purpose, they are known as biological control agents or natural enemies. These organisms include invertebrates, such as insects, mites and nematodes; vertebrates, such as birds and bats; and microorganisms, such as fungi, bacteria and viruses.

In this chapter, we will look specifically at the macroorganisms that are generally used in biological control. Microorganisms (bacteria, viruses, fungi, etc.) will be dealt with in Chaps. 9, 10, and 11. The main macroorganisms used in biological control are arthropods – chiefly insects and mites – and nematodes, which are used against other invertebrates (which will be the focus here) or weeds. One example of weed biocontrol via macroorganisms is the use of the sawfly *Cibdela janthina* (Hymenoptera: Argidae) against the giant bramble (*Rubus alceifolius*), an invasive plant on Réunion Island (see also Chap. 3).

This chapter is divided into three sections. In the first, we will delve into the taxonomic diversity of macroorganisms and the many antagonistic interactions they have with target pests. In the second, we will present integrative systematics, a discipline that is now central to the identification of and interactions between species used in biological control, regardless of the chosen strategy (classical, augmentative or conservation). In the last section, we will adopt an eco-evolutionary perspective to

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look at three cross-cutting themes that are anchored in academic inquiry, but which are absolutely relevant for research and development.

In general, the bibliographical references have been pared down as much as possible. The most frequently cited reviews or experimental research have been favoured at the risk of overlooking equally interesting but more recent references.

1.2 General Remarks on the Diversity of Macroorganisms for Biological Control

1.2.1 Taxonomic Diversity

Even when limited to macroorganisms, the natural enemies that can be used in biological control are extremely diverse. This diversity results in a very large number of species with various feeding strategies and that show multiple adaptations, from morphological to physiological and behavioural. Table 1.1 lists the main orders and families of arthropods used for biological control, together with their estimated species richness. Among insects (see Fig. 1.1), a few species of earwigs (Carroll and Hoyt 1984) and thrips (Mound 2005) are considered to be biological control agents for crops. However, most of the potential agents are found in the five main orders: Hemiptera, Neuroptera, Coleoptera, Diptera and Hymenoptera. Among hemipterans, only the suborder Heteroptera ("true bugs") includes natural enemies from two families of particular importance in crop protection, Anthocoridae and Miridae (Lattin 1999; Cassis and Schuh 2012). Among neuropterans, the best-known agents are Chrysopidae, with the genus *Chrysoperla* (Daane et al. 1996). Among coleopterans, there are two main families used in biocontrol, Carabidae (Gardiner et al. 2010; Minarro and Dapena 2003) and Coccinellidae (Giorgi et al. 2009; Kuznetsov 1999), while dipterans include three families: Cecidomyiidae, Syrphidae and Tachinidae (Stireman et al. 2006; Feener and Brown 1997; Pineda and Marcos-Garcia 2008; Cheng et al. 1992). Finally, there are several families found among hymenopterans, such as Ichneumonidae, Braconidae and several families of Chalcidoidea, namely Aphelinidae, Encyrtidae and Trichogrammatidae (Godfray 1994; Quicke 1997). Besides insects, arachnids make up a significant contingent of natural enemies. In particular, several mites from the family Phytoseiidae are used (McMurtry et al. 2013). Spiders and Opiliones also play an important but often underestimated role. Myriapods, which are less studied, must also be considered. Aside from arthropods, nematodes are the last important group of macroorganisms, which includes several entomopathogenic species from the order Rhabditida, including several from the genus *Steinernema* (Stuart et al. 2008; Shapiro-Ilan et al. 2006). We do not currently know the exact – or even approximate – number of natural enemies that could potentially be used for biological control, as many species remain undescribed and, most importantly, their biology has not been studied. However, we could venture an estimate of more than 300,000 species worldwide, and likely more than 5500 in France. By comparison, the list of potential pests for France is about 2200 species.

Table 1.1 Orders and main arthropod families with natural enemies for plant protection

Orders	Families	Number of species France (World)	Phytophagous	Feeding strategy	Larva/ adult	Key taxa
Dermaptera		**21 (1930)**				
	Forficulidae	14 (500)	Yes	Predators	L/A	*Forficula auricularia*
Thysanoptera		**260 (6174)**				
	Aeolothripidae Thripidae	17 (207) 135 (2100)	No Yes	Predators Predators	L/A L/A	*Aeolothrips* *Franklinothrips* *Scolothrips*
Hemiptera		**3670 (105,500)**				
	Anthocoridae	50 (500)	No	Predators	L/A	*Anthocoris* *Orius*
	Miridae	510 (11,130)	Yes	Predators	L/A	*Macrolophus* *Nesidiocoris* *Deraeocoris*
	Nabidae	28 (386)	No	Predators	L/A	*Nabis*
	Reduviidae	51 (7000)	Yes (rare)	Predators	L/A	
	Pentatomidae	96 (4937)	Yes	Predators	L/A	
Neuroptera		**162 (5704)**				
	Chrysopidae	51 (1415)	No	Predators	L/A	*Chrysoperla*
	Hemerobiidae	45 (591)	No	Predators	L/A	
	Coniopterygidae	23 (571)	No	Predators	L/A	
Coleoptera		**11,670 (386,755)**				
	Carabidae	1335 (40,000)	Yes	Predators (parasitoids)	L/A	
	Coccinellidae	135 (6000)	Yes	Predators	L/A	*Adalia* *Chilocorus* *Coccinella*

(continued)

Table 1.1 (continued)

Orders	Families	Number of species France (World)	Phytophagous	Feeding strategy	Larva/ adult	Key taxa
						Cryptolaemus *Harmonia* *Rodolia*
	Staphylinidae	2886 (56,000)	Yes	Predators (parasitoids)	L/A	
	Cantharidae	157 (5100)	Yes	Predators	L/A	
Diptera		**9100 (154,969)**[3]				
	Cecidomyiidae	662[4] (6470)[1]	Yes	Predators	L	*Aphidoletes* *Feltiella*
	Syrphidae	555[2] (6000)[3]	Yes	Predators	L	*Episyrphus* *Xanthandrus*
	Tachinidae	608[2] (8550)[3]	No	Parasitoids	L	*Pseudoperichaeta* *Trichopoda*
	Chamaemyiidae	28[2] (350)[3]	No	Predators	L	
	Pipunculidae	113[2] (1410)[3]	No	Parasitoids	L	
Hymenoptera		**8600 (154,067)**				
	Braconidae	988 (19,439)	Yes	Parasitoids	L	*Aphidius* *Cotesia* *Diaeretiella* *Praon*
	Ichneumonidae	3426 (24,281)	No	Parasitoids (predators)	L	*Apanteles* *Diadegma*
	Aphelinidae	62 (1078)	No	Parasitoids	L	*Aphelinus* *Encarsia* *Eretmocerus*
	Bethylidae	59[2] (2588)	No	Parasitoids	L	

	Chalcididae	46 (1469)	No	Parasitoids	L	
	Dryinidae	59 (1605)	No	Parasitoids	L	
	Encyrtidae	197 (4058)	No	Parasitoids	L	*Encyrtus* *Metaphycus* *Pseudaphycus*
	Eulophidae	413 (4969)	Yes	Parasitoids	L	
	Eupelmidae	53 (931)	No	Parasitoids	L	
	Eurytomidae	97 (1453)	Yes	Parasitoids	L	
	Mymaridae	53 (1437)	No	Parasitoids	L	
	Platygastridae	66[2] (5385)	No	Parasitoids	L	
	Pteromalidae	289 (3544)	No	Parasitoids	L	
	Torymidae	117 (900)	No	Parasitoids	L	
	Trichogrammatidae	42 (881)	No	Parasitoids	L	*Trichogramma*
Araneae		**1620 (45,752)**[5]	**No**	**Predators**	**L/A**	
Opiliones		**123 (6616)**[5]	**Yes**	**Predators**	**L/A**	
Acari (subclass)		**> 1500 (54,580)**[5]	**Yes**			
	Phytoseiidae	120 (2251)	No	Predators	L/A	*Amblyseius* *Neoseiulus* *Phytoseiulus*

Table 1.1 indicates the approximate numbers of species known in France and described worldwide as well as their dominant food sources. The figures correspond to taxonomic groups; some comprise only natural enemies (such as Anthocoridae), but others also include phytophagous species (such as Miridae, Pentatomidae, etc.). Accordingly, these figures do not always correspond specifically to natural enemies

With regard to food sources, phytophagous refers to an organism that eats plant matter other than pollen (many adult natural enemy insects consume pollen at least occasionally). “Yes” in the Phytophagous column means there are phytophagous species within the group. For zoophagous species, we specify whether they are parasitoid or predatory species (Feeding strategy), as well as the stage (larva or adult) at which the organism is beneficial for pest control

Sources: the numbers of species for France are drawn from unpublished research (Dermaptera and Hemiptera: Streito; Thysanoptera: Reynaud; Neuroptera: Michel; Hymenoptera: Delvare; Phytoseiidae: Kreiter and Tixier). Worldwide species numbers are mainly taken from the following reference: Foottit and Adler (2017). More specific references are as follows: 1. Gagné and Jaschhof (2017), 2. Gargominy et al. (2018), 3. Martinez (2020), 4. Skuhravá et al. (2005) and 5. Delfosse (2015)

Fig. 1.1 The diverse range of insect natural enemies. (**a**) *Macrolophus melanotoma*, Miridae predator on *Dittrichia.* (**b**) Dipteran larvae (Chamaemyiidae family), predator of aphids. (**c**) Seven-spotted lady beetle *Coccinella septempunctata*, predator of aphids. (**d**) Adult spiked shield bugs *Picromerus bidens* (Pentatomidae family) attacking a caterpillar. (**e**) Adult tachinid fly *Gymnosoma rotundatum*, a dipteran parasitoid of shield bugs. (**f**) Green lacewing larvae (Neuroptera: Chrysopidae) eating a fruit fly. (**g**) Adult *Eretmocerus*, a hymenopteran parasitoid of whiteflies, emerging from a *Bemisia tabaci* puparium. (**h**) *Trissolcus semistriatus*, a hymenopteran parasitoid of Pentatomidae shield bug eggs. (Photos: © J.-C. Streito, except photo E, © M. Huguet)

1.2.2 Antagonistic Interactions Between Natural Enemies and Target Pests

There are three main feeding strategies among macroorganisms that regulate plant pests: (i) predation, (ii) parasitoidism and (iii) parasitism. Predators complete their development cycle by killing and consuming multiple prey. All vertebrate natural enemies (e.g. birds, bats), dermapterans, thysanopterans, neuropterans, hemipterans and arachnids (including mites) are predators, as are most coleopterans and some dipterans and hymenopterans. In all, there are more than 220,000 predatory species worldwide; lady beetles are probably the most widely known and are discussed in greater detail in the Sect. 1.4.1 “Ecological specialization in natural enemies”. Parasitoids generally complete their entire preimaginal development at the expense of a single host, which is eventually killed (Godfray 1994; Quicke 1997). Heraty (2017) estimates the number of parasitoids described worldwide at 77,000 species (about 10% of insects), but the diversity of insects remains largely unknown and there are likely an astounding 680,000 different species. Parasitoids are mainly found in the orders Diptera (13,646 species described) and Hymenoptera (69,785 species described), although a few species are known among Coleoptera (e.g. *Aleochara bilineata*) and several other orders (Strepsiptera, Trichoptera, Lepidoptera, Neuroptera) (Heraty 2017). *Trichogramma* are an emblematic example of parasitoids used in augmentative biological control (see Chap. 4) because of their suitability for mass rearing and use on field crops. Parasitic macroorganisms include entomopathogenic nematodes, whose larvae infest their hosts, develop and multiply for several generations before killing the hosts and releasing mobile juveniles that will infest new hosts (Burnell and Stock 2000). Some natural enemies adopt hybrid feeding strategies. Some species behave as parasitoids in the preimaginal state and as predators in the adult state – sometimes on the same target species, which then becomes a host and/or prey (this is known as host-feeding behaviour, see Jervis and Kidd 1986; Heimpel and Collier 1996). As we will also see in this chapter (see Sect. 1.4.1 “Ecological specialization”), natural enemies can sometimes also exploit food sources other than their host/prey for their development.

1.3 The Underlying Challenges of Identifying Beneficial Macroorganisms

1.3.1 Identification Issues

Given the species diversity of potential natural enemies as discussed in the previous section, one of the major challenges of biological control research and development lies in identifying an organism that will be fully effective against the target pest in the desired context, while also ensuring little to no ecological impact on non-target species (see Chaps. 3 and 4 for more on non-target effects). Agricultural advisors and farmers dealing with a pest outbreak must also be able to recognize the pests and choose the right natural enemy. Errors in the identification of one or the other can lead to poor effectiveness or even failure of biological control. For example, when dealing with a whitefly outbreak, the parasitoid *Encarsia formosa* will be highly effective against *Trialeurodes vaporariorum*, but much less so against *Bemisia tabaci* and not at all against *Crenidorsum aroidephagus*. Text Box 1.1 also shows another example of an ineffective biological control strategy due to poor understanding of potential natural enemies and their ecology.

Given the species diversity of potential natural enemies (Table 1.1), most studies conducted without the support of taxonomists stop at the order or family level. Identifying the genus of natural enemies is certainly even more challenging, but not impossible for an experienced entomologist. Indeed, the most important genera are relatively few in number and well characterized. This is the case, for example, for minute pirate bugs (Anthocoridae), with two main genera of natural enemies that are easy to recognize – *Orius* and *Anthocoris* – and for green lacewings (Chrysopidae), most of whose commercial species belong to the genus *Chrysoperla*. Simplified identification keys are available, such as for wine grape natural enemies in France (Sentenac 2011). Identifying natural enemies at the species level is, however, extremely difficult, especially as new studies often reveal a previously unknown degree of diversity. This is particularly evident in "species complexes", which are characterized by species that are morphologically very similar, but genetically quite different. Such complexes have been found in predators, particularly in the genera *Macrolophus* (Text Box 1.1), *Orius* and *Nabis* in true bugs or *Chrysoperla* in lacewings. In parasitoids, they have been detected in the genera *Aphytis*, *Encarsia*, *Eupelmus* and *Trichogramma*. Ideally, each type of beneficial would be subject to a thorough taxonomic review.

1.3.2 Integrative Taxonomy: A Core Discipline

Although the notion of species was defined by Ernst Mayr (1942) based on the concept of interbreeding between individuals, the delimitation of species and higher taxonomic levels (genus, family, order, etc.) has historically been based on morphological characterization. This includes the examination of genitalia, which are relatively easily observable structures that often reflect reproductive barriers, as well as more qualitative criteria on other parts of the body (e.g. the presence of bristles, pores or patterns) and morphometric variables that can be used for statistical processing. However, morphological information alone will not suffice, particularly given the issues related to species complexes. This is why integrative taxonomy approaches are now favoured; this discipline aims to describe the units of living organisms through different complementary sources of information (according to Dayrat 2005).

While morphological characterization obviously continues to be an extremely important approach to discriminate between species and maintain links to previous (sometimes very old) research, molecular information provides crucial insights. More specifically, DNA barcoding studies, which seek to describe diversity on the basis of a few molecular markers, have become more widespread. For arthropods, an international consortium selected a portion of approximately 600–700 base pairs of the mitochondrial cytochrome c oxidase subunit I (COI) gene (Hebert and Gregory 2005). Although the approach has been criticized and limits have been identified (see, for example, Buhay 2009; Collins and Cruickshank 2013; Lis et al. 2016), it is clearly useful in identifying different taxa. In particular, the use of this marker made it possible to unequivocally distinguish between European species of the genus *Macrolophus* and species of the *Eupelmus urozonus* complex (see Text Box 1.1), where morphology had initially reached its limits. In some cases, however, this approach is not a panacea: for instance, it has not been of any help in differentiating between species of green lacewings of the genus *Chrysoperla*. As a result, other genes must be used to compile sufficient information. More recently, the development of high-throughput sequencing has led to significant strides in systematics: specific differences on several thousand markers can now be explored at a reasonable cost.

Text Box 1.1: Biodiversity, Companion Plants and Ecosystem Services: Not So Simple

Initially, most biological control solutions come from empirical observations that are then generalized. While such an approach is logical and has resulted in successful innovations, it cannot be taken for granted and requires real expertise before it can be widely promoted. In recent years, we have had the opportunity to focus on the particular case of false yellowhead, *Dittrichia viscosa*, a common Mediterranean asterid that is especially abundant in some fallow agricultural areas and urban wastelands. Generally speaking, false yellowhead has many virtues (Parolin et al. 2014): it can decontaminate soils, has pharmacological properties, produces pollen for bees, has a biocidal action on pathogens and can be used as a beneficial plant for biological control (Parolin et al. 2014; Ris et al. 2014), for which two benefits were envisaged.

With regard to olive cultivation, *D. viscosa* was considered as a host plant for the fruit fly *Myopites stylatus* (Diptera: Tephritidae), which may be parastized by hymenopteran parasitoids of the *Eupelmus urozonus* complex (Hymenoptera: Eupelmidae), which are also known to parasitize the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae). Planting false yellowhead near olive groves would thus favour the local abundance of *Eupelmus* and increase parasitism on the olive fruit fly.

For vegetable crops, false yellowhead is viewed as a potential reservoir of various sap-sucking phytophagous insects (aphids, whiteflies) that can be preyed upon by predatory bugs, particularly of the family Miridae and the genus *Macrolophus*. As such, false yellowhead could help support (over time and in numbers) natural enemy populations.

(continued)

Text Box 1.1 (continued)

The main observation from our investigations is that there is an unsuspected diversity of candidate biological control agents. With regard to olive cultivation, the complete taxonomic revision of the *Eupelmus urozonus* complex has brought to light 11 new species, two of which (*E. confusus* and *E. gemellus*) are associated with the olive fruit fly (Al Khatib et al. 2014, 2016). Moreover, at the sites studied, there is very little cross-over of *Eupelmus* communities associated with the two hosts (*E. confusus*, *E. gemellus* and *E. urozonus* for *Bactrocera oleae* and *E. kiefferi* for *Mypoites stylatus*), contrary to the initial "virtuous" scenario put forward.

Similarly, molecular and morphological characterization confirms the distinction between *Macrolophus pygmaeus* (a species also marketed for augmentative biological control) and *Macrolophus melanotoma*, two predatory bugs that have long been confused (Bout et al. 2019). At the sites studied, these two species show significant niche segregation, with *M. pygmaeus* being relatively rare on false yellowhead and restricted to crops (probably often linked to intentional releases) and *M. melanotoma* being found on false yellowhead (see Fig. 1.1) where it is particularly important.

This research tends to call into question the beneficial role of false yellowhead for the two supposed ecosystem services and the geographical area under consideration. However, these findings do not completely exclude false yellowhead as a possible contributor to crop pest regulation in other geographical areas, in association with other companion plants and via other mechanisms.

Sometimes, integrative taxonomy uses more unconventional sources of information such as specific behaviours. This is the case for *Chrysoperla* species, which can be distinguished based on the vibrations they emit during courtship (Henry et al. 1999). The study of larval stages, host range, pheromones, etc. can also reveal useful characteristics. Finally, when natural enemies are reared, cross-breeding tests can directly assess reproductive compatibility (Stouthamer et al. 1996, 2000; Benvenuto et al. 2012).

Integrative taxonomy thus combines a set of techniques that enable specialists to form an opinion on species delimitation and ultimately to recognize those species. However, while these methods are used in research, they are not meant for non-specialists because they are too complex and costly. Based on this academic research, the challenge is then to develop characterization tools that are accessible in terms of price, technical difficulty and processing times. Depending on the case, these tools may use different molecular methods (taxon-specific amplification, size polymorphism, Sanger sequencing, or even next-generation sequencing technologies) or techniques based on morphology/morphometry. Several initiatives of this type are currently underway on taxa of interest (scale insects, thrips, *Trichogramma* wasps, etc.).

1.3.3 Other Benefits of Molecular Diagnostics

The molecular methods used in taxonomy and integrative systematics can meet other needs beyond identification, such as evaluating real ecosystem services provided by natural enemies. They are used to characterize and understand the ecological relationships (species co-occurrence, food webs) within communities that depend on agricultural systems, such as identifying parasitoids within their host, prey in a predator's digestive tract or the insects caught in a trap (see Chap. 6). High-throughput sequencing has been successfully used to detect, identify and count parasitoids of oilseed rape stem weevils, species that are particularly difficult to rear (Robert et al. 2019).

These molecular identifications are based on the search for DNA in a matrix (an insect's digestive content, trap, soil, etc.), followed by comparison of the sequences obtained with correctly identified reference sequences. Currently, international databases of DNA sequences or libraries are still far from complete. For example, DNA barcoding has been performed on just 12% of described hemipterans (Wilson et al. 2017). Moreover, these databases are unfortunately sometimes marred by identification errors (see, for example, Lis et al. 2016). To eliminate errors linked to misidentification of reference sequences, France's National Research Institute for Agriculture, Food and Environment (INRAE) is developing a molecular database at its Centre for Biology and Population Management (CBGP) containing morphologically and phylogenetically validated sequences specific to crop pests and natural enemies. This database, which is currently being developed, contains more than 5000 sequences of about 500 natural enemy species (Arthemis 2021). In addition to these errors that are inherent to molecular databases, other biases have already been identified and must be taken into account to avoid hasty interpretation (Alberdi et al. 2019).

Finally, diagnostics must sometimes be approached on an infraspecific scale. This is true for certain pests (e.g. aphids) in which individuals/populations/strains present very different molecular and ecological characteristics (e.g. host races, biotypes, subspecies; see for example De Barro 2005; Carletto et al. 2009). Although examples are still limited, such rankings can also be observed at the upper trophic level, which includes natural enemies, requiring population genetics approaches.

1.4 A Deeper Look at Three Cross-Cutting Eco-Evolutionary Themes

1.4.1 Ecological Specialization in Natural Enemies

A particularly important aspect of the ecology of natural enemies is their degree of specialization in relation to prey (for predators) and hosts (for parasitoids and parasites). Once again, diversity is the rule, and natural enemies may fall anywhere on the spectrum from monophagous to broadly polyphagous and even zoophytophagous organisms.

Lady beetles, for example (Giorgi et al. 2009), show considerable variability in taxonomic status and number of prey consumed. The Asian lady beetle *Harmonia axyridis* is extremely polyphagous, consuming aphids as well as butterfly eggs and even the larvae of other lady beetles. The two-spotted lady beetle *Adalia bipunctata* and seven-spotted lady beetle *Coccinella septempunctata* have a clear preference for aphids (they can be called aphidophagous) although they may occasionally be opportunistic and consume other prey. Other species such as *Chilocorus bipustulatus* have a marked preference for scale insects (and so are described as coccidophagous) while others are aleurodophagous and specialize in whiteflies (e.g. *Delphastus pusillus* or *Clitostethus arcuatus*). The tiny *Stethorus pusillus* is acariphagous and attacks mites. Some species are virtually monophagous, such as the *Rodolia cardinalis*, which was the subject of the first documented case of classical biological control (see Chap. 3); this species feeds almost exclusively on the cottony cushion scale *Icerya purchasi*. Finally, some rare lady beetles are phytophagous, such as *Henosepilachna argus*, which eat plants from the curcubit family, or mycophagous, such as *Psyllobora vigintiduopunctata*. This latter species could even be considered as a natural enemy against leaf diseases such as powdery mildew and blight (Aberlenc 2011). This type of specialization is also observed in Phytoseiidae mites (Kreiter 2011) and other predator groups.

In parasitoids, such variability is also common. Some species are indeed extremely polyphagous and can parasitize several orders of insects: the pteromalid *Pachyneuron muscarum* attacks not only scale insects, but also psyllids, aphids and lady beetles (Delvare 2011). However, the complex and necessary adaptations that have evolved in the relationship between parasitoids and their hosts have often led to a much greater degree of ecological specialization within this group. As a result, many species have become specialized or even ultra-specialized. The torymid wasp *Torymus sinensis* is a perfect example of this: it attacks almost exclusively the oriental chestnut gall wasp *Dryocosmus kuriphilus* (see Chap. 4). Contrasting host ranges can even be observed between species that are phylogenetically similar. Thus, the *Eupelmus urozonus* species complex comprises several different species: those strictly specialized on one host species (*Eupelmus pistaciae* or *E. tibicinis*), those specialized on the family Cynipidae (*E.azureus* and *E. fulvipes*) and those capable of parasitizing different orders (*E. confusus*, *E. kiefferi* and *E. urozonus*) (Al Khatib et al. 2014, 2016).

The situation is even more complex when the organism is phytophagous. The best-known case is that of *Nesidiocoris tenuis* (Castañé et al. 2011). This highly polyphagous predatory tomato bug attacks prey of all kinds, including whitefly larvae, *Tuta absoluta* eggs and young larvae, etc. Under certain conditions, such as in Morocco or southern Spain, this species behaves as a very effective generalist predator and is released in augmentative biological control programmes (see Chap. 4). Used in greenhouses in France and northern Europe, *N. tenuis* also exhibits phytophagous behaviour and will feed on the apex of tomato plants, at times causing such significant damage that its interest as a biological control agent may be questionable. The causes of this behavioural variability (genetic determinism? environmental factors?) remain poorly understood. Less problematic, and sometimes even advantageous, cases of zoophytophagous behaviour are observed in other species of Miridae (see Chap. 4).

1.4.2 Behavioural Adaptations in Natural Enemies: Finding Targets

The taxonomic diversity and variability in feeding strategies and levels of specialization we have just touched on are accompanied by an array of adaptations, particularly in terms of behaviour. More specifically, natural enemies must be able to detect one or more targets, prey or hosts. Depending on the organism's biology, target selection is carried out by the individual itself for immediate (e.g. predatory arthropods) or delayed benefit (e.g. infectious larval stages in nematodes, and even some parasitoids whose first larval stages are mobile), or by the female for the benefit of her offspring (e.g. most parasitoid insects). Approaches likely differ between predatory arthropods on the one hand, which must consume multiple prey throughout their lives (e.g. between 50 and 100 *Aphis fabae* aphids per day for the lady beetle *Coccinella septempunctata*), and parasitoid insects and entomopathogenic nematodes on the other, which need only a single host.

In general, finding a prey or host is based on various non-exclusive types of visual, acoustic and olfactory cues (Vet and Dicke 1992; Stireman 2002; Steidle and van Loon 2003; Lewis et al. 2006; Giunti et al. 2015); olfactory cues are probably the most generic. As such, many studies have looked specifically at how natural enemies exploit chemical cues. For example, reviews by Vet and Dicke (1992) and Steidle and van Loon (2003) clearly show the various information sources that can be used and the questions regarding the origin of these detection capabilities.

Thus, natural enemies rely on three main information sources: substances emitted directly by prey or host, substances associated with by-products of hosts or prey (e.g. faeces, exuviae, honeydew or other secretions) and chemical cues emitted by plants (see Chaps. 13 and 15). In the latter case, these are the plant's constituent compounds and the compounds specially emitted by the plant in the event of attack by an herbivore pest, known as herbivore-induced plant volatiles (HIPV). HIPVs have attracted the attention of many researchers for more than 20 years as they appear to be a "win-win" mechanism for both the attacked plant and natural enemies (see for example reviews by de Boer and Dicke 2006; Turlings and Erb 2018). One particularly interesting tritrophic interaction is that involving (i) maize and its wild ancestor teosinte, (ii) herbivorous pests (notably the western corn rootworm *Diabrotica virgifera virgifera* and lepidopterans of the genus *Spodoptera*) and (iii) natural flying (parasitoid insects) or crawling enemies (entomopathogenic nematodes) (see de Lange et al. 2016; Turlings and Erb 2018 among others).

Regardless of the type of cue, questions arise as to whether the detection of targets (prey or hosts) by natural enemies is limited to innate ability, as has long been thought. Over the last 20 years or so, studies carried out on arthropods in general, and on predatory and parasitoid arthropods in particular, have unequivocally proven that they show an ability to learn (see for example de Boer and Dicke 2006; Dukas 2008; Giunti et al. 2015). Depending on feeding strategies and species, this learning is based on information perceived by the individual during its development or emergence as an adult (e.g. parasitoids), or during its first experiences in target selection (prey and hosts).

As mentioned previously, natural enemies as a whole show considerable diversity in the types of cues they perceive, the sources of information they use and the origin of their detection capabilities (innate and/or acquired). Several authors have tried to correlate the resulting behavioural strategies with the level of generalist/polyphagous behaviours of entomophagous arthropods in general and natural enemies in particular (see for example Vet and Dicke 1992; Steidle and van Loon 2003). The meta-analysis by Steidle and van Loon (2003), however, sets out very few general rules a posteriori. At most, it has been observed that specialist species are more likely to exploit host-specific chemical cues and, conversely, that generalist species are more likely to exploit generic cues. However, contrary to initial hypotheses, innate responses are no more frequent in specialist species than in generalists. Beyond academic considerations, the study of behavioural strategies is important with regard to operational applications for biological control, whether classical (see Chap. 3) or augmentative (see for example Giunti et al. 2015 and Chap. 4).

1.4.3 Diversity of Symbionts in Natural Enemies

For some 30 years now, entomological research has been trying to understand the role of symbionts, viruses and bacteria on the phenotype of their insect hosts. Natural enemies are no exception to this rule, and depending on the situation, the impact of symbionts on their hosts (here, predatory arthropods, entomopathogenic nematodes or parasitoid insects) may be (generally) positive or negative.

With regard to unquestionably beneficial impacts induced by symbionts, two exemplary cases can be cited: first, the association between nematodes and bacteria, and second, the "domestication" of viruses by certain hymenopteran parasitoids. Entomopathogenic nematodes (especially the genera *Heterorhabditis* and *Steinernema*) harbour symbiotic bacteria (the genera *Xenorhabdus* and *Photorhabdus*, respectively) whose metabolic activity is essential for host consumption (Burnell and Stock 2000; Forst et al. 1997). Following infection of the host by the infectious nematode larvae, the bacteria – until this point living within their host – are released into the hemolymph of the infected insect where they multiply and release a cocktail of insecticidal toxins, enzymes, and antibiotic and antifungal substances. All of these substances contribute directly to the host's death and its external digestion while also limiting the proliferation of competing organisms.

Once the insect is consumed, bacteria are reintegrated into the nematodes and the cycle continues. The ancient "domestication" of viruses by certain hymenopteran parasitoids is another fascinating example of mutualistic symbiosis (Herniou et al. 2013; Drezen et al. 2014). Such eco-evolutionary events have been highlighted in Braconidae and Ichneumonidae, where symbiosis enables the injection and expression of virulence factors capable of altering the immune defences of the parasitized and newly infected host. In both cases, similar processes were ultimately observed with a tight interconnection between the two partners (integration of the virus's genetic information into the parasitoid's genome; transfer of hymenopteran genes via viral mechanisms).

In addition to these two examples of well-established symbioses, some mutualisms are more "labile" (i.e. where the modalities are likely to vary between related host species or within a species). Certain endosymbiotic bacteria, particularly of the genus *Wolbachia*, can thus affect their host's normal reproduction and induce a thelytokous phenomenon, i.e. where an unfertilized female produces daughters, which are also thelytokous (Werren 1997; Stouthamer et al. 1999). The host's asexual reproduction is as advantageous for the endosymbiotic bacteria as it is for the host species. The bacteria thus maximize their transmission via this manipulation and the host population dynamics show improved performance (especially in establishment and growth rates), at least in the near and medium terms. Perhaps more anecdotally, *Wolbachia* can also induce other types of mutualism, as in the *Trichogramma* wasp *Trichogramma bourarachae* where it increases its host's fertility (Vavre et al. 1999).

With regard to the negative impacts induced by symbionts, and aside from cases of infection by "classic" pathogens (viruses, bacteria, fungi), a fairly general case is probably the induction of cytoplasmic incompatibility (CI). This is another manifestation of endosymbiotic bacteria manipulating arthropod reproduction. Unlike the induction of thelytoky, here the interests of the host and endosymbiont differ. While the endosymbiotic bacteria always benefit from the manipulation they induce, host populations are negatively impacted demographically, and even genetically, by the fact that some cross-breeding may prove sterile depending on the infection status of the male and female. As research progresses, CI appears to be a phenomenon induced by an ever-increasing diversity of endosymbiotic bacteria (genera *Wolbachia* and *Caridinium* in particular) and in the main taxa likely to provide biological control agents (parasitoid insects, predatory insects, predatory mites) (Enigl and Schausberger 2007; Duron et al. 2008; Hilgenboecker et al. 2008).

This brief overview of symbioses between microorganisms and taxa of interest for biological control shows the omnipresence of this type of interaction. This symbiotic and even microbiotic dimension must not be overlooked during biological control operations, especially at certain key stages such as choosing the candidate strain, or even the implementation of a genetic improvement approach and the long-term conservation of strains in an artificial environment.

1.5 Conclusion

The beneficial macroorganisms available for plant protection are extremely diverse and often very difficult to identify. This taxonomic diversity is accompanied by nuanced and extremely varied adaptations which ultimately have a major impact on the effectiveness of biological control strategies. As a result, correct initial discrimination of these species and their reliable routine identification are major challenges. Inaccurate characterization of natural enemies during the research and development phase or during field applications leads to suboptimal or even failed pest regulation. In turn, this can contribute to the discrediting of biological control, which has indeed happened in the past. At a time when attempts are being made to drastically reduce insecticide use, biological control solutions must be supported at the organizational level by (i) maintaining disciplinary competence in taxonomy for taxa of interest, (ii) increasing awareness and training among end users (farmers, advisors and consultants) and (iii) increasing capacities for routine diagnosis, backed by low-cost and rapid methods, and possibly supported by new stakeholders.

Chapter 2
The Biology of Introduced Populations

Xavier Fauvergue

2.1 Introduction

Several biological control strategies involve introducing natural enemies into a target environment to control pests: classical biological control (Chap. 3), augmentative biological control (Chap. 4) and autocidal control (Chap. 5). Introduction is a critical stage during which populations are vulnerable, and the conditions of introduction are those that practitioners can most easily manipulate. Analysis of classical biological control attempts suggests, for example, that a significant proportion of introduced populations fail to establish in the target environment, with two-thirds of entomophagous species (Cock et al. 2016) and one-third of phytophagous species failing (Schwarzlander et al. 2018). Thus, optimizing biological control approaches depends on understanding the underlying processes that determine introduction success. This type of research must start with population biology. The successful or failed introduction of biological control agents is measured, at the population level, on the basis of criteria such as the establishment and maintenance of the introduced population in the target environment and its population growth, dispersal, genetic evolution and impact on the target organisms. Other scales can also be considered, from the individual to the community, in order to document the basic tenets (physiology, life history traits, behaviours, etc.) and outcomes in terms of biodiversity dynamics. In this chapter, the three types of processes that are important for introduced populations will be explained so that all readers can understand them. Emphasis will be placed on classical biological control (Chap. 3), because this is the strategy for which introduction is most crucial. The sterile insect technique (Chap. 5), which is based on some of the same processes, will also be discussed briefly. To begin with, however, it seems appropriate to mention a few cross-cutting

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ideas that, once linked together, lay out the context, the problem and various possibilities for further research and application.

2.2 Ideas That Inspire

2.2.1 Science and Technology

Private companies as well as technical and research institutes can all oversee introductions of biological control agents. Classical biological control is most commonly handled by research institutes, making this method a public service in its own right. Operational progress is based on a combination of intuition, trial and error, acquisition and sharing of know-how, and research, both basic and applied. Accordingly, biological control is a field of application that draws on disciplines such as taxonomy (Chap. 1), population dynamics and genetics, community and landscape ecology (Chap. 6), and many others. If there is one deeply rooted idea in this chapter, it is that biological control benefits from these disciplines – their theoretical foundations and their empirical results – as well as from cooperation with other related fields of application, such as invasion biology and conservation biology. This interdisciplinarity generates reciprocal benefits for all involved. Classical biological control is an unparalleled framework for developing an experimental approach to understanding how small populations function, which is often impossible for threatened or invasive species.

2.2.2 Finding Equilibrium

Biological control has long advanced in step with theoretical research on the dynamics of host-parasitoid (or prey-predator) systems, the host in this context typically being an insect pest. The simple forms of these models, such as that of Nicholson and Bailey (1935), are unstable and lead to the inevitable extinction of hosts or parasitoids. The more effective the parasitoids are, the greater the instability, resulting in what Arditi and Berryman (1991) coined the "biological control paradox": in theory, it is impossible to achieve a strong and sustained reduction in the host population. This paradox has motivated several decades of theoretical and experimental research to identify stabilizing processes – for example, density-dependent behaviours such as lower parasitoid effectiveness at high densities (Bernstein 2000). Meanwhile, more recent research in invasion biology and conservation biology has focused on the dynamics and genetics of unstable populations. Indeed, many populations of interest (declining, invasive, introduced or reintroduced) clearly do not follow the classic assumptions of infinite size or equilibrium that were the basis of previous work. This is particularly true for natural enemy populations used in classical biological control, whose main characteristic is that

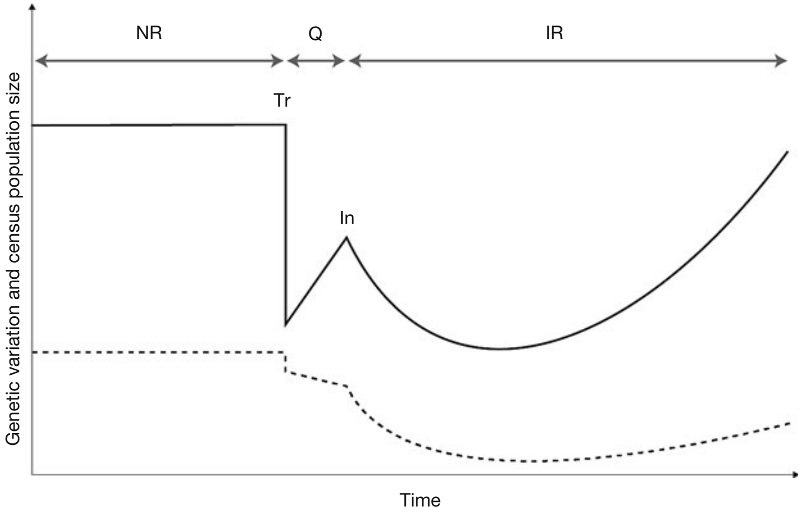

Fig. 2.1 Demographic and genetic bottlenecks in classical biological control. (Based on Fauvergue et al. 2012)
Introduced populations are characterized by major demographic and genetic disequilibria during transit (*Tr*) from their native range (*NR*) and when introduced (*In*) into the new introduced range (*IR*). The bottleneck in demographic population size (solid line) may be buffered by an episode of mass rearing in quarantine (*Q*) in the case of biological control. The effective population size (N_e dashed line), which relates to genetic diversity, decreases as a result of sampling in the native population and genetic drift during mass rearing as well as after introduction. In the absence of additional inputs of genetic material, the effective population size will lag behind demographic size and remain low even after the population has established and starts to grow

they experience genetic and demographic bottlenecks at the time of introduction (Fig. 2.1, see also Chap. 4 for disequilibrium dynamics in augmentative biological control). We must therefore identify and understand the consequences of these disequilibria in order to predict the outcome of introductions.

2.2.3 Propagule Pressure

When populations are introduced, whether planned or not and regardless of taxa, the most widespread and generally accepted observation is the positive effect of propagule pressure on the probability of population establishment (Fig. 2.2; Lockwood et al. 2005). Propagule pressure accounts for the total number of individuals introduced by multiplying the number of different introductions by the number of individuals per introduction. In practice, the effect of propagule pressure constrains the optimization of introductions: based on the total number of individuals to introduce, is it better to release a few individuals several times and in different

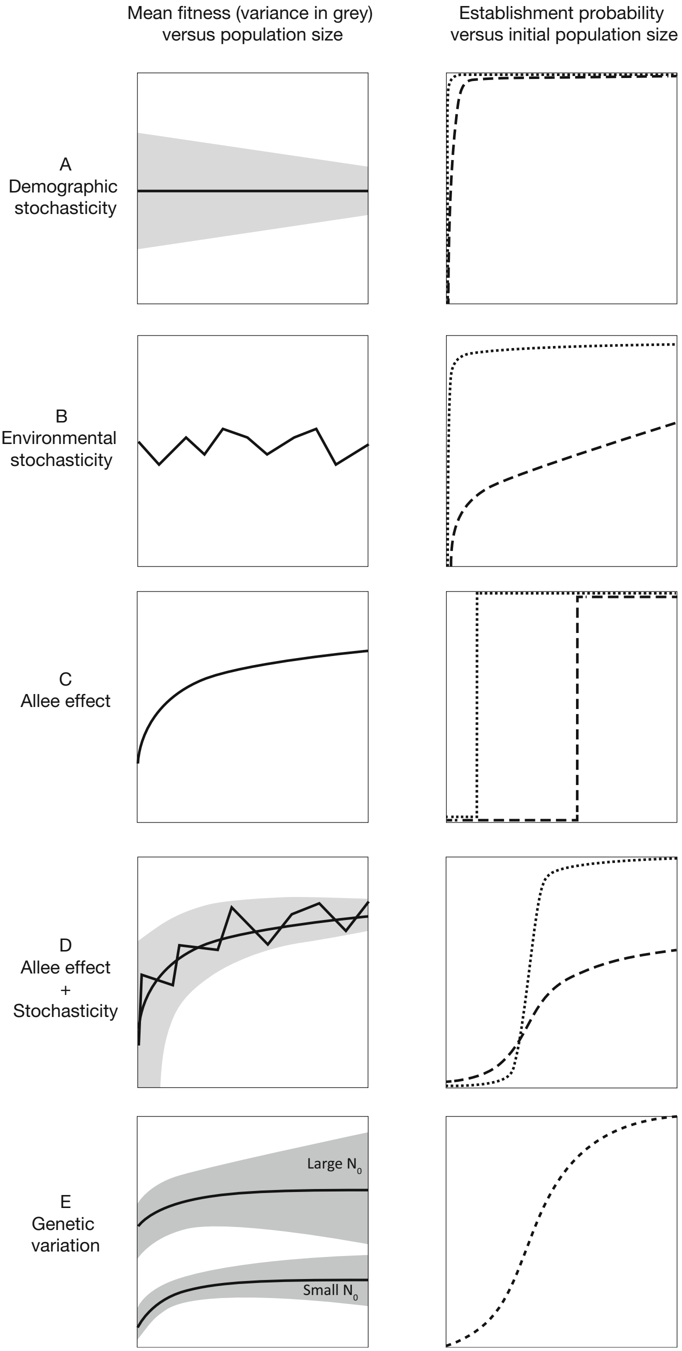

Fig. 2.2 General overview of theoretical predictions resulting from stochasticity, Allee effect and inbreeding depression in small introduced populations. (Based on Fauvergue et al. 2012)
Left panels: individual fitness versus current population size a few generations after introduction, during the establishment phase. The grey area represents variance in fitness. Right panels:

places, or release many individuals fewer times in strategic places? This question is neatly summed up in the age-old SLOSS debate (Single Large Or Several Small), which often pertains to chance events. At one extreme, if all the natural enemies are released once in a single field and a catastrophic event such as a storm or a fire occurs the following day, establishment will certainly fail. At the other extreme, the impact of chance events can be mitigated by releasing very small numbers in many places or at different times. However, none of the introduced population will persist because there are too few individuals. The answer to this question depends on the processes that occur in the introduced populations (Shea and Possingham 2000), which is all the more reason to better understand them.

2.3 The Laws of Small Numbers

In the simplest terms, population dynamics can be described as a process of births and deaths, so that the evolution of a population's size can be predicted as a function of birth and death rates b and d. For example, the discrete-time model $N_{t+1} = N_t + b\,N_t - d\,N_t$ means that the future number of individuals N_{t+1} depends solely on the current number N_t and constant birth and death rates (b and d represent the number of births and deaths per individual between successive time steps). This is a deterministic approach since any population that has N_t numbers at time t will unsurprisingly reach N_{t+1} at time $t + 1$. Taking stochasticity into account means accepting that this is not always the case. Contrary to what is implied by the above equation, in nature the rates b and d are not constants that apply identically to all time steps and to all individuals in a population, nor can the number be equated with an integer number. Two types of stochasticity emerge from this more realistic understanding.

When a birth rate is expressed (e.g. 1.674 offspring per individual per generation), it is an average that applies to the whole population. In reality, each individual produces an integer number of offspring (0, 1, 2, 3, etc.), and this number differs from one individual to another. Individual deviations from the average most often lack identifiable causes and are interpreted as a manifestation of demographic stochasticity. The key point for introduced population dynamics is that these inter-individual variations offset each other in large populations, but become perceptible

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Fig. 2.2 (continued) establishment probability versus initial population size (number of founders). The different lines represent differences in the strength of each process. (**a**, **b**) Demographic and environmental stochasticity. The short dash illustrates low stochasticity and the long dash illustrates high stochasticity. (**c**) Allee effect: the short dash shows the Allee threshold at small population size and the long dash shows it at the larger population size. (**d**) Allee effect and stochasticity; The short dash shows demographic stochasticity only. The long dash shows demographic and environmental stochasticity. (**e**) Hypothesized influence of genetic variation under a scenario with inbreeding depression and no purging of genetic load in small ones: “The dynamics of a small population are governed by the specific fortunes of each of its few individuals” (Caughley 1994). Indeed, by simple chance, several individuals in the same generation may reproduce at lower rates, but this will only have an impact on the growth rate if the population is small. Thus, the rate of increase is much more variable in small populations, and this combination of low numbers and fluctuations in the rate of increase leads to a higher probability of extinction (Fig. 2.3).

Demographic stochasticity is compounded by environmental stochasticity, which refers to the random fluctuations in the average population growth rate from one generation to the next. Environmental stochasticity is generated by unpredictable biotic or abiotic variations, such as a harsher winter that may cause an atypical increase in the average mortality rate, or variability in available resources affecting reproduction. Since all individuals in the population are affected, large populations are also impacted by this type of stochasticity, with the growth rate becoming as variable as in small populations (Fig. 2.3). Nevertheless, as with demographic stochasticity, the probability of extinction in the case of environmental stochasticity is higher in smaller population sizes, simply because zero population size is reached more quickly (it would take many generations of bad luck to reduce a large population to zero). Catastrophic events, such as fires, floods, etc., are an extreme form of environmental stochasticity: all individuals are impacted and the high probability of local extinction is not offset by the population size.

As such, stochasticity is a key process in defining a minimum viable population size (Shaffer 1981). This process is therefore taken into consideration both for the conservation of threatened species (Lande 1988) and for biological control (Fauvergue et al. 2012). Stochasticity results in a positive relationship between the number of individuals released and the probability of establishment in theoretical models (Fig. 2.2; Grevstad 1999b), and could therefore explain the recurrent observations of this relationship in the field (Hopper and Roush 1993). However, other processes, such as the Allee effect or inbreeding depression, lead to the same relationship (Fig. 2.2 and below); accordingly, the effect that the number of individuals introduced has on the probability of establishment cannot be explained by one of these processes alone. The positive effect of the number of repeated introductions on the probability of establishment (Hopper and Roush 1993) is a more specific clue to environmental stochasticity: if conditions vary from one introduction to another, several repeated introductions make it possible to buffer the effect of occasionally unfavourable conditions. In practice, introductions of biological control agents are often repeated (e.g. recent releases of *Torymus sinensis* against oriental chestnut gall wasps, Borowiec et al. 2018), a sign that practitioners have an intuitive understanding of environmental stochasticity.

Experimental introductions reveal the influence of stochasticity. On the one hand, estimated growth rates always vary from one environment to another, and a significant share of these variations cannot be explained by manipulated factors or density (Fauvergue and Hopper 2009; Fauvergue et al. 2007; Grevstad 1999a; Memmott et al. 2005). On the other hand, stochasticity is the most straightforward hypothesis to explain certain establishment failures observed in small population numbers

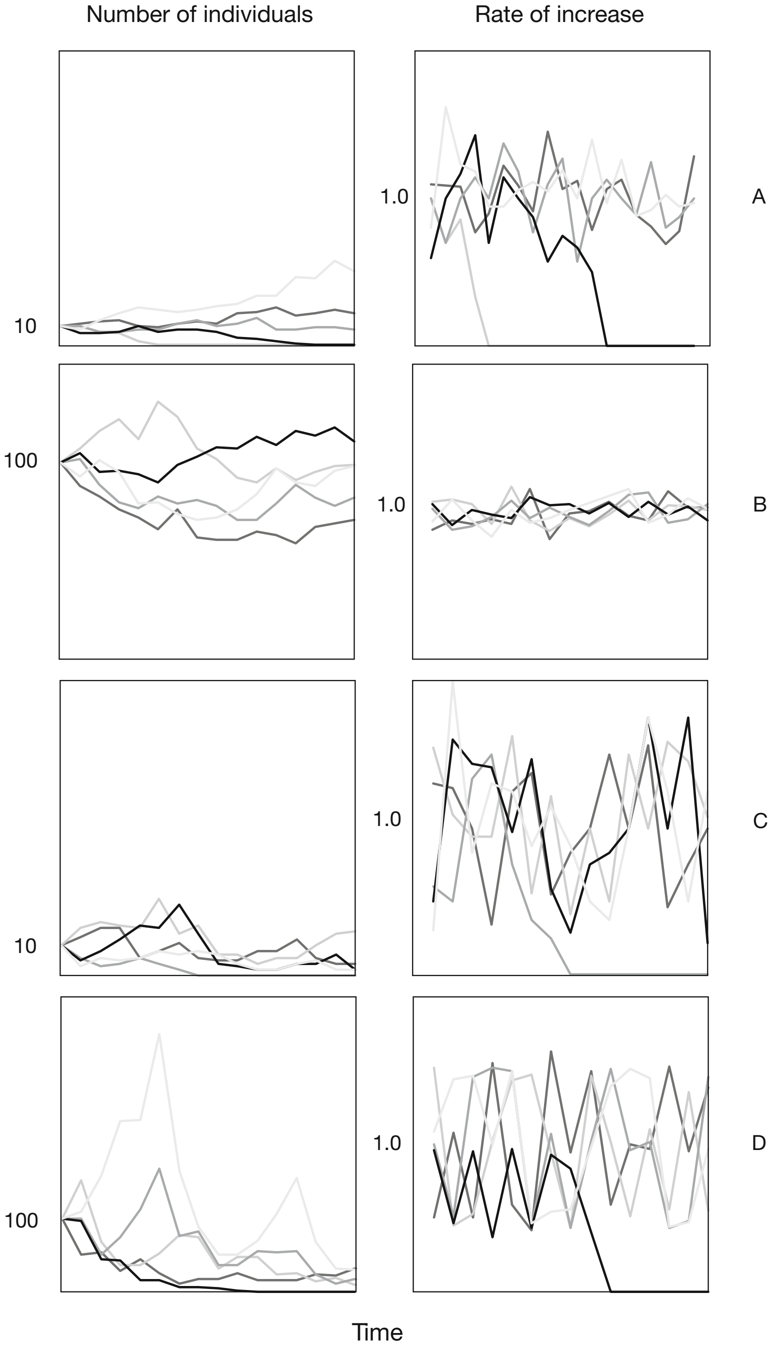

Fig. 2.3 Examples of stochastic effects on the dynamics of introduced populations
Each line represents a simulated population with an initial number of 10 or 100 individuals (5 repeated populations for each case). In the case of demographic stochasticity (*A*, *B*), each individual produces 1, 2 or 3 offspring per generation (*b*). The mortality rate *d* is constant at

(demographic stochasticity, Fauvergue et al. 2007; environmental stochasticity, Memmott et al. 2005).

2.4 Lost Benefits at Small Numbers: The Allee Effect

2.4.1 Definitions

The Allee effect is a phenomenon that was originally described in the 1930s, when the American ecologist Warder Clyde Allee observed small groups of beetles breeding in jars of flour. Growing awareness about the ongoing sixth mass species extinction has brought this phenomenon back to the fore, and publications on the topic have risen steadily since the 1990s. The Allee effect is now considered to be one of the major processes that influences small population dynamics and is taken into account in population management for declining, invasive, introduced and reintroduced populations.

The Allee effect is defined as a decrease in survival or reproduction in populations that are declining in size. For example, at low densities, sexual partners become scarcer and may have greater difficulty locating each other. As a result, the share of unfertilized females not producing offspring rises, and the population growth rate falls.

The Allee effect is defined at two levels and at two intensities (Stephens et al. 1999; Taylor and Hastings 2005). At the individual level, the *component Allee effect* refers to a decrease in one or more fitness components when the population size decreases (Fig. 2.2c). This decrease is generally caused by a change in cooperative behaviours, such as mate seeking, antipredator behaviours, rearing young, foraging, etc. At the population level, the *demographic Allee effect* refers to a decrease in the population growth rate when the population size falls. A demographic Allee effect is always caused by a component Allee effect, but a component Allee effect does not always result in a demographic Allee effect: the positive effects of cooperation are often offset by the negative effects of competition, so that a drop in numbers may have opposite consequences (for example, less mating but more food), and therefore not affect mean fitness. Furthermore, a demographic Allee effect is defined as weak when the growth rate remains positive, even for the smallest populations. When this happens, small populations continue to grow, but at a slower rate than when the

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Fig. 2.3 (continued) 2. This means that on average the population is stable ($b–d = 0$). The graphs show that random samples of b for each individual in each generation generate instability. This instability is all the greater when the population is smaller (A versus B), even to the point of extinction. In the case of environmental stochasticity (C, D), the average birth rate of the population is randomly sampled at each generation (1.5, 2.0, 2.5). The rest of the simulation follows the same procedure for sampling the individual birth rate used for demographic stochasticity. Variations between generations are higher and extinctions can occur even in initially large populations (D)

Allee effect is absent. A demographic Allee effect is considered to be strong as soon as the growth rate becomes negative below a threshold point, known as the Allee threshold. It is a precarious balance: any population whose numbers fall below this threshold will be deterministically driven to extinction.

2.4.2 Allee Effect and Classical Biological Control

In classical biological control, the significance of the Allee effect on the probability of establishment is a crucial question that has not yet found an answer that applies to every situation. Several models have been developed to predict the consequences of a mate-finding Allee effect on the dynamics of introduced populations (Grevstad 1999b; Hopper and Roush 1993). Whether through a spatially explicit deterministic approach, or a stochastic approach without the spatial aspect, an Allee effect results in an initial population size threshold below which populations will not establish (Fig. 2.2c, d). The Allee effect is therefore one of the hypotheses put forward to explain why the probability of establishment of biological control agents decreases when fewer individuals are introduced (Hopper and Roush 1993).

Meta-analyses show that terrestrial arthropods constitute the taxonomic group for which there are both the largest number of studies on the Allee effect and the highest proportion of studies revealing a significant Allee effect (Kramer et al. 2009). In nonsocial insects, truly cooperative behaviours may exist (Wertheim et al. 2002) but they are rare. However, reproduction is often reduced at low density: among the 34 behavioural studies that tested the relationship between density and mating success, just over half show a mate-finding Allee effect characterized by a positive density effect on mated females (Fauvergue 2013).

A few classical biological control operations have made it possible to directly test the Allee effect on introduced populations and its consequences on establishment success. The results are sparse and insufficient to draw general conclusions. Two experimental introductions of parasitoid insects suggest that density has no effect on reproductive success, and a negative effect on the population growth rate, i.e. exactly the opposite of what would be expected for an Allee effect (Fauvergue and Hopper 2009; Fauvergue et al. 2007). In biological control of weeds, introductions of psyllids for broom control in New Zealand show that smaller populations are somewhat less likely to establish (Memmott et al. 2005). However, the observed growth rates, independent of the initial numbers, suggest stochastic effects, without an Allee effect (Fig. 2.2a, b). Only introductions of leaf beetles to control purple loosestrife in the state of New York showed a demographic Allee effect (Grevstad 1999a), probably caused by low-density mating problems. Thus, the hypothesis that the effect of the introduced number on the probability of establishment could be explained by an Allee effect is not yet fully supported by the scant existing data.

2.4.3 Allee Effect, Autocidal Control and Mating Disruption

The Allee effect is also an important factor in control methods that aim to limit reproductive success, whether by releasing sterile males (SIT, see Chap. 5) or via mating disruption using sex pheromones (see Chap. 17). The theory, which has been widely debated in recent years, is that by limiting reproductive success, a pre-existing component Allee effect can be intensified, with the possible consequence of raising the Allee threshold (Fauvergue 2013). A population manipulated in this way could then fall below a higher Allee threshold and thus decline to the point of extinction (Liebhold et al. 2016; Tobin et al. 2011). These concepts were developed in tandem with attempts to better control gypsy moths in North America. In this species, a decrease in mating success at low density (Tobin et al. 2013) seems to cause a strong demographic Allee effect (Tobin et al. 2007), and these effects are leveraged in mass mating disruption efforts to slow invasion. Nevertheless, theoretical models have far outpaced actual data, especially data that could prove the causal relationships between the component Allee effect, the demographic Allee effect and population persistence. However, these ideas offer exciting scientific possibilities, such as the development of combined and synergistic autocidal control strategies (Blackwood et al. 2018) and new research projects that straddle the line between population biology and autocidal control of major pests such as *Ceratitis capitata* and *Drosophila suzukii*.

2.5 Lower Genetic Diversity in Small Populations

A population is a group of individuals, each carrying a combination of gene variants, called alleles. Depending on the number of individuals in the population, their fitness and the mode of reproduction, these different alleles are passed on across generations. Thus, the demographic bottleneck that occurs when natural enemies are sampled from their native range and released into the introduced range inevitably affects allelic distribution.

The genetic processes associated with introductions are more complex in their nature and effects than demographic processes, but can nevertheless be broadly categorized according to three major mechanisms:

1. The founder effect results from the random selection of a certain proportion of the alleles present in the native population or in subsequent reared populations; this effect leads to a decrease in allelic richness in the introduced populations.
2. Genetic drift is the result of the random selection of alleles, repeated over generations, caused by the stochastic nature of cross-breeding in finite populations. While all alleles continue to be passed on in an infinite population, drift leads to the random fixation or loss of certain alleles (and thus a decrease in allelic richness). The smaller the population, the more quickly this occurs.

3. Inbreeding (reproduction between related individuals) is all the more frequent when populations are small. This increased inbreeding raises the probability that an individual will carry two identical alleles of a certain gene; in this homozygous state, these alleles are expressed, and if they are unfavourable for the individual carrying them, they produce a decrease in fitness called inbreeding depression (Hedrick and Garcia-Dorado 2016). To a lesser extent, founder effects and drift also contribute to an increase in homozygosity (the fewer alleles there are, the more likely they are to end up in the homozygous state).

Thus, introduced populations are generally characterized by lower genetic diversity, either through the total number of genetic variants or through the differentiation of these variants at each gene within the genomes.

Genetic analyses show that small natural populations are generally characterized by low heterozygosity (Frankham 1996). The trend is similar in populations introduced for biological control, with lower genetic diversity than in native populations. Bottlenecks have a greater impact on allelic richness than on heterozygosity (Fauvergue et al. 2012). The relationship between genetic diversity, individual fitness and population dynamics is much less clear. Indeed, theories suggest positive effects of heterozygosity (Luque et al. 2016), but meta-analyses have shown variable findings and weak effects (Chapman et al. 2009). In biological control, evidence is again rare: only introductions of *Aphelinus asychis* in the United States show an effect of the initial population size persisting over several generations and therefore suggest a genetic influence (Fauvergue and Hopper 2009). A commonly accepted idea is that a large number of natural enemies, particularly in parasitoid hymenopterans (see Chap. 1), escape this relationship because of their particular mode of reproduction, called haplodiploidy. In these species, only the females are diploid (two alleles present per gene) whereas the males (from unfertilized oocytes) are haploid (only one allele per gene). This characteristic would allow for a continuous purging of deleterious recessive alleles in males, and therefore a reduction in the genetic load causing inbreeding depression (Henter 2003).

The decrease in genetic diversity in introduced populations may also lower their evolutionary potential in the introduced range. There are many review articles on invasion biology that cover this topic. For biological control, see the article by Szücs et al. (2019).

2.6 Conclusion

Three main types of processes could explain the relatively low probability of establishment of introduced populations: stochasticity (demographic and environmental), the Allee effect and the decline in genetic diversity. On the basis of these theoretical concepts and data, invasion biology, conservation biology and, more recently, biological control comprise three fields of application that can support the development of a “biology of introduced populations”, a sort of robust and documented meta-discipline that has been briefly covered here.

One recurring question deals with the relative importance of the different processes discussed in this chapter (Frankham and Ralls 1998; Lande 1988; Spielman et al. 2004). This is not a purely abstract epistemological question: if one of these processes proved to have a greater impact on small populations, it would merit greater research efforts and a more systematic consideration in biological control operations. For the time being, however, the data have not yet definitively settled the issue, whether in biological control or in other scientific fields that study small populations. The most likely scenario is that the different processes interact and reinforce each other. Once strong stochastic or catastrophic events have occurred, introduced populations would then be the source of demogenetic feedback. The concepts of genetic Allee effects and extinction vortex (Luque et al. 2016) formalize and integrate this feedback and therefore appear to be the most promising areas for future research.

Chapter 3
Classical Biological Control

Nicolas Borowiec and René F. H. Sforza

3.1 Introduction

Among today's many biological control strategies, classical biological control (CBC) is the oldest – it dates back to the nineteenth century, which explains its name. This biological control method is based on the intuitive idea that fighting an invasive population requires finding a natural enemy from its native range. CBC is therefore closely linked to biological invasions for two reasons: first, because the main principle of CBC is to combat invasive insects or plants by introducing specific natural enemies from the same native range; and second, because introductions of exotic biological control agents for CBC can be considered a "planned biological invasion" (see Chap. 1; Fauvergue et al. 2012; Marsico et al. 2010), and therefore used to analyse the factors for establishment success and failure in introduced populations. Thus, the initial idea opens the door to a lengthy scientific, technological and sociological process.

In this chapter, we will discuss the different stages of this process through selected examples. The starting point of this journey is the search for natural enemies of species considered harmful because they negatively impact crops. These natural enemies, or biological control agents, are considered "beneficial"; they are used for the benefit of human activities, while the fauna and flora of the invaded areas also benefit from this sustainable and environmentally friendly control method. We detail the different stages of this long journey, which goes from studying the literature on pests and potential biological control agents to field exploration and specimen collection. We will then discuss laboratory and natural condition assessments through recent examples. But first, we will start with a short history and definition of our discipline.

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3.2 Definition, History and Evolution

Biological control is based on humans' exploitation for their own benefit of a natural relationship between two living organisms: on the one hand, a target harmful organism (which can be a crop pest, weed, pathogen, livestock parasite, etc.), and on the other, a biological control agent (e.g. predator, parasitoid, parasitic herbivore, pathogen). There are a variety of biological control strategies (see Chap. 1). Classical biological control is defined as "the intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control" (Eilenberg et al. 2001). The initial stage of this strategy consists in conducting specific surveys in the target pest's native range. Once collected (see the section "Foreign exploration"), these biological control agents must then be exported and kept in appropriate quarantine conditions in order to identify them, evaluate the possibilities for rearing them, and study some of their biological traits (effectiveness, specificity; see the section "Importing exotic material and performing laboratory evaluations"). Some of these characteristics must then be validated in conditions that are similar to the field (e.g. in greenhouses) and release strategies must be optimized. If the results are sufficiently convincing, the biological control agents are used on a larger scale (see the section "Environmental release of biological control agents").

The first CBC project was developed to control an invasive plant, with the introduction in the late eighteenth century in southern India of a scale insect native to Brazil (*Dactylopius ceylonicus*; Dactylopiidae) to control prickly pears of the genus *Opuntia* (Cactaceae). This sap-sucking insect led to complete control of prickly pears and was later relocated to southern India (1836) and later Sri Lanka (1865). When it comes to insect pests, the American entomologist Charles Riley is credited with developing modern CBC. Following the accidental introduction of the cottony cushion scale from Australia (*Icerya purchasi*: Margarodidae) into citrus orchards in California in 1868, Riley organized a survey in Australia. He was convinced that the harmlessness of this scale insect in its country of origin was due to native natural enemies. Various entomophagous insects were reported, including the lady beetle *Rodolia cardinalis* (Coccinellidae). Forty thousand lady beetles were imported. They were released in California, but also mass reared for redistribution to farmers. Spectacular results were achieved within two years. Cottony cushion scale numbers were stabilized below an economically acceptable threshold, with a 200% increase in citrus production in the following years. Then came the lengthy programme (70 years) to combat gypsy moths (*Lymantria dispar*: Lymantriidae) native to Europe, which were ravaging North American hardwood forests. Several million European parasitoids, including ten major species, were successfully released from 1907 onwards (McManus and Csoka 2007).

The United States of America (USA)(including Hawaii), Australia, South Africa, Canada and the countries of Oceania (Table 3.1) are at the forefront of CBC programmes for weeds, but more than 70 countries have already conducted at least one programme, mainly in Asia and Africa. One of Europe's first programmes dates

Table 3.1 Number of phytophagous biological control agents released in classical biological control of invasive plants over 100 years on all continents

Country/region	Released species	Invasive target plants
United States (including Hawaii), Canada	224	54
Australia	202	56
South Africa	103	51
Oceania[a]	58	19
New Zealand	53	23
Asia	42	18
Africa	38	15
Caribbean	16	13
South America	14	10
Western Europe	4	4

Based on McFadyen (1998) and Winston et al. (2014)

[a] Excluding Australia and New Zealand

back only to the late 2000s (Shaw et al. 2009) with the authorization in 2010 to introduce the psyllid *Aphalara itadori* (Psyllidae) into Great Britain to control Japanese knotweed, *Fallopia japonica* (Polygonaceae). Since then, an insect in Portugal and a fungus and a mite in Great Britain have been authorized and introduced to control *Acacia* sp. (Fabaceae), *Impatiens* sp. (Balsaminaceae) and *Crassula* sp. (Crassulaceae), respectively.

With regard to insects, the USA is the global leader for CBC introductions (1956), followed by Australia (343), Canada (290), the United Kingdom (240) and New Zealand (212) (Cock et al. 2016). However, these figures must be qualified if we consider the evolution of the number of introductions carried out worldwide: after plateauing between the 1950s and 1970s, introductions fell steadily until the 2000s, when they reached levels comparable to those of the nineteenth century (Fig. 3.1). What is remarkable is that although the number of introductions has been very low in recent years, establishment success is higher than ever, which suggests a significant improvement in methodologies. Currently, more than half of CBC introductions for insect control results in successful establishment of the biological control agents, compared to one third or less a few decades ago (Cock et al. 2016).

Although the establishment success of biological control agents has risen, there is still considerable room for improvement in terms of practices. Only 10% of introductions result in effective pest control, despite good establishment rates. To maximize establishment and control success, there is likely a high need for prior knowledge of the pest and/or its natural enemies in its native range. Accordingly, it is possible to distinguish two main categories of CBC programmes.

First, there are operations that can be described as "transfer" operations. They are characterized by the fact that the target pest has already impacted many areas and that considerable data on its biology and natural enemies are known (see Text Box 3.1 on the recent case of the oriental chestnut gall wasp). The principle is to transfer and adapt the approach already developed elsewhere to the new country. Although

Fig. 3.1 Evolution of the number of introductions for classical biological control worldwide (1870–2010). (Based on Cock et al. 2016)

success is not guaranteed, these "transfer" operations can be considered low risk if carried out properly. International scientific collaborations are particularly important, since biological control agents that have proven useful in some countries are later very often used in others. For example, *Rodolia cardinalis* has been used successfully in more than 50 countries (Cock et al. 2010).

Text Box 3.1: Example of a "Transfer" Operation: Using *Torymus sinensis* to Control Oriental Chestnut Gall Wasps

As its name suggests, the oriental chestnut gall wasp *Dryocosmus kuriphilus* (Cynipidae) is a hymenopteran that induces galls on chestnut trees (*Castanea* spp.; Fagaceae), which can result in substantial economic losses for associated sectors (e.g. chestnut farming, beekeeping). This pest, which is native to China, first invaded Asia (Japan, Korea, Nepal) before colonizing other continents. After arriving in Italy in 2002, it took just 15 years for this insect to colonize the entire European chestnut production area. Surveys carried out in China by Japanese researchers led to the discovery of a parasitoid specific to oriental chestnut gall wasps: *Torymus sinensis* (Torymidae). Introductions of this parasitoid wasp effectively controlled pest populations in Japan in the 1970s (Moriya et al. 2002); introductions of *T. sinensis* were then made in the USA. In Europe, *T. sinesnsis* was first introduced in Italy in 2005, with effective control of the pest achieved after eight years (Quacchia et al. 2014), followed by other European countries (France, Slovakia, Hungary, Portugal, Spain). In France, experimental introductions combined with post-release monitoring showed that pest populations had stabilized below the

(continued)

Text Box 3.1 (continued)
damage threshold 5–6 years after the initial releases. The CBC programme also showed that *T. sinensis* had maximum success in establishment (100%), regardless of the tested introduction treatment (Borowiec et al. 2018). Because other countries had previous success using this parasitoid, the operation was set up more quickly in France, which explains why effective control occurred faster than in earlier projects in Italy and Japan. This example of a CBC programme can be held up as not only an agricultural success in France, but also an achievement in terms of scientific production, communication with the general public and positive interactions among all the stakeholders that were involved (public research and technical institutes, experimental stations, producers' unions, organizations conducting public health surveillance, government agencies, etc.). Finally, this operation underlines the major role of international collaborations (China, Spain, France, Hungary, Italy, Japan, Slovakia, Portugal) in the effective transfer and implementation of a classical biological control method.

There are also "groundbreaking" operations with a high degree of uncertainty due to the lack of data. These operations require the implementation of all the usual stages of a CBC programme (surveys in the native range, evaluation of biocontrol candidates in the laboratory and field release), which makes these operations more complex, longer and more costly. For example, at least eight species of a parasitic hymenopteran currently under study in Europe (France and Switzerland) were brought back from Japan to be tested in a biological control project against the fruit fly *Drosophila suzukii* (Drosophilidae). A parasitoid of the genus *Ganaspis*, whose exact systematic position is now being investigated, shows a clear preference for the target (Girod et al. 2018). The findings are identical for CBC of weeds; one example includes surveys throughout the Mediterranean basin in search of specific herbivores against French broom (*Genista monspessulana*; Fabaceae). The psyllid *Arytinnis hakani* (Psyllidae) and the weevil *Lepidapion argentatum* (Brentidae) are under study (Kerdellant et al. 2019).

3.3 Foreign Exploration

In some ways, going on a field trip is like setting off on an adventure, and sometimes the adventure is just around the corner. But often, when dealing with CBC of invasive populations, the logistics can become much more complex when it comes to surveying other continents. Before leaving home, a team obviously needs to know where to go! When researching harmful organisms, scientists generally will not have to start from scratch in terms of knowledge unless they are the first to tackle a new pest. Usually a team somewhere in the world will have investigated the taxonomy, biology and ecology of the target pest. Foreign exploration therefore always starts with a data collection phase, which can be difficult when the data are in a different language. Collaboration with colleagues in the local country is essential. These colleagues often provide access to insect collections and herbaria, and facilitate contacts with local regulatory bodies. To refine the preliminary research, climate maps between introduced and native ranges can be compared to target the areas to be surveyed. Population genetics studies (phylogeography) can be carried out on the plant or insect specimens that are brought back to identify the areas to be surveyed. For example, by sampling the vine mealybug *Planococcus ficus* (Pseudococcidae) throughout the Mediterranean basin and comparing their DNA sequences with those of invasive populations in Californian vineyards, Middle Eastern populations proved to be the source of Californian introductions (Daane et al. 2018). Such a posteriori reconstructions of invasion routes have also been carried out for other invasive insects such as *Harmonia axyridis* (Coccinellidae) (Lombaert et al. 2010) and *Drosophila suzukii* (Fraimout et al. 2017). Genetic methods can also be used to verify the identity of the target pest, which in some cases may be part of a group of morphologically indistinguishable species (see Chap. 1). For example, a gall-inducing eucalyptus pest in France had begun causing serious problems. Preliminary investigations (morphological, molecular and ecological) pointed to a new species, *Ophelimus mediterraneus* (Eulophidae), that was very similar to a well-known and initially suspected target pest, *Ophelimus maskelli*. The parasitoid used in biological control of the latter is likely unsuited to controlling the new *O. mediterraneus* species (Borowiec et al. 2019). All the data acquired through the literature, contacts made in the countries of origin and preliminary laboratory studies are therefore critical in assessing the feasibility and complexity of a CBC operation.

Another prerequisite before going into the field is to learn about current legislation concerning the collection of biological material in the target country. This is all the more true since the Nagoya conference in 2010, which led to the adoption of the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (the concept of Access and Benefit Sharing, or ABS). A total of 92 parties, including France and the European Union, have signed this protocol, which requires signatories to implement specific regulations for better sharing of the potential benefits related to using living organisms (FRB 2017). However, the level of national regulation varies considerably from country to country. In the USA, for example, a non-signatory country, there is no national ABS legislation in force to date, while Spain's national legislation requires an official collection permit before accessing genetic resources. Regardless of the specific legislation in the country, the collection of macroorganisms or the transfer of breeding strains from a foreign laboratory should always be subject to a material transfer agreement at minimum. This is a contract that details the terms and conditions for the use and valuation of the collected resources, often accompanied by other official documents in countries with specific ABS legislation in place. Whatever the conditions, international collaboration and prior contact with local resource persons are indispensable to know all official steps that must be taken before collecting and exporting any material.

Prior to any foreign exploration, a careful assessment should be made of the equipment needed on site, which mainly includes sampling and observation equipment (magnifying glasses, forceps, nets), and storage equipment (tubes of all sizes, 90% ethyl alcohol for preserving specimens, ziplock bags, travel herbarium, etc.). Another important thing to plan for is the type of containers that will be used for return transport. They must be airtight to preserve the integrity of the material and ensure maximum containment. The planned collection methods, whether active (e.g. sight hunting, collection of potentially parasitized hosts, etc.) or passive (e.g. Malaise or sticky traps), will determine how bulky and cumbersome the equipment to be transported will be.

The choice of biocontrol candidates to be collected is more complex than it might seem. Within the native range of a species, many natural enemies can coexist: for example, the Asian lady beetle *Harmonia axyridis* has several dozen natural enemies (Ceryngier et al. 2018), while the Japanese knotweed *Fallopia japonica* has more than 200 (Shaw et al. 2009). Gathering information from the literature can provide a good working basis, as can preliminary surveys to update these data when possible. Of course, collection periods must align with the life cycles of the target pests, which may produce only one generation per year (e.g. oriental chestnut gall wasps) or up to several generations per year (e.g. *Drosophila suzukii*).

For insect pest control, parasitic hymenopterans are preferred, which generally have a narrower host range than predators (Sforza 2021). These parasitoids are most often parasitoids of eggs, nymphs, pupae or, in rare cases, even adults. Among these parasitoids, endoparasitoids are generally better adapted to the target pest because their feeding strategy requires them to bypass the host's immune defences. Once again, natural enemies with the greatest specificity as possible to the target pest should be favoured. The collection plan is organized by surveying the areas where the target host (plant or insect) is reported and by collecting samples. Because parasitoids are a special case, potentially parasitized pests are collected in the hope that parasitoid adults will emerge before returning. More specifically, the specimens collected are eggs (single or clusters), larvae showing signs of parasitism (cysts containing the developing parasitoid, such as with leafhoppers and psyllids) or mummies (e.g. with aphids, scale insects). If no prior information is available on the natural enemies in the native range, then specimens at different larval stages must be collected, fed and kept alive until the end of their development to allow the emergence of possible parasitoids. For example, surveys carried out in Asia to collect parasitoids of *Drosophila suzukii* have mainly consisted in collecting fruits attacked by this pest (cherries, blackberries); supplementing these fruits with an artificial diet so the nymphs can continue their development; retrieving and isolating the pupae obtained in specific containers; and then monitoring the emergence of adult parasitoids. Several parasitoid species have been collected and reared in France and Switzerland (Girod et al. 2018). When looking for herbivorous insects, all parts of the attacked target plants (pods, seeds, stems, leaves, apex, collar, roots, etc.) should be inspected. The actual insect (egg, larva, adult) or the part of the plant in which the herbivore is found (pod, gall, stem, root) is then collected.

3.4 Importing Exotic Material and Performing Laboratory Evaluations

The type of material brought back from foreign exploration depends on the study objective. Generally speaking, initial surveys are carried out to describe the communities associated with the target pest, particularly at the morphological and genetic level (see Chap. 1). For this process, the insects or mites should be brought back in 70% or 90% ethyl alcohol for identification at the laboratory. However, if the aim is to have material to initiate rearing or laboratory tests, live insects must be brought back.

Importing can be a lengthy process depending on the country. For example, since 2012, France requires an application for the introduction into a confined environment to be submitted and approved by the Ministry of Agriculture – besides the various required collection authorizations and export documents – before any insects collected abroad for biological control can be brought into the country. This document lists the organisms that will be imported and indicates the objectives of the research. It also describes the containment conditions in detail (e.g. quarantine facility) in which these insects will be kept. Before each import of live material, a request for an official letter of authorization must be made to the regional food service of the administrative region where the quarantine facility is located.

A quarantine facility is indispensable for working on live exotic organisms. It provides suitable working conditions for the study of the biology of a plant or animal species while protecting the environment. To ensure proper containment, these facilities have solid, liquid and gaseous waste treatment capabilities; restricted and secure access; remote monitoring systems etc., and are subject to very strict working procedures. The Ministry of Agriculture must approve quarantine facilities in France. The USDA European Biological Control Laboratory (Montpellier, France) is one such facility; the stinkbug *Bagrada hilaris* (Pentatomidae) has been routinely reared there since 2016. This global pest of Brassicaceae crops (cabbage, broccoli, cauliflower) is not found in continental Europe. An egg parasitoid, *Gryon gonikopalense* (Scelionidae) introduced from Pakistan (Martel et al. 2019), is also reared in this facility, where scientists can study the relationship between an exotic insect pest and an exotic parasitoid. Similarly, the “Entomopolis” quarantine facility at INRAE’s Sophia Antipolis centre is designed to rear regulated organisms (exotic and/or quarantined insects). For example, parasitoids of *Drosophila suzukii*, of Asian origin (China, Japan), have been under study there since 2016.

Once the species (pest or natural enemy) has been identified, and before considering introducing it into a new environment, its biology must be understood to accurately assess certain biological parameters (known as life history traits), mainly demographic, such as those related to reproduction, the number of generations per year, and the survival of the different stages of development (egg, larval and adult stages). These parameters are measured under standard temperature, hygrometry and photoperiod conditions, but the variation of these factors can also be used to evaluate the degree of phenotypic plasticity. For example, a parasitoid that completes its preimaginal development at both 10 °C and 30 °C will likely acclimate to various climates. Many other life history traits can be studied, such as dispersal ability (measured in wind tunnels or greenhouses) or the attractiveness of certain chemicals emitted by the target pest (kairomones) or by the attacked host plant (synomones) (see Chap. 15). These parameters will then be used to assess as accurately as possible the effectiveness of the natural enemy, its chances of establishment, its dispersal ability, as well as any possible non-target effects. The host range is one parameter used for the risk assessment associated with the introduction of an exotic biological control agent (see section "Environmental release of biological control agents"): Is the biological control agent a specialist, developing only on the target host or other related species, or rather a generalist and capable of developing on many different hosts? Specificity tests are used to evaluate this parameter.

Various guidelines are available to help estimate the potential impacts of exotic species introductions (Kuhlmann et al. 2006; van Lenteren et al. 2006). The main criteria to be considered when selecting non-target species for testing the specificity of a biological control agent are (i) ecological similarities with the target species, (ii) phylogenetic or taxonomic proximity to the target species and (iii) "safeguarding" considerations (e.g. protected or economically important species). Various practical constraints (species availability, rearing difficulties) must be taken into account to refine the list of species to be tested. Once the constraints have been determined, the tests are carried out step by step: first, laboratory tests are performed in no-choice conditions (presence of the non-target species only and assessment of physiological suitability), then in choice feeding conditions (preference for the target species compared to one or more non-target species), and finally in semi-natural conditions (e.g. in a greenhouse) in order to consider other behavioural aspects, such as short-distance attraction. The results of these tests are then used to quantify the risk of non-target effects. Estimating this risk is easier when a large amount of data has been acquired, which is generally the case for "transfer" operations. For example, in 2017, INRAE obtained approval to release the exotic parasitoid *Mastrus ridens* (Ichneumonidae) in France to control codling moth *Cydia pomonella* (Tortricidae). The dossier submitted for evaluation was based on data produced by other user countries (the USA, Argentina, Chile, New Zealand, Australia) as well as on additional specificity tests set up at INRAE (25 non-target species tested in all) (Borowiec et al. 2016).

3.5 Environmental Release of Biological Control Agents

If one or more promising biological control agents are identified during experiments in the laboratory and in semi-natural conditions (in terms of potential effectiveness and specificity), the next stage is to move on to the field evaluation phase. Releasing biological control agents into the environment is usually subject to legislation, but rules vary considerably from one country to another. Australia was one of the first countries to introduce specific legislation (as early as 1908), as were the USA, Canada and New Zealand (Hunt et al. 2008). In Europe, each country remains in charge of its own legislation. For example, of 19 European countries surveyed in 2004, only eight had specific legislation governing biological control (Ibid.). In France, a decree was issued in 2012 relating to the introduction of exotic macroorganisms useful to plants into the territory (in a confined environment) and into the environment. Since that date, any intention to release an exotic biological control agent into the environment requires a dossier to be submitted, including a detailed assessment of the expected risks and benefits, assessed by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the French Directorate-General for Food (DGAL). If the dossier is approved, a decision co-signed by the French Ministries of Agriculture and the Environment is published and authorizes the introduction. The systems set up outside the European Union are more or less identical, with compulsory approval of dossiers validating multi-annual scientific research work on the specificity and effectiveness of the biocontrol candidate to be released. In the USA, experts mandated by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS, the equivalent of ANSES in France), must approve a request for a permit to release a biological control agent.

Approval for environmental release is based on a cost and benefit assessment related to using the exotic organism. Generally, the benefits (economic and ecological) are most often derived from the level of control obtained, and are closely linked to reduced pesticide use (which in turns produces economic, environmental and health benefits). Costs are related to possible non-target effects and can be direct (i.e. directly impacting a non-target species), or indirect, by affecting a third species associated with the non-target species within a food chain. This is the case, for example, of the thistle seed weevil *Rhinocyllus conicus* (Curculionidae), which was introduced into the USA in the 1970s to control the musk thistle *Carduus nutans* (Asteraceae), an invasive plant native to Europe. The weevil now feeds on several North American thistle species of the genus *Cirsium* and induces indirect effects on the phytophagous communities associated with thistles in North America (Gassmann and Louda 2001).

Despite such occasional non-target effects, biological control is considered a safe approach. Over the last 140 years, more than 6100 introductions of around 2400 different insect species targeting some 600 pests have been made in nearly 150 countries (Cock et al. 2016). With regard to weed biological control, at least 1555 introductions of 468 species have targeted 175 different plants (Winston et al. 2014). For both plants and insects, the 99% biosafety level is often proposed (Suckling and Sforza 2014; van Lenteren et al. 2006). However, the rare non-target effects (e.g. prickly pears and thistles in the USA) are an argument in favour of specific regulation of biological control agents (Louda et al. 2003). Nevertheless, introductions of exotic organisms for CBC are negligible compared to the unintentional introductions of invasive alien species caused by the explosion of global trade (Fig. 3.2).

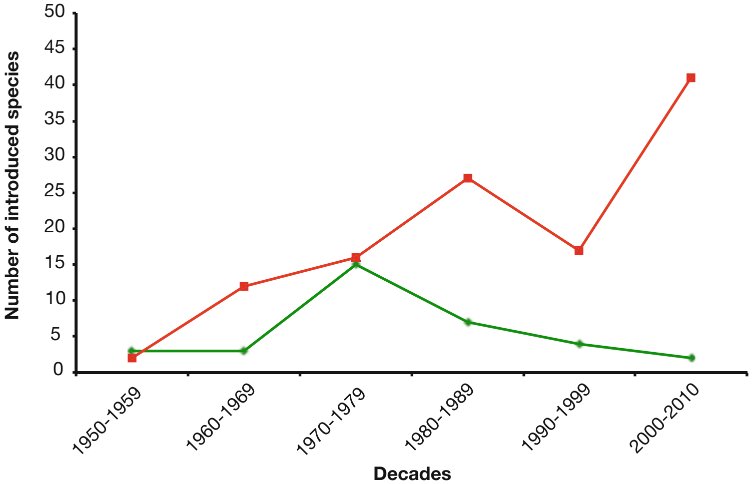

Fig. 3.2 Trend in the number of exotic species introduced in mainland France (1950–2011). (Source: Borowiec et al. 2011)
Major crop pests (*red line*) and entomophagous insects used in biological control (*green line*)

Once all the necessary authorizations are obtained, the next stage is the field evaluation phase. Field introductions are most often restricted by logistical constraints: first in the laboratory, since before considering releases, the production capacities of the biological control agents must be precisely defined; and then in the field, because the difficulty of the release operations depends on the size of the experimental system and the geographical area covered. Given that establishment failures observed in CBC are nonnegligible (see section "Definition, history and evolution"), it is advisable to include different implementation modalities as much as possible in order to better understand the factors of success or failure of these introductions (Fauvergue et al. 2012). For example, the decision might be made to release different numbers of individuals at the sites, as in the case in France with the use of *Neodryinus typhlocybae* (Dryinidae) to control the citrus flatid planthopper *Metcalfa pruinosa* (Flatidae) (Fauvergue et al. 2007); to carry out single or multiple releases, e.g. the use in France of *Torymus sinensis* to control the oriental chestnut gall wasp *Dryocosmus kuriphilus* (Cynipidae) (Borowiec et al. 2018); or to introduce populations of biological control agents with different levels of genetic diversity, such as with the introduction of *Psyttalia lounsburyi* (Braconidae) in France to control the olive fruit fly *Bactrocera oleae* (Tephritidae) (Malausa et al. 2010). These various constraints mean that release operations require significant human, financial and logistical resources.

Before any introductions, field surveys must be conducted in the target area to have as accurate an inventory as possible of the previous situation. This is called pre-release monitoring. After the introductions, post-release monitoring will then be carried out to clearly characterize the effectiveness of the introduced biological control agents, their potential non-target effects and the effect of the tested introduction methods. Post-release monitoring often includes sites where no introductions have been made in order to assess the effectiveness and safety of the biological control agents, as well as their dispersal ability. Since the probabilities of recapturing the biological control agents vary according to the methodology implemented and the organisms used, post-release monitoring should continue for a sufficient period of time (at least 4–5 years). Similarly, because the detection of potential non-target effects is often linked to a decrease in pest populations (the main resource that becomes limiting for the biological control agents), long-term monitoring is essential (8–10 years).

3.6 Conclusion

The way CBC projects are implemented has hardly changed in 140 years. Raising the necessary funds to launch the project, surveying the pest's native range, rearing potential biocontrol candidates and evaluating their specificity are still the key phases of any classical biological control programme today. However, the diagnostic tools (see Chap. 1) now available to evaluate and verify the taxonomic and biological parameters of organisms (plants, insects, mites, pathogens) and various modes of transport have revolutionized the discipline.

CBC is now a well-established plant protection approach. However, new projects require sufficient new technological, human and financial resources for up to 10 years, which can be an obstacle to implementation. This is all the more significant given that CBC operations are generally carried out by public research stakeholders with limited budgets, which means they must respond to calls for projects in an increasingly competitive environment. But while securing funding for CBC research is challenging, the benefits it brings to agriculture and the environment are real and should encourage public and private funding. The recent case of the oriental chestnut gall wasp (see Text Box 3.1) is a perfect example.

CBC successes are numerous and well documented, which lends greater legitimacy to the practice. For example, the successful introduction of a European rust to control skeleton weed in Australia resulted a reduction in herbicide inputs of more than US$12 bn, with a cost-benefit ratio of 1 to 112 (Mortensen 1986). In South Africa, the annual cost of plant invasions is US$6.5 bn, while the benefit of CBC on ecosystem services such as water and biodiversity is estimated at $21 bn (de Lange and van Wilgen 2010). The list is long and shows how investing in CBC has never been a better solution for the present and the future in a world contaminated by 70 years of chemical control (Hoddle 2004).

Chapter 4
Augmentative Biological Control Using Entomophagous Arthropods

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4.1 Background and Definitions

Augmentative biological control is based on the repeated introduction of biological control agents into agricultural crops. These agents are mass-produced in commercial insectaries, with the aim of eradicating pest populations in the short to medium term (van Lenteren 2012). In this chapter, we will focus more specifically on the issues related to the use of entomophagous arthropods (insects, mites) – i.e. predators or parasitoids – and entomopathogenic nematodes used against phytophagous arthropods.

An augmentative biological control programme aims to quickly reduce the pest population or maintain low levels of infestation throughout the growing season by directly introducing natural enemies from an exogenous source into the cropping system. This type of control is particularly apt when natural enemies are absent or are unable to persist naturally in the crop and surrounding environment to prevent damage to the plants. For example, this may occur when the natural enemies are unable to survive locally between growing seasons or when their densities are too low (isolation from the crop, short growing season). The goal is therefore to artificially increase the natural enemy populations to densities that allow for satisfactory pest control (Sivinski 2013).

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4.1.1 The Blurred Line Between Inoculation and Inundation

The term augmentative biological control traditionally encompasses two methods of introduction: inundation and inoculation (Eilenberg et al. 2001). Inundation control aims to quickly eradicate pests by releasing massive numbers of natural enemies, while inoculation control seeks a more sustainable regulation through the temporary establishment of natural enemy populations over several generations. For both methods, introductions are repeated over time when pests reappear, or according to a determined schedule. Strictly speaking, in inundation control, phytophagous populations are controlled exclusively by the introduced biological control agents, whereas in inoculation control, the offspring of the introduced agents ensure control (Eilenberg et al. 2001). In practice, the line between inundation and inoculation biological control is somewhat blurred because introduced organisms are generally capable of both reproduction and predation/parasitism. The different strategies of augmentative biological control using macroorganisms thus form a continuum ranging from seasonal inoculations of small numbers of natural enemies to intense campaigns of regular mass releases (Hajek and Eilenberg 2018). Along this continuum are various preventive practices in which biological control agents are released on a regular basis to ensure a permanent presence to keep pests in check as soon as they appear (Messelink et al. 2014; Hajek and Eilenberg 2018).

4.1.2 A Brief History of Augmentative Biological Control

Ancestral forms of augmentative biological control emerged as early as the second century AD in China. Chinese botanist Ji Han reported in *Nanfang caomu zhuang* (“A fourth century flora of southeast Asia”) the trade in nests of predatory ants (*Oecophylla smaragdina*) that farmers bought at markets and introduced into citrus orchards for pest protection. Modern forms of augmentative biological control date back to the very beginning of the twentieth century, with the production and introduction of a hymenopteran parasitoid species (*Metaphycus lounsburyi*) and a predatory beetle species (*Chilocorus circumdatus*) to control scale insects from the Coccidae and Diaspididae families, respectively. However, it was not until the early 1970s that many new arthropod species began being used for augmentative biological control programmes (van Lenteren 2012). Worldwide, the number of species rose from around ten to 170 by the early 2010s (Cock et al. 2010), and has nearly doubled since then (van Lenteren et al. 2018), although the market is dominated by only a few dozen species. Some that were commercially available for a while have been discontinued.

Augmentative biological control strategies are now used in many crops around the globe, such as maize, cotton, sugar cane and soya beans. However, this type of biological control is most used in crops with high added value grown in greenhouses and under cover (vegetables, ornamental plants) or open fields (strawberries, grapes).

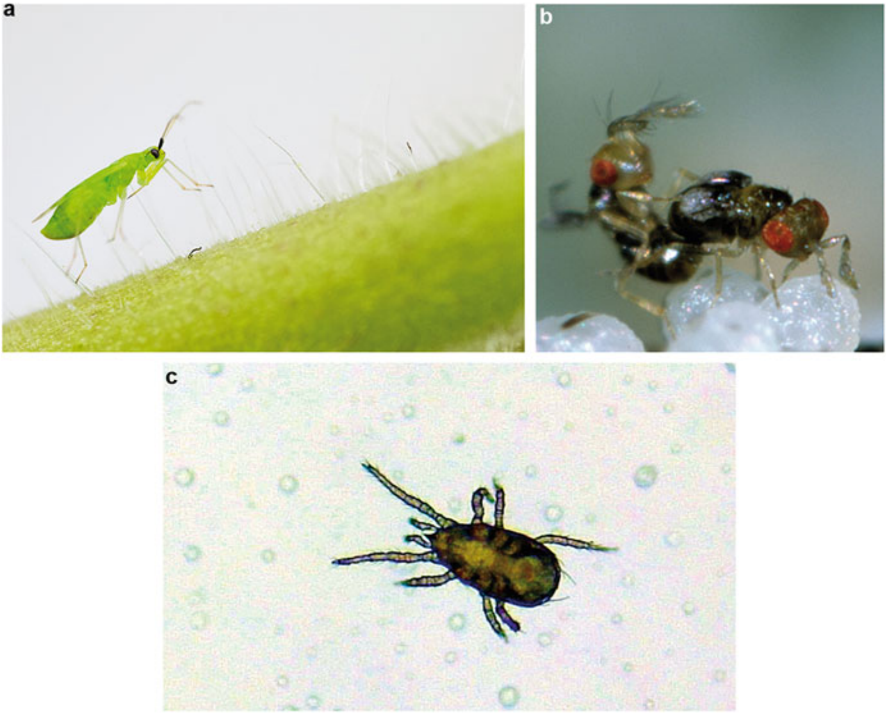


Fig. 4.1 Three major augmentative biological control agents: (**a**) *Macrolophus pygmaeus* (predatory insect), © A. Bout/INRAE. (**b**) *Trichogramma brassicae* (parasitoid insect), © J.-C. Malausa/INRAE. (**c**) *Neoseiulus cucumeris* (predatory mite), © L. Étienne/INRAE

These crops account for about 80% of the steadily growing augmentative biological control market, which is today worth around US$400 to US$600 m (van Lenteren et al. 2012, 2018), while the global biocontrol market has an estimated value of US $2.8 bn. The targeted pests are mainly thrips (40% by value of commercialized macroorganisms), whiteflies (30%), spider mites (12%) and aphids (8%). Parasitoid insects, especially hymenopterans, have long comprised the majority of natural enemies used in augmentative biological control, but this has changed since the mid-2000s with the rapid development of the use of predators, mainly mites but also hemipterans, in protected or open field crops (van Lenteren et al. 2018) (Fig. 4.1).

4.2 Current Challenges

The need to rear biological control agents on a massive and sustainable scale for later introduction into crops requires a business model and relatively large investments in production infrastructure.

4.2.1 Mass Production of Biological Control Agents

One of the most important prerequisites for the implementation of augmentative biological control is the capacity to produce a very large number of organisms (Morales-Ramos and Rojas 2003). Setting up this type of facility on an industrial scale is a complex process that first requires the coordination of multiple skills and disciplines. Mass production entails major investments, both technical, with the installation and development of specific rearing equipment for the different species, and human, with the training of specialized personnel. Large-scale commercial use of biological control agents began almost 60 years ago with the production of *Phytoseiulus persimilis*, a predatory mite, against phytophagous mites of the Tetranychidae family (van Lenteren and Woets 1988).

Mass production of most parasitoids and predators requires prior control of the production of their host(s) or prey, which are mainly phytophagous arthropods. In many cases, producing these hosts or prey is the most technically difficult aspect, and is in fact the limiting factor for biocontrol agent production. Moreover, host plants (or an alternative to them) must also be produced at this stage. In terms of profitability, these constraints will at the very least double production costs, and only the production of entomophagous organisms actually generates income (Van Driesche and Bellows 1996). This constraint can be partly removed by searching for alternative hosts, but above all by developing rearing environments, especially for phytophagous hosts and prey. This has led to a kind of natural selection of the biological control agents that are produced and marketed: commercial insectaries have mostly opted for species that can grow on host/prey that are easy to rear in large numbers. The use of artificial environments for the direct rearing of biocontrol agents is always a major challenge. Production on artificial growth media, when possible, often appears to be of lower quality compared to production on natural hosts and prey (Grenier and De Clercq 2003; Riddick 2009).

To simplify production, biocontrol companies sometimes select candidates with zoophytophagous tendencies, i.e. predators that can also consume plant material and therefore be easily produced in the absence of prey, on plants or in an artificial environment. Examples include Phytoseiidae mites such as *Neoseiulus californicus* or *Amblyseius swirskii* (Messelink et al., 2008), and even heteropterans such as *Macrolophus pygmaeus* or *Orius insidiosus*.

4.2.2 Business Models

The term augmentative biological control may refer to biocontrol strategies using macroorganisms or microorganisms. The regulations concerning these two categories of agents differ. Unlike microorganisms, which are regulated at the European level (see Chap. 11), macroorganisms are subject to national legislation. For instance, France is the first European country to have introduced (in 2014) a definition of biocontrol that includes the use of macroorganisms in its regulatory framework. Meanwhile, with the entry into force of the Convention on Biological Diversity (1993), access to and the use of biological resources now require benefit sharing with the country of origin. These regulations have limited, from the 2000s onwards, the diversification of commercialized species and their penetration into markets outside their country of origin (van Lenteren et al. 2018). Along with this regulatory context, a strong preference has been observed for native species, which now account for three quarters of new biological control agents placed on the market (Cock et al. 2010).

Macroorganisms accounted for about 16% of extended biocontrol products by value marketed worldwide in 2017 (IBMA France 2017). The players holding this market share have been around a long time; more than half of the active companies to date were founded between the 1970s and the 1990s. Around 500 companies currently market macroorganisms for biocontrol applications, but only ten of them are small and medium-sized enterprises or larger. The European market for macroorganisms for biological control is dominated by three of these specialized companies: Koppert (founded in 1967 in the Netherlands), Bioline AgroSciences (resulting from the 2016 merger of Bioline, an English company founded in 1979, and the Biotop subsidiary of the French group InViVo, founded in 1991) and Biobest (founded in 1987 in Belgium). These three companies originally adopted a classic business model of selling their own production – predatory mites for Koppert, *Trichogramma* wasps and mites for Biotop and Bioline, and pollinating bumblebees for Biobest – before diversifying their approach by expanding their product portfolios, especially through distribution. In France, some crop grower cooperatives, such as Savéol and its subsidiary Savéol Nature, have set up and maintain their own insectaries to meet their needs, an organizational model that can be found in other parts of the world, especially in Latin America (van Lenteren et al. 2018). Alternative approaches to marketing macroorganisms for biological control involving public funds and sometimes combined with private capital are also available, particularly in Asia and Latin America. For example, the publicly funded Okanagan-Kootenay Sterile Insect Release (OKSIR) programme, launched in 1992 in Canada, relies on a tax levied on general property owners and apple and pear growers to finance the mass production, processing and release of sterile codling moths (see Chap. 5 for more on the sterile insect technique).

4.2.3 Non-target Effects

As with any control method, the issue of possible non-target effects and their relative importance in relation to the expected and observed benefits inevitably arises. There are generally two types of non-target effects, depending on whether they occur within or outside of the relevant agricultural system.

Various non-target effects are possible within agricultural systems themselves. The biological control agent may be less specialized or more polyphagous than initially assessed and may attack other pest species. In this case, the non-target effect is positive. A more problematic case is when the biological control agent negatively impacts the crop it is meant to protect. This may occur in the case of omnivorous predatory species (Coll and Guershon 2002). However, this is not necessarily a prohibitive characteristic as it may allow the persistence of the biological control agent if the target pest is temporarily unavailable, and can even facilitate their production. Finally, the most frequent cases of negative non-target effects within the agricultural system include cases of complex ecological processes leading to interactions between biological control agents, whether they are used to control the same or different targets. Intraguild predation occurs when several biological control species feed on a common resource as well as on each other (Rosenheim et al. 1995). These are trophic interactions that are common in natural ecosystems, but which can also occur in agricultural systems, such as when several species are deliberately introduced for initial complementarity, or when an organism introduced for biological control by augmentation interacts with natural enemies that are spontaneously present. For example, Snyder and Ives (2001) report that some predatory beetles of the genus *Pterostichus* consume both healthy aphids (*Acyrthosiphon pisum*) and parasitized aphids, which impairs the dynamics of the parasitoid wasp *Aphidius ervi*. Theoretical studies as well as laboratory and mesocosm experiments and in situ observations show highly variable consequences of intraguild predation on the population dynamics of the different organisms, and therefore ultimately on the effectiveness and durability of control.

Non-target effects outside the relevant agricultural system are related to the dispersal of the biological control agents, which depends on their own abilities (flying, walking, passive dispersal) and growing conditions (open field, open-roof or closed greenhouses). The contrast between greenhouses with favourable microclimates and resource abundance, and generally unfavourable external conditions may sometimes be enough to prevent dispersal (Hart et al. 2002). However, this compartmentalization between cultivated and other habitats cannot be ruled out by default, especially in the case of inundative releases. Among the few studies on this topic, some conducted in Switzerland have assessed possible non-target effects of inundative releases of *Trichogramma brassicae* against the European corn borer *Ostrinia nubilalis* (Kuske et al. 2003). The findings highlighted (i) the dispersal of a significant portion of the *Trichogramma* wasps outside the release plot (first 50 metres), (ii) a relative predominance of *T. brassicae* during the first days after release, and (iii) a more durable residual presence. However, the authors concluded that this will likely not seriously affect native *Trichogramma* or non-target host species. It is of course difficult to generalize from such a case study, especially as the longer-term evolutionary consequences of these introductions on natural populations of *T. brassicae* have not been estimated. The problem of non-target effects takes on a whole new dimension when the candidate biocontrol agents are exotic species (van Lenteren et al. 2003). The problem is then similar to that of classical biological control (see Chap. 3).

Generally speaking, the issue of non-target effects, and more specifically those impacting non-target species, may give rise to debate within the scientific community and beyond. Indeed, although it seems quite obvious that augmentative biological control cannot be considered an ecologically neutral act, it is viewed differently depending on whether a “precautionary” or “principle of innovation” is favoured. However, these debates should be put into perspective by considering the risks of current chemical-intensive practices, whose non-target effects on non-target species – including humans – are well proven.

4.3 Ways to Improve Augmentative Biological Control

4.3.1 Genetic Improvement of Biological Control Agents

For most agricultural resources (crops or livestock), genetic improvement has a proven track record in improving phenotypic traits that impact performance. This potential lever was therefore quickly identified to optimize augmentative biological control methods. Genuine successes are, however, rare. While there may be many reasons for this relative failure, at least three of them deserve special attention. First of all, the market for macroorganisms used in biological control is very fragmented and profits are limited. This situation constrains investment possibilities in research and development, particularly in genetic improvement. In addition, several companies that produce biocontrol agents are reluctant to develop genetic improvement programmes because of the time required, the expected benefits and the lack of legal protection against unfair competition. Finally, at the biological level, questions arise about which traits should be selected (Hopper et al. 1993; Roderick and Navajas 2003): classic phenotypic traits (size, potential fertility, longevity), behavioural traits (dispersal ability, exploratory tendency, resource exploitation strategies) or particular abilities (diapause allowing storage, thermal stress resistance, pesticide tolerance). However, the situation appears to be evolving (Lommen et al. 2016) and companies producing biocontrol agents are gradually acquiring skills that will enable them to address the issues involved in enhancing and protecting biological material and the related expertise.

Meanwhile, new molecular genetics and genomics methods and tools can be used to characterize biological material with a previously inaccessible degree of accuracy (Cruaud et al. 2018; Lindsey et al. 2018), resulting in unprecedented traceability capacity and the prospect of selection programmes based on coupling between molecular markers and phenotypic traits. Finally, there is a growing awareness among public and private R&D stakeholders of the suboptimal quality of historical strains used for mass rearing. One example of this is recent work carried out jointly by INRAE and Bioline AgroSciences to optimize the effectiveness of *T. brassicae* against the European corn borer *Ostrinia nubilalis* using genetic levers.

4.3.2 Improving Mass Production

Solutions must still be identified and developed to adapt the mass production of biocontrol agents to current and future needs (Leppla et al. 2004). The vast majority of current commercial insectaries rely on accumulated knowledge from relatively small-scale production. However, the biocontrol industry could benefit from other insect production sectors. For example, silk production has provided important resources for the development of biological control in China, enabling the mass production of *Trichogramma* spp. from *Antheraea* spp. eggs (silkworms) to control lepidopteran crop pests. Similarly, important developments are now expected in terms of automated production, which can leverage the technologies adopted by producers of insects for animal feed or human food. Automation should help reduce production costs while guaranteeing optimal quality monitoring and standardization of the biocontrol agents that are produced – two major challenges that must still be addressed for augmentative biological control (van Lenteren 2012). It should be noted, however, that the quality of biocontrol agents is also determined by the transport and distribution logistics chain, and is not limited to production aspects alone.

4.3.3 Resource Supplementation

In some cropping systems, introduced populations of biocontrol agents may have difficulty establishing or persisting because the organisms do not have all the food sources they need. For example, prey or host densities may be temporarily too low to support natural enemy populations, supplementary food sources may be absent or of poor quality, or the biocontrol agents may lack oviposition sites or shelter. Regardless of the reason, the survival or reproduction of the biocontrol agents is impacted, which reduces the effectiveness of control and requires frequent reintroductions, resulting in higher costs (Huang et al. 2011; Messelink et al. 2014). Important improvements can be made through food supplementation, i.e. providing the missing resources through crops. One of the oldest techniques is the use of banker plants, which consists in introducing companion plants that are not harvested but which support alternative prey or host populations and help maintain biocontrol agent populations (Huang et al. 2011). In addition to the phytophagous insects they harbour, these plants can also provide biocontrol agents with alternative or complementary foods, such as pollen, nectar or sap (Messelink et al. 2014). Nevertheless, in very intensive cropping systems such as greenhouse systems, the competition for productive space is such that this solution is rarely used. Methods based on food supplementation directly on crops of alternative hosts or prey or complementary foods are now being developed. For example, sterilized lepidopteran eggs or artemisia cysts are used to support natural enemy populations in different crops. The introduction of pollen, which has long been difficult because of the harvesting costs, has recently undergone a massive development, namely following the marketing by the company Biobest of broadleaf cattail pollen as an alternative food for predatory mites. Broadly speaking, the development of low-cost alternative hosts or food is seen as a major challenge to improve augmentative biological control methods (Messelink et al. 2014). Finally, other types of supplementation are based on the introduction of oviposition sites or shelters in crops that allow better reproduction and survival of juvenile natural enemies. These techniques are being developed especially for predatory mites with fibres applied to plant leaves. Combined food and shelter supplementation are perfectly compatible and even appear to produce synergistic effects in different crops (Pekas and Wäckers 2017).

4.3.4 Population Dynamics

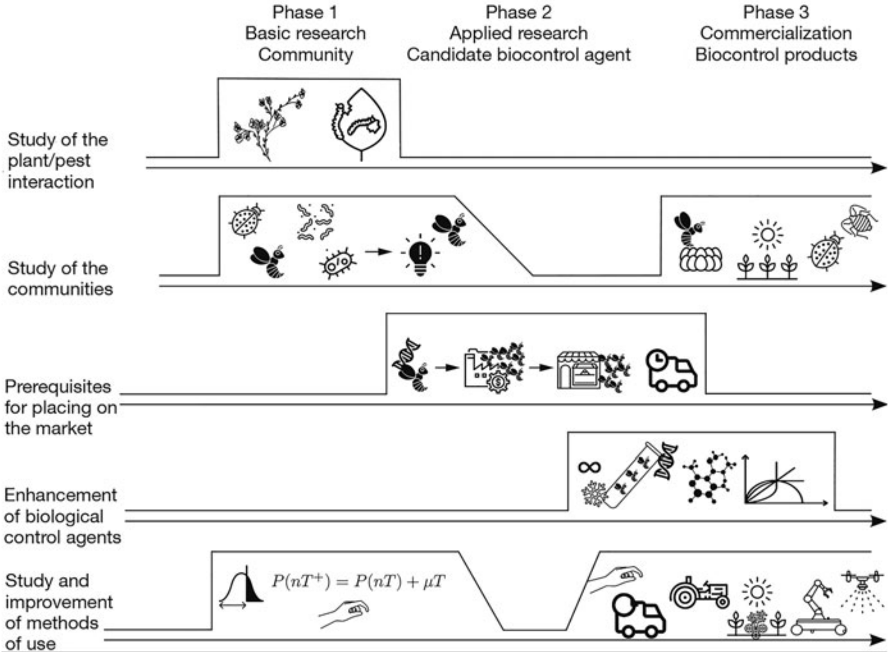
In contrast to classical biological control, which aims to achieve a long-term equilibrium between pest and natural enemy populations (see Chaps. 2 and 3), augmentative biological control raises questions about the unbalanced dynamics of systems that are regularly disturbed by introductions of biological control agents. Several theoretical studies have thus highlighted interactions between the introduction strategies of natural enemies over space and time, and the intrinsic biological characteristics of these populations. For instance, the presence of positive or negative density dependence (i.e. the influence of the abundance of natural enemies on their population growth) or the type of dispersal modulate the effectiveness of a given introduction strategy. Thus, when natural enemies interfere with each other – a common occurrence in predatory mites – the most effective strategies are based on frequent introductions of small numbers of agents (Nundloll et al. 2010). More generally, these theoretical studies underscore that the successful implementation of augmentative biological control hinges on detailed knowledge of the biological processes in the populations involved. They can also guide users towards better strategies for releasing a particular natural enemy or, when technical or cropping constraints come into play help users choose the most suitable biological control agents. For example, the high dispersal capacity of biological control agents has long been considered as a selection criterion. This is now being called into question by studies highlighting the potentially deleterious nature of excessive dispersal (Heimpel and Asplen 2011).

4.3.5 Entomovectoring

One last way to improve the use of biological control agents, or their usefulness in crop protection, is to develop strategies based on a technique known as entomovectoring. This technique consists of having one or more elements transported or distributed by an insect. This element may be another arthropod (insect or mite), a bacterium, or a natural or synthetic substance involved in or unrelated to the biocontrol solutions. Current practices entail distributing plant protection or pollination solutions using pollinating insects, such as bumblebees that are commonly introduced for pollination of different crops grown under cover or in orchards. The best-known examples are the distribution of an antifungal against *Botrytis cinerea* by bumblebees introduced in strawberry crops (solution proposed by the Lallemand group) or a *Bt* biopesticide (Biobest solution). One of the advantages is that these solutions can be distributed quickly, easily and specifically to the targeted locations, all in small quantities. Recent work has also focused on leveraging the zoophytophagous characteristics of certain predators, such as *M. pygmaeus*, to distribute biocontrol microorganism-based solutions, bacterial toxins or natural defence stimulators directly into the plant's tissues. These predators would then provide a second layer of protection against fungal pathogens, without compromising their primary function. In addition to increasing the benefits of these enhanced predators, these developments also help reinforce the interest of natural defence stimulators, which are sometimes still too costly and can induce phytotoxicity. These approaches thus offer prospects for a multi-layered biocontrol approach.

4.4 Conclusion

The development and promotion of augmentative biological control methods depends not only on scientific and technical considerations, but also on social (training, advice), economic (absolute or relative costs compared to competing practices), regulatory (authorization/withdrawal of plant protection products, legislation on exotic organisms) and even legal considerations (protection of know-how and biological material) (see Fig. 4.2). Accordingly, an ambitious development of this strategy must involve concerted efforts at different levels. For example, at the scientific level, sufficient time and financial means must be allocated to first correctly identify/evaluate candidate biocontrol agents, and then verify their effectiveness and harmlessness in real-world use. From a zootechnical standpoint, it would make sense to improve mass production techniques, which are still highly dependent on human labour and therefore expensive. In this respect, progress could be achieved through converging interests and generic innovations, not only in terms of other biocontrol strategies – especially the various autocidal control methods, which also require mass production (see Chap. 5) – but also with other insect production activities for animal or human consumption. Finally, further consideration must be given to the



Stakeholders	Phase 1	Phase 2	Phase 3
Agronomic partners (technical institutes, experimental stations, etc.)	+ (monitoring)	++ (validation)	++ (validation)
Public laboratories	+++	++	
Biocontrol companies	+	++	+++
Other private companies			++

Fig. 4.2 Workflow diagram for developing an augmentative biological control programme
The activities carried out are divided into three phases for which the main actors and their levels of involvement are indicated

business models underpinning the production and marketing of biological control agents. Recent merger and acquisitions in the private sector will hopefully result in more substantial investment in research and development operations. Regional initiatives, based on joint partnerships (public and private organizations) and efforts to go beyond the usual responsibilities of certain traditional stakeholders, could be a complementary or alternative solution.

Chapter 5
Sterile Insect Technique: Principles, Deployment and Prospects

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5.1 Introduction

The sterile insect technique (SIT) is a pest control method that aims to gradually reduce insect reproduction. Mass-reared sterilized males are released in large numbers into the agricultural landscape, where they mate with wild females; the females then lay sterile eggs, producing embryos that die in early development. SIT is generally one important component of an area-wide integrated pest management programme (AW-IPM). The aim is therefore no longer to protect individual fields but rather entire regions, which requires cooperation among many stakeholders. This approach is also sometimes used to eliminate insects from large areas or to prevent colonization by new exotic species (Hendrichs et al. 2005). SIT has been used for operational control programmes on around twenty insect species (Hendrichs et al. 2005; Klassen and Curtis 2005; Vreysen et al. 2007; Text Box 5.1), mainly Diptera as well as Lepidoptera and Coleoptera species. It is currently being developed for insects of interest in Europe, such as the fruit fly *Drosophila suzukii*, the olive fruit fly *Bactrocera oleae* and the tiger mosquito *Aedes albopictus*.

The sterile insect technique (SIT) is generally separated from biological control sensu stricto. It does, however, rely on the mass release of living macroorganisms to

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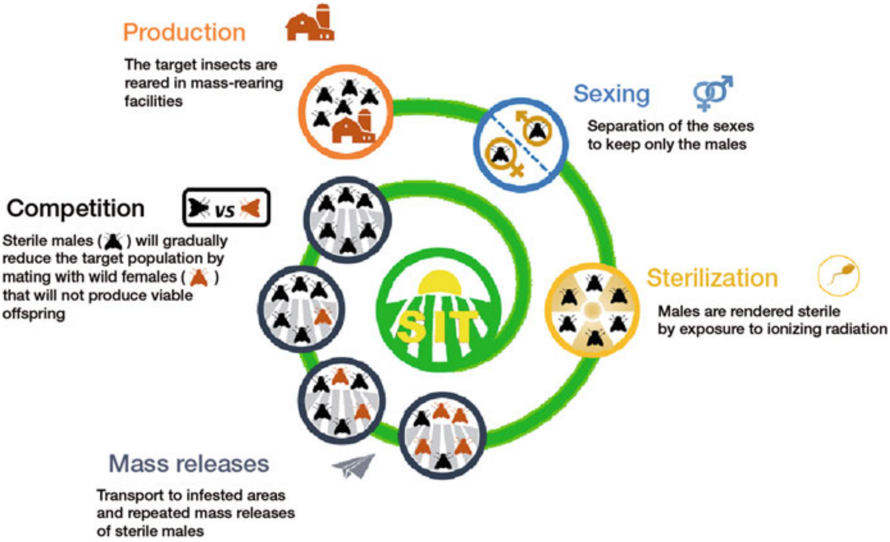

Fig. 5.1 Diagram of the main steps of the sterile insect technique
The goal is to reduce the reproduction of a target species through mating between wild females and males reared under controlled conditions and then sterilized. The sex-separation step during the rearing phase (in order to release only males) is optional. Likewise, releases can take place on an area-wide scale and in confined conditions (e.g. greenhouses)

reduce the abundance of a pest, with the only peculiarity being that sterile individuals are the natural enemies of their own species (hence the term *autocidal* control). SIT therefore has a legitimate place in extended biocontrol.

This chapter describes the main principles of SIT and highlights both its potential and its limitations (Fig. 5.1). We will also explain various future research avenues for SIT and its conditions of use, while underscoring the need for transdisciplinary cooperation among researchers in the biological sciences and humanities and social sciences as well as key stakeholders.

5.2 Technical Basics

5.2.1 Mass Rearing the Target Insect

A prerequisite for SIT on a target insect is the ability to mass produce enough sterile insects to release over large areas. For example, as part of SIT programmes to control the Mediterranean fruit fly *Ceratitis capitata*, the El Pino mass rearing facility in Guatemala can produce up to two billion sterilized insects per week (Parker 2005). Several years of research and development are usually required to develop a suitable rearing process. Good knowledge of the ecology, physiology, genetics and behaviour of the target insect is necessary to develop equipment and efficient procedures. Sustainable rearing operations must keep costs low while ensuring maximum quality of the released males.

5.2.2 Sex Separation

It is generally preferable to release only sterile males for two reasons. First, in many insect species, only females are responsible for crop damage (e.g. laying their eggs in fruit) or pathogen transmission. Releasing females, even sterile ones, can therefore sometimes pose an unacceptable risk. Additionally, the presence of sterile females during releases may make the campaign less successful, since they could also mate with sterile males (Rendón et al. 2004). Sex separation is ideally done as early as possible in the production process to reduce rearing costs.

When no mechanical/visual technique allows for reliable sorting (e.g. relying on sexual dimorphism), alternative methods, such as genetic sexing strains (GSS), can be developed. GSS are based on genetic variations that are either naturally present in certain wild populations or randomly induced by low doses of ionizing radiation, which confer a different phenotype or resistance to certain chemical or physical conditions (e.g. high temperatures). If these genetic variants are positioned on the male sex chromosome, they confer a different sensitivity to the two sexes that may enable separation. Chemical or physical treatments of eggs, larvae, pupae or adults will eliminate females before sterilization and release.

5.2.3 Sterilization

For the so-called classical SIT, insects are sterilized by exposing the pupae or adults to precise doses of ionizing radiation (X-rays or gamma rays). The sterilizing effect of X- and gamma radiation is similar. However, X-ray irradiators are increasingly emerging as the best alternative for safety reasons, as they do not require radioisotopic sources.

Exposure to certain doses of ionizing radiation causes sterility because it damages reproductive cells, which are more sensitive than somatic cells (Bakri et al. 2005). This is why the insects are sterilized but survive the radiation. However, the exposure can sometimes significantly reduce the insect's vigour, especially when administered at high doses or an early developmental stage (Bakri et al. 2005). To limit somatic lesions, sterilization should be carried out as late as possible in the insect's development, when the number of cell divisions is minimal. The choice of radiation dose is an important element in a SIT programme, where a balance must be struck between minimum acceptable sterility levels and male competitiveness.

The general public sometimes fears that insects sterilized using ionizing radiation would be radioactive. It is worth mentioning that the effects of radiation cease as soon as the operation is completed and leave no trace of residual radioactivity in the insects.

5.2.4 Releases and Monitoring

Population densities and fluctuations, along with potential movement of the target wild insects in the field, determine the appropriate scale and modalities for population management integrating SIT. A detailed understanding of the relevant territories, ecosystems and agricultural systems is indispensable. The level of isolation is crucial when choosing the release site, and its area will be determined by its topographical configuration and insect supply capacity. Accordingly, the sterile insects are progressively released over an increasingly large area in successive phases (Hendrichs et al. 2005).

The release of sterile males preferably starts at a time of the year when the density of the target species is lowest. If necessary, insecticides can be used prior to release to reduce the wild population size and increase the sterile male to wild male ratio. In some cases, releases may also be preceded and accompanied by releases of parasitoids of the target pest (see Chap. 4) to increase the effectiveness of the operation (Barclay 2005; Jang et al. 2008).

An effective entomological monitoring system is key to determine the quantity and frequency of releases and evaluate their performance. Depending on the programme, the ratio of sterile to wild males varies from 7:1 for tsetse fly control to 100:1 in some New World screwworm management contexts (Vreysen 2005). The critical ratio is based on many factors, including the spatial distribution in the habitat, the propensity of sterile males to disperse, their sexual competitiveness, and the density of the wild target population. Mathematical models can estimate the parameters for SIT success according to different biological factors and anticipate release and integrated management strategies (Barclay 2005; Bliman et al. 2019).

5.3 Conditions of Application

The suitability of SIT for a given species and environment depends on many biological, ecological, economic and political factors (Lance and McInnis 2005). Each new programme starts by acquiring background knowledge before implementation begins (Fig. 5.2). The biology and ecology of the species determine how to mass produce it and obtain competitive sterile males in the field. The socio-ecological context of the target regions will influence the logistical possibility of using this approach (initial density of wild populations, isolation of sites, agricultural network, stakeholder cooperation). These data will help to define the AW-IPM strategy (see Hendrichs et al. 2007). An interesting example of a collaborative pest management programme to control a complex of four species of fruit flies took place in Hawaii in the early 2000s. The strategy combined various tools and involved multiple stakeholders. By collaborating, they were able to take all the different constraints and solutions into account, facilitate cooperation between stakeholders and locally eliminate the pest populations across the region, as well as reduce the use

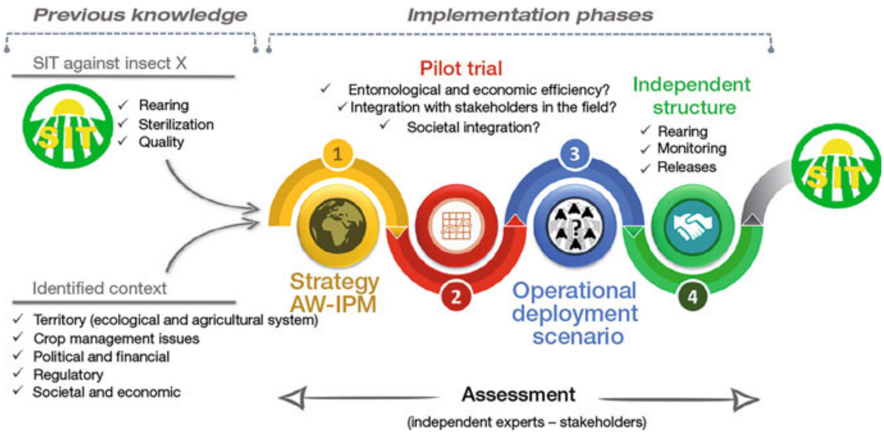

Fig. 5.2 Phases in the design and implementation of a new sterile insect technique (SIT) programme

Prior understanding of the technical modalities of adapting SIT to an insect and the context in which it is used are prerequisites when designing an implementation strategy. This strategy will then be rolled out in four progressive stages: (1) the development of an area-wide integrated pest management strategy that will be tested during (2) a small-scale pilot trial; the entomological and socio-economic results will inform the design of (3) a large-scale operational deployment scenario. This last phase will require (4) the creation of an independent entity handling the whole chain of SIT-related services. Regular evaluations at each phase are crucial to ensure the reliability, efficiency, cost-effectiveness and sustainability of the programme

of organophosphate insecticides (Mau et al. 2007; Jang et al. 2008). Pilot trials and small-scale co-development are fundamental phases to validate and improve the technical aspects as well as to test the feasibility of partnership implementation, economic projections and stakeholder perceptions (Fig. 5.2; Hendrichs et al. 2007). Rigorous efforts must be made when choosing the site and conducting the trial as its success will have a decisive impact on whether the programme moves forward to a larger scale and long-term operational phase.

When SIT is used for area-wide protection, economic and political stakeholders (farms, municipalities, etc.) must work together to make the programme a success. As a result, many of these programmes are generally commissioned and managed by government or territorial authorities (Dyck et al. 2005). An entity tasked with handling the production, monitoring and release operations generally allows for better tracking and management, and therefore better programme responsiveness, than having many entities involved (Vreysen et al. 2007). Successful SIT programmes over large areas, such as in the case of the New World screwworm from the northern United States to Panama (Wyss 2000) or fruit fly control in Central America (Reyes et al. 2007), has thus been based on strong regional coordination and cooperation among farmers or producers and regulatory authorities.

However, this technique is not feasible to manage all insects. Certain biological characteristics are incompatible with its operating principle. This is the case for insects reproducing by parthenogenesis or those with ephemeral and synchronous reproduction periods, a highly sedentary lifestyle, long-distance migratory behaviour or long life cycles. Likewise, SIT is not suitable when the males (sterile and mass released) may themselves be harmful (e.g. grasshoppers, cockroaches) or vectors of pathogens via their feeding method (e.g. leafhoppers).

5.4 Strengths and Weaknesses of SIT

5.4.1 Strengths

A major advantage of SIT its specificity: only target species are affected directly by sterile releases. The reproduction and survival of other organisms found in the ecosystem or agricultural system are not affected, unlike with methods such as pesticides, whose active agents have a broad spectrum of action. Remarkably, the reduction in fertility induced by SIT ends once no further sterile males are released, making it a self-limiting method. It is therefore possible to stop the effect of a SIT programme if it no longer meets expectations. However, prolonged action requires continuous production of insects. This has significant economic consequences for those funding the campaign, but it can facilitate the creation of a sustainable sector.

While some insect control techniques lose their effectiveness over time (such as when resistance evolves), SIT becomes more effective as target insect numbers decline (Hendrichs et al. 2007). As the ratio of sterile males to wild males rises, the likelihood of wild females mating with sterile males also increases. Thus, the cost of a SIT-based AW-IPM decreases over time. Finally, a methodical SIT programme can keep insect densities low enough to allow the export of produce to regulated markets.

This is the case, for example, in British Columbia, where apples protected by the OKSIR programme for the codling moth *Cydia pomonella* are exported to Asia. The programme initially only sought to locally eradicate the pest in the production area, but the inability to sufficiently control infested private gardens and fruit imports, along with insufficient resources to expand the release area, forced the board of administrators to review the programme's long-term objective (Bloem et al. 2007). The eradication of a target population from an entire region has been achieved in several contexts with different organisms, following the rigorous implementation of a set of preventive and control measures (Hendrichs et al. 2005). Quarantine measures are essential to prevent the return of pests to the region concerned.

5.4.2 Limits

The specificity of SIT can also be considered a drawback since only one species can be managed at a time. When used in place of a single broad-spectrum solution (e.g. an insecticide), users may have to deploy as many campaigns as there are insect species to regulate. If several insects share similar ecological niches, such as certain tephritid fruit flies or mosquitoes, controlling a single species may have a marginal effect on the nuisance users experience.

Moreover, because SIT acts on reproduction and not on insect survival, it has a more gradual effect on target pest abundance. Managing crisis situations (e.g. outbreaks or epidemics of insect-borne diseases) often requires other, more immediate solutions, such as spot treatments with insecticides.

The spatial scale of SIT is another important factor, as most programmes aim to protect large areas. Sometimes SIT can be used on isolated or confined plots; however, inconsistent spatial release may not be cost-effective, as in the case of the onion fly in the Netherlands (Dyck et al. 2005; Klassen and Curtis 2005; Hendrichs et al. 2007). If insects are also widely present outside the crop areas to be protected, SIT could fail on a territorial scale but be effective in closed environments, such as greenhouses or net-protected plots. This would likely occur with the fruit fly *D. suzukii*, which breeds year round on many wild plants (Poyet et al. 2015).

5.5 Future Research Avenues

Several possibilities have been explored to increase the effectiveness of approaches using SIT. They can be divided into two types: solutions aimed at improving technical performance and those that combine SIT with other methods.

5.5.1 Technical Improvements

Since SIT is based on mating sterilized males with wild females, eliminating females during the production process is a major way to improve profitability. Using genetic sexing strains often requires cross-breeding with local populations to ensure the competitiveness and sometimes sexual compatibility of released insects with their wild counterparts.

The quality of the released insects is also extremely important. Radiation doses can be adjusted to minimize the deleterious effects on males while maintaining a satisfactory level of sterility (Parker and Mehta 2007). Many studies are also looking into insect production conditions, and particularly insect diets. The composition of nutrient media has received a lot of attention, and the application of probiotics is a promising avenue to enhance the general vigour of males (Augustinos et al. 2015). Symbiotic microorganisms (e.g. extracellular bacteria) are believed to be involved in the mating choices of some flies in the laboratory (Sharon et al. 2010) and could therefore also be a factor of success. Finally, nutritional, hormonal or semiochemical (olfactory attractants) supplements are sometimes used on adult males before release to improve their performance (Pereira et al. 2013). Males of *C. capitata*, for example, are usually scented with ginger oil, which significantly increases their attraction level.

The breeding of wild species in captivity leads to a form of domestication, i.e. adaptation to artificial environmental conditions. This can reduce the ability of released males to mate with wild females as the traits favourable to males in factory cages may diverge from those expected by wild females. Thus, SIT programmes need to ensure that wild traits (behaviour, competitiveness) are maintained through different strategies of regular cross-breeding with wild strains. Ongoing research is evaluating the possibility of maintaining the reproductive characteristics of wild insects in production facilities or voluntarily selecting for wild females' preferred male sexual characteristics (McInnis et al. 2002).

5.5.2 Integration and Synergy

Combining SIT with other methods of insect population control is an appealing and promising strategy, whether to optimize the management of a single pest or to implement integrated management of several pests. The way different methods interact and their potential effect on Allee dynamics (see Chap. 2) are discussed by Suckling et al. (2012). For example, mathematical modelling indicates that solutions based on SIT or its variants could bring population sizes below viability thresholds and thus cause extinction (Fauvergue 2013; Blackwood et al. 2018).

Synergistic interactions include the combination of parasitoids (see Chap. 4) and SIT, where the former are effective at high densities, while SIT has a greater effect at low densities. This strategy has been tested on fruit flies (Rendón et al. 2006; Jang et al. 2008) and the codling moth *C. pomonella* (Botto and Glaz 2010). "Mobile" mating disruption is also an interesting approach which involves the release of sterile males of one species to which a sex pheromone (see Chap. 15) of another species has been applied (Howse et al. 2007). For example, sterile males of *C. capitata* can disrupt sexual activity in populations of the lepidopteran *Epiphyas postvittana*, enabling successful integrated management of both species (Suckling et al. 2011).

Following a similar principle, entomovectoring (or boosted SIT) consists of associating released sterile insects with pathogenic microorganisms or toxic substances that are then transmitted to the wild insects with which they interact (see also Chap. 4). The results are encouraging (Howse et al. 2007; Flores et al. 2013) and the doses of substances or pathogens used are much lower than those required in conventional spray applications. However, data on the potential ecosystem consequences of entomovectoring associated with SIT are needed to ensure the long-term safety and acceptability of this approach.

5.5.3 The Incompatible Insect Technique (IIT): A Complementary Approach

An alternative to sterilizing males by exposure to ionizing radiation is to release males carrying intracellular bacteria – usually of the genus *Wolbachia* – which interfere with their host's reproduction. These bacteria are very common in insects, with nearly two thirds of species thought to be infected (Hilgenboecker et al. 2008). They only infect arthropods and nematodes, and are transmitted from mother to offspring. *Wolbachia* are used for population control because they trigger a process called cytoplasmic incompatibility (CI). CI results in the non-viability of offspring when males infected with the bacteria mate with females that either do not carry the bacteria or carry a different variant. Releasing males infected with CI-inducing *Wolbachia* could therefore have a similar effect as releasing sterile males (Bourtzis 2008). Promising initial results have been obtained on mosquitoes (Crawford et al. 2020) and similar studies are under way on the fruit fly *D. suzukii* (Cattel et al. 2018). However, the sexing method must be foolproof: if there are any females in the releases, the bacteria could invade the host populations, rendering the technique ineffective. This risk could be prevented by combining IIT and SIT because, in many insect species, females are sterilized at lower doses of radiation than males, thus allowing the release of better performing males while ensuring the harmlessness of the females (Nikolouli et al. 2018). This approach was effective in reducing mosquito population densities in a recent pilot test in China (Zheng et al. 2019).

5.6 Environmental and Sociological Implications

There are few rigorous studies to date that evaluate the ecosystem consequences (positive or negative) of SIT deployment. Because of the specific effect of SIT and the resulting lower need for plant protection products, its use is often considered beneficial for the environment and the human populations living nearby. This idea is reinforced by the fact that the technique mainly targets exotic insects or insects that proliferate as a result of human activities.

However, variations in target insect population densities – due to large releases or the effectiveness of SIT itself – could produce indirect effects by affecting the species interacting with the target insect. For example, the predators of an insect controlled by SIT could suffer from the depletion of their prey if it is a major food source for them (at immature stages). Conversely, abundant releases of sterile insects increase the population density of the target insect, which can raise food competition with other species. In turn, this higher population density could benefit parasites and generalist predators, boosting their pressure on other hosts and prey, a phenomenon known as apparent competition (Holt and Bonsall 2017). Studies on these phenomena, which are not specific to SIT, are lacking in the context of crop protection.

Finally, the possibility of gene flow between released insects and wild populations raises questions. This apparently paradoxical scenario is plausible because the rate of male sterility is not always 100%. The consequences of the introgression of new alleles in relict wild populations would depend on the genetic characteristics of the released insects, related to their origin or exposure to ionizing radiation.

As far as human populations are concerned, the stakes of using SIT may go beyond ecosystem issues. Thus, using SIT to replace other technical solutions requires significant shifts in practices, including changes in insecticide use, the need for regular population monitoring and appropriate responses, tolerance to large numbers of the insect to be managed by SIT, and coordination between economically independent stakeholders (e.g. farmers). In Senegal, the eradication of the tsetse fly, a vector of livestock diseases, via SIT has broadly impacted the agrarian economy and land values: the livestock system has shifted to more productive breeds, farmers' incomes have increased, and pastoral pressure on ecosystems has decreased (Bouyer et al. 2014). The public (e.g. local residents and consumers) are also concerned by the use of SIT, but these issues are still poorly documented.

5.7 Insects and Society

5.7.1 Transdisciplinary Synergy

The societal context in which SIT is emerging in France and other European states – which are undergoing an agroecological transition and questioning widespread biocide use – underscores the sustainable, ethical and performance issues associated with this technique. SIT programmes require cooperation among many stakeholders; identifying them and their roles in evaluating and co-developing SIT-based solutions is vital, especially as debates emerge regarding the ethical dimensions related to the eradication of an insect from a particular territory (Bouyer et al. 2019).

Understanding the full range of issues related to SIT requires broad consultation and breaking down disciplinary siloes. For example, new environmental questions emerged when the relevant stakeholders began evaluating SIT in France. When stakeholders have diverging interests, pinpointing each of their constraints and promoting cooperation to create responses can lead to solutions that are acceptable to all. Thus, active stakeholder participation in the long-term management of SIT programmes has proved essential (Hendrichs et al. 2007; Vreysen et al. 2007). According to Mau et al. (2007), the Hawaiian IPM programme (of which SIT is one of many components) succeeded because it relied on a decision-making process based on consensus and mutual trust, with the involvement of a local coalition of stakeholders and a technical group guiding the programme. This broad partnership, with experts from various fields, was forged through consultation, awareness-raising and popularization activities carried out by the consortium that led the project. These efforts involved farmers, local residents, technicians (in charge of implementing the programme), institutions (to support the regulatory aspects), and the private sector, which provided technical solutions.

5.7.2 Regulatory Framework

The International Standard for Phytosanitary Measures (ISPM) No. 5 defines the sterile insect technique as a “method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species”. A sterile insect is defined as “an insect that, as a result of a specific treatment, is unable to reproduce”.

ISPM 3 provides guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms, including sterile insects. The regulatory framework governing the import and use of sterile insects is different in each country. For example, in France, when it comes to unregulated organisms (and which are not subject to quarantine or compulsory control measures), the sterilization of insect pests means they can be labelled as macroorganisms that are “beneficial to plants”. For the import of sterile insects from another territory (in the regulatory sense; for example, mainland France and Corsica are considered separate territories), the petitioner is required by official decree to apply for authorization to bring the macroorganism into the territory and introduce it into the environment (Coutinot 2014). Sterilized insects from a population indigenous to the territory of introduction fall outside the scope of the decree.

The method used for sterilization determines the regulatory framework. To date, sterilization by exposure to ionizing radiation is the only method that falls within the French regulatory framework for biocontrol macroorganisms. There is currently no regulatory framework in place authorizing the use of IIT or insect sterilization by transgenesis in Europe (beyond the Cartagena Protocol regulating the use of living modified organisms).

5.7.3 Economic Integration

While SIT is often deployed at regional scales and funded by government bodies (Dyck et al. 2005; Vreysen et al. 2007), the business models for its use in Europe have yet to be established. While some sterile insects are produced by private companies (e.g. De Groene Vlieg in the Netherlands, Bio-Fly Ltd. in Israel and FruitFly Africa in South Africa), the majority of rearing facilities operate as public programmes or public-private partnerships.

Many area-wide SIT programmes (see Text Box 5.1) have reduced agricultural insect pest populations below the economic threshold, thus benefiting farmers and local residents. The economic benefits associated with area-wide SIT programmes include reduced crop losses, lower pesticide expenditure and access to new markets (Enkerlin 2005), and depend on the type of agriculture practised. Any cost-benefit analysis of insect management solutions must integrate all externalities before comparing them with their alternatives (Bourguet and Guillemaud 2016). The benefit-cost ratio of SIT programmes against the fruit fly *C. capitata* worldwide varies between 2.8 and 400, depending on how they are integrated with other control tools and the scale of implementation (Enkerlin 2005). SIT is sometimes used as a preventive measure against the establishment of an exotic species. This is the case with the release of sterile males of *C. capitata* in California, which receives many inocula from Latin America. The benefits of preventive and consistent releases can then far outweigh the estimated cost of an insect infestation (Enkerlin 2005).

Text Box 5.1: A Short History of SIT

The idea for SIT came about in the 1930s and 1940s through work by two scientists named Knipling and Serebrovsky (Klassen and Curtis 2005). It was first used against the New World screwworm *Cochliomyia hominivorax* (leading to its eradication in Curaçao in 1954). The programme was gradually expanded across the southern United States, Mexico, Central America and Panama over more than 50 years and is still operational (Wyss 2000). The tsetse fly *Glossina austeni*, a vector of the trypanosome parasite, was also eliminated from Zanzibar (Vreysen et al. 2000) using this approach. SIT has been quite successful in suppressing or eliminating local fruit fly populations, such as the Mediterranean fruit fly in Chile and Argentina and the melon fly *Bactrocera cucurbitae* in the Okinawa archipelago in Japan and Hawaii (Vreysen et al. 2007). There are many ongoing operational programmes deploying SIT[1] to reduce or maintain fruit fly or lepidopteran pest populations below an economic threshold.

In Europe, Spain established a rearing facility in the 1990s to support a regional *C. capitata* management programme for citrus crops near Valencia (Hendrichs et al. 2005). An onion fly rearing facility in the Netherlands supplies growers directly for plot management. Croatia also uses SIT against *C. capitata* to protect fruit production in the Neretva River valley (Bjeliš et al. 2016). Finally, Italy also recently performed small-scale pilot projects.

In France, a national working group called Collectif TIS was formed in 2018. It brings together scientists, institutional representatives, private operators and stakeholders from both the agricultural and public health sectors with the aim of supporting the study and possible deployment of SIT in France. Collectif TIS aims to work with all stakeholders to co-develop a robust framework while respecting all points of view and maintaining transparency. The topics are interdisciplinary and focus on issues such as technical implementation, environmental and societal concerns, regulations, governance and consultation.

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[1] The World-Wide Directory of SIT Facilities (DIR-SIT, IAEA) lists sterile insect rearing facilities and operational programmes using SIT across the globe.

5.8 Conclusion

This chapter has been written by biology and ecology researchers at a time when SIT in France and some other European countries is still in its infancy (Text Box 5.1). The technique is currently being studied for several insects of agricultural and public health importance, but no official programme is yet in place. We hope that this transdisciplinary approach combined with technical and human transparency – which we believe is inseparable from SIT deployment – will help more fully integrate these new issues and ensure our project's success for ethical, sustainable and efficient management of insects interacting with humans.

Part II
Stimulating Natural Pest Control in Agricultural Landscapes: Theoretical and Operational Insights into Conservation Biological Control

Adrien Rusch

One of the major challenges behind the development of agroecology and biocontrol methods is understanding the ecological processes at work in trophic interactions between the different species in a crop and the surrounding environment. These processes must be better understood to promote biological control of pests and diseases while reducing the environmental footprint of agriculture.

This part of the book focuses mainly on conservation biological control strategies, which aim to promote the activity, abundance and diversity of natural enemy species already present in the environment in order to enhance natural pest control.

The three chapters in this part provide an overview of the theoretical and operational knowledge of community and landscape ecology in relation to regulating insect pests and weeds. The first chapter explains the theoretical knowledge on the processes at work within trophic interaction networks, the methods and tools available to characterize these networks, and the conceptual framework for understanding species assemblages. The second chapter summarizes current knowledge on the effects of agricultural practices and landscape structure on insect pest control. Finally, the last chapter takes stock of the issues of natural weed control in agricultural landscapes. These last two chapters provide a non-exhaustive but relatively broad overview of the direct ways to promote natural enemies and limit the development of insect pests and weeds in agricultural landscapes on multiple spatial and temporal scales.

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Chapter 6
Community Ecology, Food Webs and Natural Pest Control

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6.1 Introduction

Conservation biological control consists in managing the environment through agricultural practices or agroecological infrastructures at different spatial scales to optimize the ecosystem services that help naturally control pests and diseases. Two complementary strategies can be leveraged: a top-down approach, where natural enemies such as predators, parasitoids and parasites provide pest control (this corresponds to a more strict definition of biological control via an indirect impact, i.e. via predation or parasitism), or a bottom-up approach that directly impacts pest populations by limiting their access to resources and favourable habitats (this type of effect is included in a broad definition of conservation biological control). Implementing these two strategies requires considering the pest to control as one taxon among many evolving within a particular environment and community. It also requires an understanding of the effects of environmental change (e.g. changes in practices and land use, climate change) on communities and interspecific interactions.

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A “community” corresponds to the assemblage of populations of living organisms within a given habitat or area. This definition can vary according to the object of study; it may relate to an environment (e.g. a community of grassland insects) or a function (e.g. a community of decomposers or predators). This ecological perspective of the organisms within an agricultural system clarifies how they function and makes it possible to analyse the ecological processes that influence pest and disease demography, i.e. natural pest control services. This chapter will outline current knowledge and concepts from community, network and landscape ecology that shed light on the way natural enemy and pest communities interact in agricultural landscapes. Accordingly, our focus will be on ecological processes that are part of top-down biological control, i.e. predation by natural enemies of crop pests.

Many studies have explored the relationship between community diversity and the level of function or service it provides. Current scientific consensus holds that higher community diversity generally increases the average level of ecosystem functions (e.g. the average rate of herbivore regulation rate or organic matter decomposition), as well as the ecosystem’s spatial and temporal stability (Cardinale et al. 2012). However, there are many examples where this positive correlation between biodiversity and ecosystem functioning is not observed; there may be no link at all, or even a negative correlation between these two components. This is explained by the high variability of interspecific interactions and by differences in the spatial or temporal scales. Given this information, the first scientific challenge involved in successfully stimulating natural pest control is to characterize the underlying mechanisms of the relationships between natural enemy community structure (e.g. abundance, diversity, functional composition) and natural pest control in agricultural systems.

The assemblage of species within a natural enemy community in a given habitat (e.g. a field) varies over time and space. It depends on environmental factors, known as “filters”, which can be biotic or abiotic in nature and come into play on different spatiotemporal scales (Fig. 6.1). On a regional scale, abiotic filters correspond to elements such as the climate and types of habitats in the landscape (e.g. crops, wet meadows, primary forests, etc.) and their configuration, which determine the pool of species potentially present in each habitat type. At the scale of each habitat, abiotic filters correspond to such aspects as the disturbance regime (e.g. farming practices) or the microclimatic conditions involved in selecting a species subset from the regional pool. The dispersal of organisms between habitats enables them to respond to variability in the environment and to complete their life cycles, which may be complex and dependent on several types of habitats or resources. Finally, and still within a habitat, biotic filters correspond to non-neutral interactions between species – i.e. interactions that affect their demography – such as competition, predation or facilitation (see section “Types of interactions within communities” and Fig. 6.1). The group of communities present within each habitat type at the landscape scale and interacting individuals is called the “metacommunity” (Text Box 6.1 and Fig. 6.1). Within this theoretical framework, the second challenge with regard to stimulating natural pest control is identifying the factors operating at the local and landscape scales that determine the local composition of the community present within an agricultural habitat.

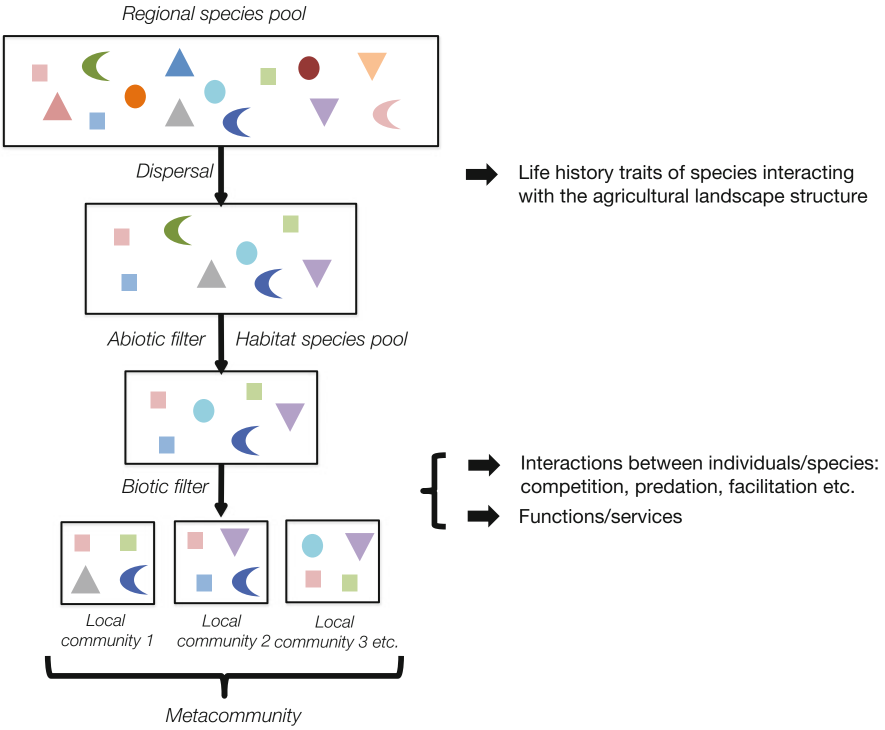


Fig. 6.1 Conceptual representation of the process of species assemblages from the regional species pool to local assemblages
The local community within a habitat is the result of biotic and abiotic filters acting on larger spatial and temporal scales. Local communities linked by the dispersal of individuals comprise the metacommunity

Natural pest control therefore depends on the composition of the communities present and on the interactions between organisms at the habitat scale (i.e. the field). These interactions also depend on the metacommunity composition at the surrounding landscape scale. In this chapter, we will take a deeper look at the relationships between these different filters and biological compartments to explain the levels of natural pest control in order to better manage them (Fig. 6.1).

6.2 Types of Interactions Within Communities

Within a community, the various interactions between organisms are organized in network form. With regard to natural pest control, the networks we are most interested in here are the so-called "antagonistic" networks, i.e. those that benefit certain species to the detriment of others. Examples include food webs, which

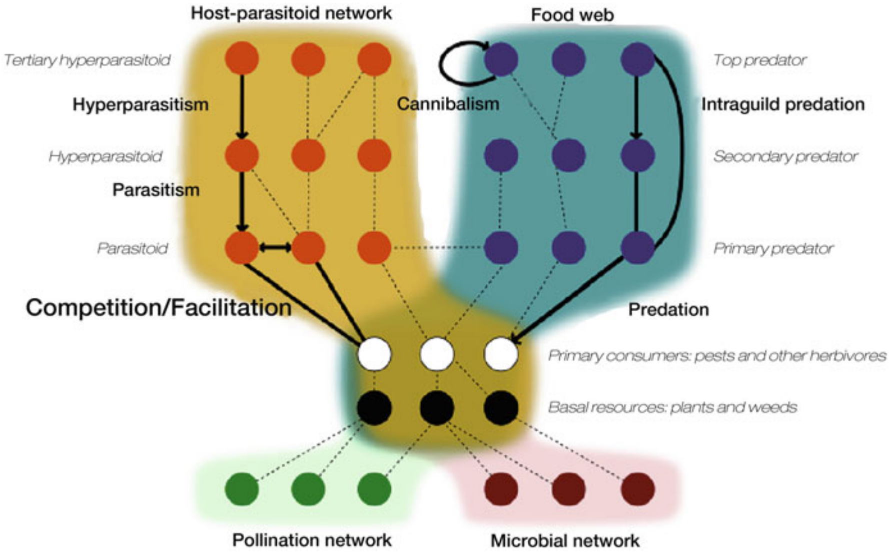


Fig. 6.2 Schematic diagram of the interaction networks between natural enemies (predators or parasitoids), phytophagous insects (insect pests) and primary producers (plants)
The ecological processes involved are shown in bold. Pests and their natural enemies are included among other taxa in this diagram. The host-parasite network, which is also involved in controlling pests, is not represented here for the sake of clarity. Other networks can be added, such as pollinator networks (mutualistic interactions) or microorganism networks

include a set of prey and predators, and "parasitic" networks, which include host-parasitoid or host-pathogen interactions. Antagonistic networks differ from so-called "mutualistic" networks in which interactions benefit different biological compartments such as networks of plants and pollinators that are dependent on each other. Some species, such as plants or bees, may be part of multiple networks (Fig. 6.2).

Within each trophic level, different types of interactions between species or between individuals of the same species can be established. First, there may be indirect and negative interspecific interactions via resource competition (e.g. water, sunlight, minerals, prey). Second, facilitation-type interactions can also take place between organisms at the same trophic level. These are positive interactions benefiting at least one of the two interacting organisms. A classic illustration of facilitation in conservation biological control is the case of lady beetles and ground beetles in cereal fields. Lady beetles attack and eat aphids on the crops; some of the aphids fall to the ground, where most are then eaten by ground beetles. In this case, the total number of aphids killed by the two species of natural enemies is greater when both are present in the crop: the lady beetles facilitate predation by the ground beetles. Another motif regularly found in food webs is intraguild predation. Intraguild predation occurs when a predator preys on another predator (or parasitoid), which can then influence the final number of phytophagous insects that are consumed. For example, certain environmental conditions have been shown to increase the intraguild predation rate between birds and flying predatory insects, which in turn decreases the level of control of lepidopteran larvae attacking cabbage (Martin et al. 2013). Other studies have quantified intraguild predation rates between different species – particularly between ground beetles and spiders, and even between parasitoids (hyperparasitism, see Text Box 6.1) – in agricultural landscapes and have shown that this is a fairly recurrent motif. When individuals of a given species prey on each other, this is called cannibalism.

These different interspecific interactions, which may have positive, negative or neutral effects on pest control levels, explain why the effect of a community's composition on predation levels may be greater than, equal to or less than the sum of the individual effects of each species on an insect pest. These interactions must be qualified and the environmental characteristics that determine them must be identified to manage natural pest control services in agricultural landscapes.

6.3 Correlations Between the Horizontal Diversity of Natural Enemy Communities and Natural Pest Control

Within ecosystems, it is generally accepted that there is a positive correlation between community diversity – whether genetic, taxonomic or functional – and the levels of functions or processes performed by that community (Cardinale et al. 2012). This positive effect of diversity on ecosystem functioning is linked to several mechanisms: niche complementarity among species (Loreau and Hector 2001), which can be described by the complementarity of species' functional traits (McGill et al. 2006), and the sampling effect (Loreau 1998; Fig. 6.3).

Niche complementarity is based on the competition exclusion principle, which says that species must occupy different niches to coexist, and as a result, species with different traits can use a resource more efficiently than species with similar traits (Loreau and Hector 2001; Gross et al. 2017). The sampling effect assumes that the greater the number of species in a given ecosystem, the greater the probability that a species that uses a resource very efficiently (therefore performing a function) will be present in that community. This variability in resource use efficiency is also called the "species-identity effect" (Straub and Snyder 2006).

Moreover, an increase in biodiversity is also generally associated with an increase in the spatial or temporal stability of ecosystem functioning. This phenomenon is attributed to two mechanisms: species asynchrony and the portfolio effect (Loreau and de Mazancourt 2008; Isbell et al. 2009). More diverse species assemblages tend to be more productive (i.e. they have a higher biomass) and therefore mean productivity is more likely to be achieved over time (Isbell et al. 2009). Furthermore, the temporal succession of a given niche's occupation by different species ensures the continued provision of a function over time (Loreau and de Mazancourt 2008). Additionally, the portfolio effect is where a system's characteristics provided by a

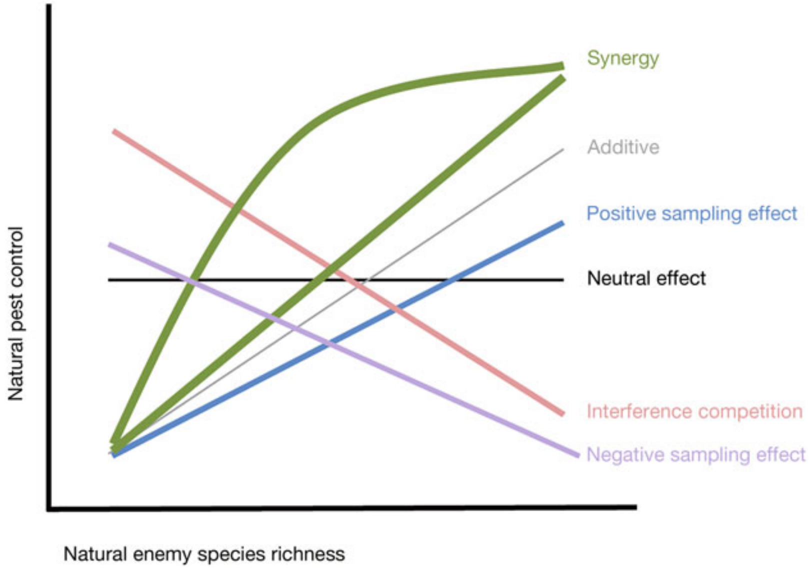


Fig. 6.3 Possible correlations between natural enemy species richness and natural insect pest control. (Based on Letourneau et al. 2009)
The direction and type of correlation depends on different ecological processes

group of species are increasingly stable as the number of species rises due to statistical averaging (Doak et al. 1998). For example, plant diversity has been shown to increase the interaction network stability at higher trophic levels because, although some arthropod populations decline, overall functioning is more stable (Haddad et al. 2011).

Although there is broad consensus on the positive correlation between biodiversity and ecosystem functioning, there are many empirical cases following experimentation or modelling that show neutral or negative correlations, particularly between taxonomic diversity and natural pest control. For example, in 30% of cases, an increase in natural enemy diversity is associated with a decrease in herbivore control (Letourneau et al. 2009). These effects have often been attributed to antagonistic biotic interactions between species or an unfavourable sampling effect at the ecosystem level. This suggests that our ability to predict ecosystem functioning, and especially natural pest control, solely on the basis of the taxonomic diversity of communities remains limited and that we must consider different processes to understand the determinants of natural pest control in a more mechanistic way.

Not all species in a community contribute in the same way to the processes they support. Community composition, and particularly functional diversity (i.e. trait diversity), plays an important role in the natural pest control process, although relatively few studies have sought to analyse these effects. Thus, we know that some predator traits or behaviours make it possible to predict trophic interactions in ecosystems, such as hunting methods, individual biomass or home ranges.

For example, one study demonstrated that the average body size in a community of predatory arthropods can explain the levels of natural aphid control in cereal fields (Rusch et al. 2015). This work shows that neither the abundance nor the species richness of predators are good indicators of pest control levels, but that the distribution of body sizes in the community makes it possible to identify situations that limit natural pest control, particularly through intraguild predation.

A highly uneven relative abundance has also been shown to reduce natural herbivore control compared to communities where the relative species distribution was more even. Although the effects of the species richness, functional diversity and evenness of natural enemies have been explored (Letourneau et al. 2009; Crowder et al. 2010; Greenop et al. 2018), few studies have looked at the effect of their composition (Fig. 6.4). There is no scientific consensus on the relative contributions of dominant and rare species to a function (Grime 1998; Mouillot et al. 2013). The "mass ratio" theory suggests that dominant species influence ecosystem functioning to the extent of their relative abundance (Grime 1998). Meanwhile, it has been suggested that, due to functional complementarity, the least abundant or even rare species would contribute significantly to ecosystem functioning (Mouillot et al. 2013).

6.4 The Effect of Vertical Diversity of Communities on Natural Pest Control

In the previous section, the structure-function link between community and control levels is discussed from a "horizontal" angle between natural enemies and the control of primary consumers (which include phytophagous insect pests). This initial approach can be supplemented by a more "vertical" view of the system, by integrating the interactions between multiple trophic levels (Fig. 6.2). This perspective offers a more holistic approach, which is often more similar to ecological mechanisms and provides the keys to understanding the highly variable correlations between natural enemy diversity and pest control levels. The organization of interactions between species across different trophic levels – i.e. the structure of interaction networks – impacts population and community dynamics. In this section, we will explain why it is important to produce knowledge on the vertical structure of trophic interaction networks to gain insight into the natural pest control process at the landscape scale. We will also show how the variation in community diversity, such as the addition or disappearance of species, can affect natural pest control.

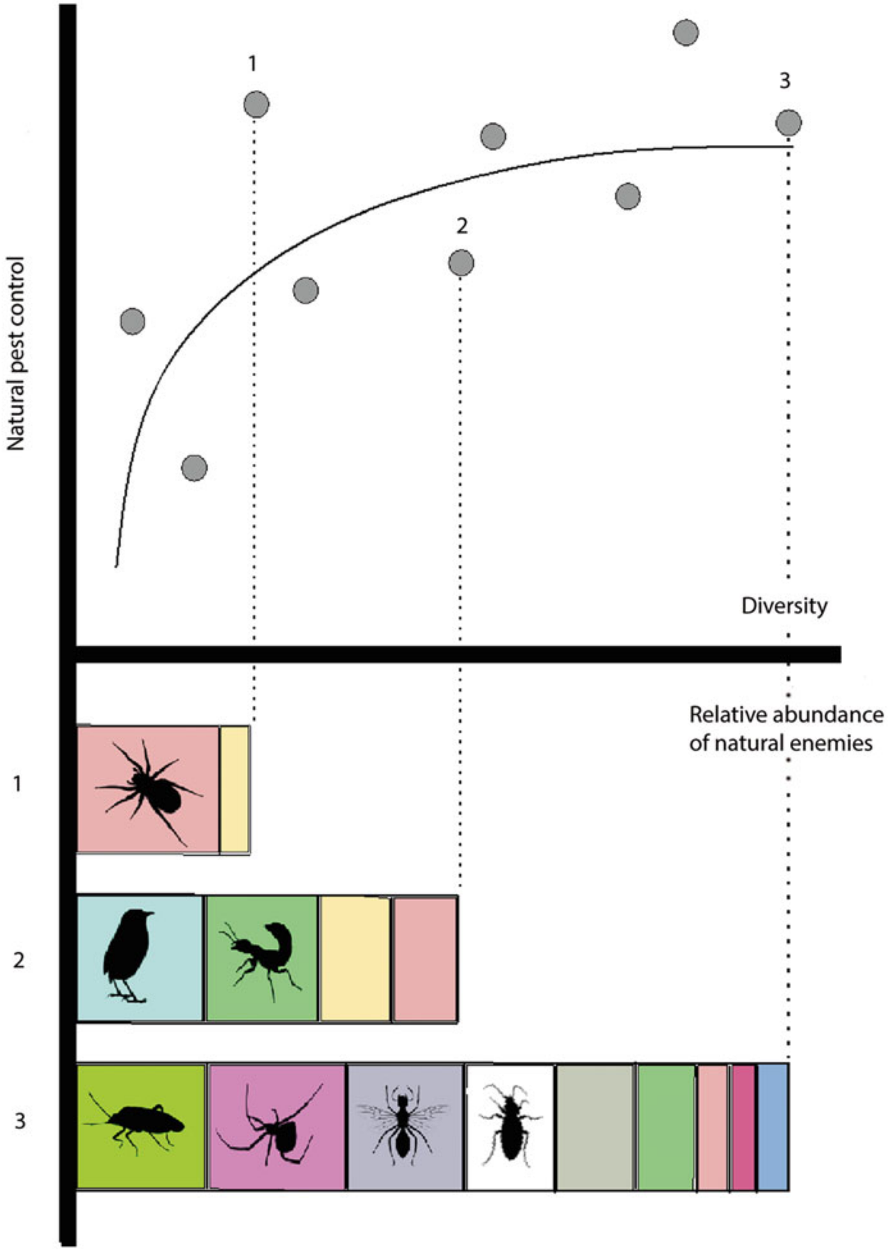


Fig. 6.4 The correlations between biodiversity and ecosystem functioning depend on community composition. (Based on Bannar-Martin et al. 2018)
Some species are more efficient than others in providing a function, while the type of interspecific interactions determines the level of natural pest control

6.4.1 Trophic Cascades

By studying species of agricultural interest, such as insect pests, within their trophic chain, we can consider both top-down and bottom-up constraints on these populations, i.e. control coming from higher trophic levels due to predators or parasitoids, and control coming from lower trophic levels due to limited resources (Fig. 6.2). Various studies have shown that increasing natural enemy abundance in cultivated fields results in a higher level of biological control of herbivores such as aphids, which in turn increases yields (Liere et al. 2015). However, within a network, changes related to one species can have ripple effects on all the others, sometimes with counter-intuitive consequences. For example, the loss of one species may imply secondary losses of other species with which it interacts, even indirectly. Conversely, the addition of a new species can have similar consequences, as cases of invasive species illustrate. This trophic cascade phenomenon has sparked numerous studies on network resilience to biodiversity loss (Dunne et al. 2002) with a view to determining the most sensitive structures and keystone species, which are those with most critical effects. These issues are especially relevant in a context of global change, where the pace of extinctions and invasions is accelerating (Pereira et al. 2012). However, these trophic cascade effects can be moderated by the dietary flexibility of certain species, which will change their feeding behaviour based on local conditions (Hawlena and Pérez-Mellado 2009). Agricultural examples illustrate the counter-intuitive effects of increased predator diversity, where one natural enemy engages in intraguild predation and turns away from its usual prey (insect pests) to consume other natural enemies. For example, ground beetles consume more spiders in organic crops than in conventional crops in response to a change in resource availability (Roubinet et al. 2017). In contrast to trophic cascade effects, there are many examples in the literature of strong positive bottom-up effects of increased diversity of primary producers on the diversity and abundance of higher trophic levels, as well as on the natural regulatory function of phytophagous insects (e.g. Scherber et al. 2010).

6.4.2 Trophic Structure and Network Stability

The way trophic relations are organized between species (i.e. the trophic structure) will determine both the functioning and sustainability of the agricultural system. To study this often very complex structure, it must be condensed or broken down into its different components (see Text Box 6.1 on descriptive metrics of network structure). Thus, a range of complementary metrics can be used to characterize (i) the average structure based on the number of trophic interactions and levels, (ii) the breakdown of interspecific network interactions, and (iii) the internal arrangement of interactions in relation to each other, especially the nestedness of interactions and the grouping of interactions into compartments. These metrics measure different structure aspects, but are covariates in relation to each other.

Network structure measurements have revealed recurrent topological patterns that withstand variations in species richness (the “scaling law”, described by Cohen and Newman 1985), suggesting general constraints on the structure of interaction networks, and possibly universal mechanisms in ecological network assembly (see for example Montoya et al. 2006). Different regular structures have been identified and debated, including the law governing the decrease in complexity with diversity (i.e. how new interactions are formed when network diversity increases, or how networks are assembled).

Network stability (or persistence) when affected by disturbances is a central issue in ecology. On the one hand, it shows that ecosystem functions are resilient to disturbances, particularly those caused by humans; on the other, theoretical work has shown that complexity destabilizes systems, thus calling into question the aspects that determine the existence of these very complex ecological components (Landi et al. 2018). The complexity-stability debate is being fuelled by numerous studies showing how particular structures bring stability to the system, as opposed to a random structure. For example, the over-representation of certain trophic modules and the high proportion of low-intensity interactions could attenuate fluctuations in prey-predator dynamics. Nested structures could protect against temporal fluctuations in the abundance of specialist species, while modular structures could mitigate the spread of disturbances. Establishing interspecific interactions according to the value of certain traits – such as the body mass ratio between predators and prey – can also have a stabilizing effect (Brose et al. 2006). Despite the importance of understanding the structure of trophic interaction networks and the links between network complexity and stability, there are relatively few examples applied to natural insect pest control in agricultural landscapes. Research in this area is severely limited by a lack of access to quality and complete data on individual diets, and therefore on the structure of interaction networks. Quantifying trophic interactions is a difficult task and requires different methods, such as direct observation of interactions, gut content analysis via dissection or molecular markers, the use of exclusion protocols or sentinel prey, or the use of isotope or fatty acid biomarkers (see Birkhofer et al. 2017). However, studies in agricultural environments suggest a decrease in structure complexity in areas that are highly modified by human activities (Tylianakis et al. 2007), with impacts caused by farming practices and primary resource diversity, particularly on predator feeding behaviour (Mollot et al. 2014).

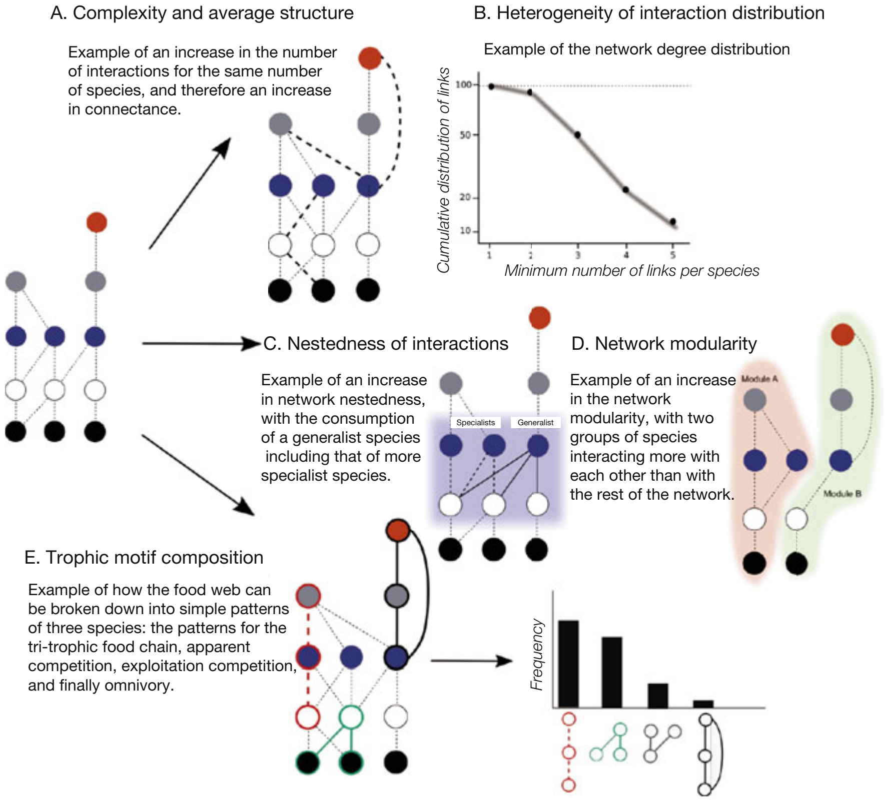
Text Box 6.1: Characterizing the Trophic Network Structure

Ecological networks represent the interactions (i.e. links) between species (i.e. nodes) in a binary way (presence/absence of links) or quantitative way (link intensities). Their complex structure can be measured using a range of complementary metrics to capture the different aspects of this structure. Many of these metrics were originally developed to account for a binary structure, but have been adapted to take into account the heterogeneity of interaction intensity.

(continued)

Text Box 6.1 (continued)

Some metrics represent the average structure complexity, such as the number of species involved *S*, the total number of links *L*, the average number of links by each species, i.e. link density *LD*, the length of the trophic chains, the proportions of species from different trophic levels, and the connectance *C* (Fig. 6.5a). Connectance is one of the most widely used and synthetic metrics since it represents the proportion of interactions among all those that are theoretically possible (i.e. if all species interact with each other), and thus reflects the average network complexity. Other types of metrics take into account the internal organization of interactions and quantify their heterogeneity (such as degree distribution, i.e. the cumulative distribution of the number of interactions per species, Fig. 6.5b), nestedness (Fig. 6.5c) or aggregation (such as modularity or compartmentalization, Fig. 6.5d). Finally, another approach is to break the network down into trophic motifs (Fig. 6.5e), which shows the frequency of patterns.



A. Complexity and average structure

Example of an increase in the number of interactions for the same number of species, and therefore an increase in connectance.

B. Heterogeneity of interaction distribution

Example of the network degree distribution

C. Nestedness of interactions

Example of an increase in network nestedness, with the consumption of a generalist species including that of more specialist species.

D. Network modularity

Example of an increase in the network modularity, with two groups of species interacting more with each other than with the rest of the network.

E. Trophic motif composition

Example of how the food web can be broken down into simple patterns of three species: the patterns for the tri-trophic food chain, apparent competition, exploitation competition, and finally omnivory.

Fig. 6.5 Illustration of various metrics to characterize the interaction network structure

6.4.3 Modelling Ecological Network Assembly

Theoretical models, especially mechanistic models, are essential for testing hypotheses on network assembly and functioning, or for studying their emerging properties. This type of modelling can also compensate for a lack of empirical data, one of the major limitations in studying interaction networks.

The study of trophic relationships began by modelling predator-prey dynamics (Lotka-Voltera models) before being extended to more diverse systems within trophic motifs limited to a few species. These models provide information on the fine-grain functioning of simple trophic motifs, such as apparent competition, but can be disconnected from the functioning of complete networks. The complexity of these systems leads us to consider other types of modelling that focus on network assembly, structure emergence, and network stability against disturbances.

By simulating species interactions, network assembly models can predict the theoretical structures expected based on different hypotheses, and then compare them with empirical network structures. This allows researchers to test the mechanisms governing interspecific interactions and produce theoretical networks that are similar to empirical networks. Many assembly models have been put forward, from those based on a static network (i.e. which do not include species population dynamics) to dynamic and evolving network models. The pioneering static model is the cascade model (Cohen and Newman 1985), followed by the so-called niche model (Williams and Martinez 2000), which can generate a network using only the number of species and connectance (see Text Box 6.1). More recent modelling work has attempted to produce realistic, probable networks based on partial species information such as relative abundance (Canard et al. 2014), or certain traits such as species body size (Gravel et al. 2013).

Assembly models generate a fixed structure, and rarely consider species population dynamics. In fact, some models attempt to describe trophic community dynamics, such as the multi-species bioenergetic model (Williams and Martinez 2004). Both types of modelling can be complementary to produce theoretical dynamic networks, as shown for example by Brose et al. (2006), who use empirical allometric scaling based on species size and trophic level to combine the static and bioenergetic models of food web structure.

6.4.4 Research Possibilities on Interaction Networks

The structure of agricultural ecological networks is closely linked to the functioning of agricultural systems, and establishing clear relationships between these two components is a major challenge for agroecological research. Linking ecological network structure to natural pest control levels can help answer questions about the type of natural enemies that are most effective at controlling pest populations, and thus help determine precise objectives regarding optimal community structure to promote regulation processes. For example, there is no consensus on whether specialist or generalist natural enemies are better for agricultural systems. Additionally, by studying natural pest control within ecological networks, traditional agricultural science studies can be scaled up since researchers can look at the entire ecological system rather than focusing on one particular or pair of species. Studying interaction networks in agricultural landscapes also makes it possible to analyse the synergies between different ecosystem functions that support agricultural production, which is essential to agroecology. These networks can highlight existing connections between cultivated habitats and the surrounding landscape. Advanced studies on ecological networks also allow the simultaneous study of several types of interactions within the same network (e.g. herbivory and pollination, Fontaine et al. 2011; Kéfi et al. 2012), which can shed more light on the ecological functioning of a cultivated field.

6.5 Metacommunities and Landscape Ecology

6.5.1 Metacommunity Theory

Beyond the local biotic and abiotic filters discussed in the introduction, each community is the result of spatial dynamics at the metacommunity level (i.e. at the regional scale, Leibold et al. 2004). The influence of these spatial dynamics on local community composition depends on three factors: (i) the spatial heterogeneity of the matrix habitat (composed of cultivated and uncultivated areas in an agricultural landscape), (ii) the dispersal of organisms within this matrix and (iii) the variability of traits between organisms, or their “equivalence” (Logue et al. 2011).

The intensity of these three factors’ influence on local community composition determines the extent to which deterministic processes (adequacy between environmental characteristics and the species niche) or stochastic processes (processes linked to the demographic parameters specific to each species) impact local community assemblages. It is now clearly established that neither deterministic nor stochastic processes alone can explain local community compositions, and that community assembly depends on both (Chase and Myers 2011). Thus, research questions tend to focus on the intensity of these three factors and the extent to which each of the four paradigms proposed by Leibold et al. (2004) explain local assemblages and impact ecosystem functioning (Bannar-Martin et al. 2018). These four paradigms are presented below along a continuum, with strong stochastic processes on one end and strong deterministic processes on the other (Fig. 6.6).

The null model (Fig. 6.6) suggests that the trait variability between individuals is nil at the metacommunity level and that local function levels mainly correlate to individual abundance, and not to niche occupation by the different species (neutral model; Hubbell 2001). In this case, the probability of finding a species in a field is independent of the intensity of local filters such as agricultural practices within a field, because it is stochasticity (i.e. population demographic processes: death, birth,

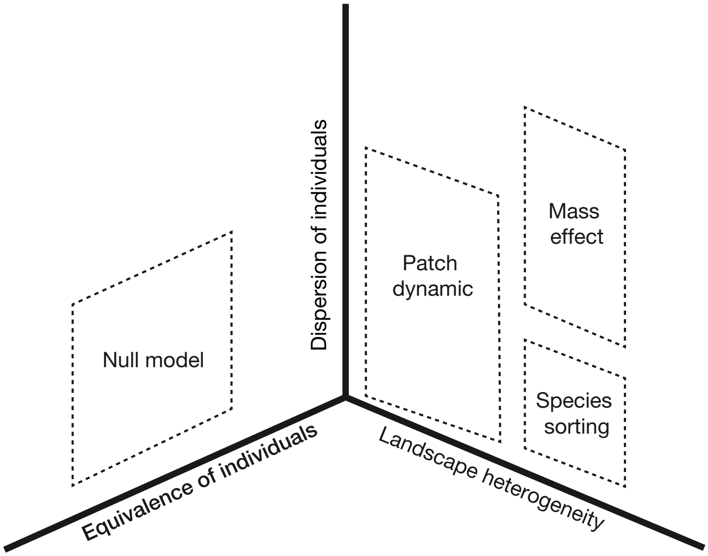


Fig. 6.6 Diagram of the four paradigms derived from metacommunity theory to show the influence of spatial dynamics on local community composition

immigration, emigration) that determines local community composition. According to this paradigm, efforts to maintain biodiversity to ultimately promote ecosystem services such as natural pest control must be made on a regional scale, and not at the local level, due to the predominance of stochastic processes over local community assembly.

The "patch dynamic" paradigm (Fig. 6.6) is an extrapolation of the theory of island biogeography put forward by MacArthur and Wilson (1967) and indicates that local community composition results from a balance between competition and dispersal. This model suggests that habitat filters are not very discriminating, so interspecific competition is relatively low and allows less competitive species to coexist with more competitive ones. In this case, stochastic effects are therefore lower than in the null model.

In contrast to the patch dynamic paradigm, the "mass effect" paradigm (Fig. 6.6) suggests that the landscape context has a structuring effect, with habitats having different and stronger filters. Dispersal allows less competitive species to thrive in habitats where they have little competition. The local assemblage therefore contains species that have adapted to the local environment along with less adapted species. The effect of stochasticity is thus further reduced and depends on the organisms' dispersal capacities (Pulliam 1988; Mouquet and Loreau 2003). This paradigm is supported by all the studies that examine the influence of the landscape context (e.g. the effect of semi-natural habitats) on community composition and ecosystem function in agricultural landscapes (see Chap. 7).

Finally, at the deterministic end of the continuum, in the “species sorting” paradigm (Fig. 6.6), dispersal is low and habitat heterogeneity has a strong impact on local community assemblages. Local communities should therefore strongly reflect potentially available niches. This paradigm lies at the heart of functional ecology and suggests, for example, that local environmental conditions (e.g. farming practices) have a strong impact on community composition. In this paradigm, biodiversity conservation efforts to promote ecosystem services (such as natural pest control) should be made at the local level.

With regard to analysing natural enemy community assemblages, metacommunity ecology provides a conceptual framework for considering the structuring effect of regional biodiversity and landscape composition on local species assemblages, which influences the average level and variability of the natural pest control at field scale. This framework also helps us understand the extent to which local practices will impact the composition of natural enemy communities, and thus determine the most relevant scales for agricultural management. Below we discuss landscape ecology approaches that explore how landscape heterogeneity affects species assemblages at the local scale.

6.5.2 Landscape Ecology: Landscape Organization Patterns and Ecological Processes

Landscape ecology can offer tools to characterize how landscape organization patterns affect ecological processes, such as when trying to describe their temporal evolution, to compare different landscape types, to predict the consequences of potential changes or to evaluate optimal land management strategies. Landscapes, particularly agricultural landscapes, are inherently heterogeneous as they generally incorporate habitats that vary in space and time (e.g. due to crop rotation in landscapes dominated by annual crops). Accordingly, we will consider two different and complementary dimensions to describe the heterogeneity of these landscapes: composition and configuration. Composition heterogeneity describes the diversity of abundance and land use types in landscape compositions and does not explicitly take into account the spatial dimension, i.e. the arrangement of landscape elements in relation to each other. Assessment metrics include the proportion and number of different land use types, heterogeneity indices such as the Shannon index or the relative distribution of land use types in the landscapes. Configuration heterogeneity more explicitly includes the spatial dimension of the landscape elements because it seeks to consider the arrangements of the habitat patches in relation to each other. To describe this dimension, it is possible, for example, to characterize the shape or size of the patches, the isolation of the patches from each other, or the fragmentation and connectivity of landscape elements.

In addition to calculating indices reflecting the compositional or configurational dimensions, we can integrate biological parameters of species and describe landscapes more functionally beyond their structure to predict species distribution within landscapes. For example, the dispersal abilities of the individuals/species studied can be integrated into the configuration index calculations so the relative values of the different land use types can be weighted according to organisms' home ranges, or to calculate low-cost paths for a given species according to its life history traits. These different metrics for composition or configuration can generally be calculated at the habitat patch, habitat class or landscape scale.

Several essential parameters also come into play when representing and analysing landscape structure, namely:

- grain size: refers to the spatial or temporal resolution and indicates the finest resolution at which an object has been measured;
- area under consideration: refers to the spatial or temporal dimension of the study area;
- thematic resolution: refers to the different land use types used to describe landscapes (Burel and Baudry 2003; Turner et al. 2001).

A large number of studies that attempt to explain population dynamics, local species assemblages or specific functions, such as natural pest control, have emerged since the 1990s. The vast majority of these studies seek to analyse how metrics relating to the structure of the surrounding landscape, briefly mentioned above, help explain pest population dynamics or the level of pest control measured at field scale. Chapter 7 provides an overview of the knowledge produced by these approaches.

6.6 Conclusion

Stimulating natural pest control within agricultural landscapes involves first answering two main sets of questions. On the one hand, we must understand how communities of natural enemy species interact with each other and to what extent their composition impacts pest demography. Current scientific consensus holds that a diverse community of natural enemies tends to support natural pest control. However, this chapter makes it clear that this is not always the case, and that “horizontal” and “vertical” approaches must be combined to understand the emergence of positive, negative or neutral interspecific interactions at different trophic levels. While it is generally accepted that multiple biotic and abiotic filters operating at different spatial scales can explain species assemblages within local communities, some questions remain largely unanswered about the relative contribution of deterministic and stochastic processes in local species assemblages. Community ecology, network ecology and landscape ecology are shedding light on these issues, but there are still many questions that need to be resolved to enhance predictions regarding natural pest control.

Chapter 7
Agroecological Management of Insect Pests from Field to Landscape

Adrien Rusch

7.1 Introduction

The idea of modifying the environment at the field scale or larger spatial and temporal scales to limit insect pest populations has been around a while. There are many historical examples of what we would call agroecological engineering today, such as the use of weaver ants by Chinese farmers to control citrus insect pests around 1200 AD, or when Pliny the Elder and Dioscorides noticed in the first century AD that certain plants such as wormwood had repellent virtues against insect pests. Managing insect pest populations by modifying the environment is obviously closely linked to the birth and development of agriculture. Throughout history, man has sought to control the factors limiting food production, and especially the crop losses associated with insect pests. Farmers have traditionally used techniques available to them at the field scale. Researchers have only relatively recently shifted their attention to the broader spatial dynamics of insect pest populations and begun to understand that environmental factors influencing population levels can operate on much larger scales than a single crop field. Agroecology research has been striving to produce detailed knowledge on the mechanisms governing insect population dynamics and biological regulation processes by their natural enemies in agricultural landscapes. The aim of this work is to manage these processes in order to reconcile agricultural production and environmental concerns. This chapter provides an overview of current knowledge on the effects of farming practices and landscape structure on insect pest management by considering conservation biological control approaches.

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7.2 Principles of Conservation Biological Control

Conservation biological control aims to maintain pest populations below a harmful threshold by combining direct approaches (e.g. olfactory or visual disturbance of the host plant's location) and indirect approaches that favour natural enemy abundance, diversity, development and activity to control pests (see Chap. 6). There are three types of natural enemies: (i) predators that feed directly on insect pests or disease vectors (in this case, vertebrates or invertebrates), (ii) parasitoids that lay their eggs in or on their host and kill their host during their development, and (iii) pathogens, which are microorganisms capable of injuring or killing their host. This chapter mainly deals with predators and parasitoids.

Implementing conservation biological control strategies requires a detailed knowledge of species biology and ecology, such as their essential needs for survival and reproduction, their dispersal abilities and their behaviour. Natural enemies encompass a wide range of species with highly varied life history traits and ecologies. However, they are generally mobile species that use several resources located unevenly across different habitats during their life cycle. Understanding the dispersal abilities of the species present and their life cycles is crucial in order to gain insights into their dynamics over space and time, which can then inform successful conservation biological control strategies. But first, we should go into a little more detail about the key resources for natural enemies involved in natural pest control.

Predatory and parasitoid arthropods need three types of resources to thrive in agricultural landscapes: refuges, nectar or pollen sources, and alternative hosts or prey (Landis et al. 2000). Most predators and parasitoids need refuges, such as overwintering or summering sites to protect themselves from poor weather or environmental disturbances related to farming practices (e.g., pesticide applications, mowing, soil tillage). Some natural enemies need additional food resources such as pollen or nectar as well as alternative prey or hosts to survive when their preferred prey (insect pests) are not present (or in insufficient numbers), or when pests are not at the life stage where they would be preyed upon by the natural enemies. Providing refuges, food resources and alternative hosts helps to maintain sufficient levels of natural enemy populations and promotes the establishment of natural pest control relatively early in the year. Semi-natural habitats are generally home to a higher proportion of neutral or beneficial arthropods than arthropod pests. It is often said that nine out of ten natural enemy species require an uncultivated habitat at some point in their life cycle, whereas this is the case for only one out of every two pests.

Refuges can be special habitats created either within the cultivated field or in the landscape environment. Within cultivated fields, grass and flower strips and cover crops can all provide refuges for many species as well as favourable microclimates. Semi-natural habitats, such as woodlands, hedgerows, meadows and field margins, are often places that provide shelter and food for predators in a relatively stable way over time because these habitats are subject to less disturbance than cultivated habitats. Thus, wooded habitats often offer a more moderated microclimate than the middle of a crop field, and can protect predators and parasitoids from extreme temperatures during the growing season (Landis et al. 2000). Grassy habitats, such as natural meadows, provide overwintering sites for many species of spiders, rove beetles, ground beetles, lady beetles and neuropterans (Sarthou et al. 2014).

Pollen and nectar are essential for many species of natural enemies. For example, parasitoid hymenopterans feed on floral and extrafloral nectar. Providing sugar resources to parasitoids has been shown, both under laboratory conditions and in experimental plots, to generally increase the longevity and fecundity of females, and thus their potential to parasitize their host (Wäckers et al. 2005). Studies have shown that nectar availability determines the longevity and fecundity of female parasitoids of *Diadegma semiclausum* (Hellen, 1949) (Hymenoptera: Ichneumonidae), and the associated parasitism rate of the diamondback moth *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) is higher when female parasitoids have access to nectar sources (Winkler et al. 2006).

Providing alternative hosts or prey is particularly important during periods of low host and prey densities in crop fields and ensures that resources are present throughout the growing season. This is especially true for generalist predators which, by definition, have a broader diet than specialists. For instance, it has been shown that if wheat aphid populations colonize fields later in the season, then the predatory lady beetle *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) becomes dependent on aphid populations located in semi-natural habitats (Bianchi and van der Werf 2004). As a result, lady beetles are especially vulnerable to periods of food limitation in cultivated fields when the availability of prey in semi-natural habitats is low.

To sum up, it is important to have a detailed understanding of the needs, responses and behaviours of natural enemies in changing environmental conditions to tailor natural pest management strategies to each specific situation. Providing food resources, alternative hosts and refuges helps maintain natural enemy communities in and around cultivated fields, and preserving, restoring and creating habitats that offer these resources across time and space are key to promoting natural pest control. Below we detail the effects of different agricultural practices on natural enemies and insect pest control at the plot and cropping system scales.

7.3 Effects of Farming Practices at Field Level

7.3.1 Plant Diversity over Space and Time

Plant community diversity at field level has a major effect on upper trophic levels, and thus on insect pests and their natural enemies. Many syntheses of current knowledge have shown that cultivated fields with higher plant diversity tend to have a higher abundance and/or diversity of natural enemies, lower densities of herbivores and lower damage resulting from insect attacks compared to monoculture fields (Letourneau et al. 2011). These beneficial effects of plant diversification on natural pest control at field level result from two complementary mechanisms: the action of natural enemies and the direct effect of heterogeneous resource distribution on insect pests. As explained above, diverse vegetation cover hosts a more abundant and richer community of natural enemies due to a greater diversity of potential resources (Langellotto and Denno 2004). A diverse plant cover will generally harbour smaller populations of phytophagous insects and suffer less damage because of the lower probability of a given pest species to locate its host plant (Root 1973). This effect is mainly attributed to chemical confusion or physical disturbances, as well as to changes in the physiological state of plants due to interspecific interactions.

On a small spatial scale, positive effects that can be explained by at least one of the two above mechanisms have been shown for different diversification strategies, such as combined crops, trap crops, the push-pull strategy and flower strips. The scientific literature is full of many examples, observed across the globe, of the positive effects of plant diversification on limiting insect pest pressure. For example, push-pull strategies to protect maize and sorghum crops from various lepidopteran pests have proved very successful and significantly increased yields in East Africa (Cook et al. 2007a). These strategies combine species mixtures to repel pests and lead them away from the main crop towards trap plants, which are chosen because they attract parasitoids and thus increase the parasitism rates of pests. Such strategies have also been used on oilseed rape and potato pests (Cook et al. 2007a; Martel et al. 2005).

Plant diversity over a longer time-frame – i.e. beyond the field level for a given year – and thus as it pertains to crop sequencing can also be an important element in managing insect pests, weeds and pathogens. The basic principle, developed empirically by farmers, is to break the pest cycle by rotating host crops in a field (Ratnadass et al. 2012). Studies have also suggested that the abundance, activity, reproduction and diversity of natural enemies such as ground beetles tend to increase with longer crop rotations combined with reduced fertilizer and pesticide use, suggesting higher levels of biological control in such systems (Büchs et al. 1997). However, further studies are needed to confirm this.

7.3.2 Nitrogen Fertilization

The physiological state of plants, and more specifically their nitrogen status, plays an important role in pest population dynamics and survival, notably by influencing plant resistance, the pest's choice of host plant and the plants' compensatory abilities. Two hypotheses have been put forward about the link between host plant quality and herbivore population levels: the plant stress hypothesis and the plant vigour hypothesis (Price 1991; White 1984). The plant stress hypothesis states that physiologically stressed plants are subject to more attacks by phytophagous insects because of the plant's nutritional state or a decline in their resistance mechanisms. Conversely, the plant vigour hypothesis suggests that more vigorous plants would be subject to greater attacks by herbivores, as they would prefer them as better quality food sources. There is evidence in the scientific literature that these two hypotheses are valid for different species, but literature reviews indicate that there are more cases where phytophagous insects respond positively to more vigorous or more fertilized plants than the opposite (Butler et al. 2012). However, this depends on particular life history traits of the species. Sap-sucking insects appear to show a more marked response to the nitrogen status of crops than chewing insects.

The fertilization method can also impact natural enemy communities, although this subject has not been well covered in the scientific literature. Reincorporating crop residues can replenish the system with different nutrients, including nitrogen. This practice generally has positive effects on predator communities, namely by increasing the organic matter in the soil (which in turn positively affects decomposer communities), as well as by providing important microhabitats for different species.

Nitrogen fertilization can also have effects on higher trophic levels, such as on parasitoid performance. For example, diamondback moth parasitism rates on cruciferous crops are lower in moth populations that have developed on less fertilized plants (Sarfraz et al. 2009). These phenomena show the interest of taking trophic interactions into account in order to fully understand the direct and indirect effects of nitrogen fertilization and host plant quality on insect pest attacks.

7.3.3 Tillage Practices

A common approach in agroecology is to change tillage practices, which has known effects on pest management, including on phytophagous insects. Organisms respond to tillage in highly variable ways and depending on taxa, but the abundance and number of species of soil fauna generally tend to increase with reduced tillage intensity. Different variables can also impact natural enemies and phytophagous insects, from tillage intensity to the equipment used, the frequency of operations or the period of time when tillage is performed. Deep tillage will have a strong impact on biological communities, namely by modifying microhabitat quality, the soil's physicochemical structure and prey availability for predators. Tillage can also have direct lethal mechanical effects, as well as indirectly force organisms to migrate or expose them to predation. The effects of tillage on natural enemies and pest control can therefore be equivocal. For example, deep and intensive tillage is generally an effective practice for controlling slug populations, with direct effects on slug mortality and indirect effects through changes in habitat structure. However, we also know that intensive tillage is rather negative for natural enemy populations, and that reducing or maintaining crop residues on the surface increases natural enemy activity and even biological control. For instance, Tamburini et al. (2016) recently showed under real cropping conditions that conservation rather than conventional tillage increases the natural regulation level of wheat aphids by 16%. The potential underlying mechanisms that explain this positive effect are: (i) the presence of physicochemical barriers linked to crop residues that directly disturb pests' movement and ability to located the host plant; (ii) reduced competition between natural enemy species due to a more complex environment that is favourable to microhabitats; (iii) greater resource and alternative prey availability; (iv) more favourable microclimatic conditions and greater availability of organic matter reducing predator mortality.

7.3.4 Organic Farming

If we go beyond the effects of individual farming practices at the crop management sequence scale, the question arises regarding the overall sensitivity of cropping systems to pest attacks and their ability (or lack of) to support natural herbivore control. To answer this question, we can look at organic farming, which imposes specifications that support ecological processes such as natural control. It is now well established that organic farming practices at the field scale favour the abundance and species richness of many taxa, from plants to mammals and birds, compared to conventional farming. Several recent meta-analyses have shown that the abundance and number of species increase by an average of 30% in organic fields (Bengtsson et al. 2005; Tuck et al. 2014). These studies show that insects, plants and birds in particular respond positively to organic farming practices. Furthermore, a recent global meta-analysis shows that organic farming practices increase the levels of pest control services provided by natural enemies and that infestation levels in organic fields are ultimately not any higher than in conventional fields (Muneret et al. 2018). These findings indicate that organic farming practices promote natural pest control processes that can be just as effective as conventional farming methods in managing pest populations. In addition to these local effects, recent work has shown that the effects of organic farming are modulated by the landscape structure, and especially the presence of semi-natural habitats and how farming practices are implemented across the landscape (Muneret et al. 2019). The following section outlines current knowledge on the effects of the landscape environment on natural enemy communities and insect pest control.

7.4 Biological Pest Control at the Landscape Scale

7.4.1 Transition Areas Between Cultivated and Non-cultivated Habitats

Transition areas, known as ecotones, between cultivated and non-cultivated habitats can offer insights into how individuals move on wider spatial scales. Individual movements – whether by natural enemies or phytophagous insects – between cultivated and non-cultivated habitats take place in a bidirectional way and are largely determined by the available resources. The direction and strength of the flows appear to depend mainly on differences in primary productivity between habitat types, on resource phenology (food or refuge), as well as on the relevant taxa, which may seek complementary or substitute resources during their life cycle. Arthropods living in agricultural landscapes show a variable degree of specialization for cultivated or semi-natural habitats. Species are found along a continuum and range from those confined solely to natural or semi-natural habitats (called stenotopic species) to those specialized in cultivated areas. A large majority of organisms lie somewhere between these two extremes and rely on both cultivated and uncultivated habitats to varying degrees during their life cycles; most species will need a semi-natural habitat at least once during their lifetimes.

The scientific literature contains many examples illustrating the role that transition areas between cultivated and non-cultivated habitats can play in the dynamics of insect pests and their natural enemies. For instance, research in Australia demonstrated that vine rows near woodland margins had more lady beetles and parasitoids, along with higher rates of predation and parasitism of a moth species, than central vine rows (Thomson and Hoffman 2013). Similarly, a study conducted in South Africa showed that the distance of mango plantations from natural vegetation patches was a key factor in the abundance of Tephritidae fruit flies, leaf-galling flies and pathogenic fungi (*Fusarium* spp.) (Henri et al. 2015). Other studies have highlighted flows of predatory or parasitoid natural enemies between different managed agroecological areas, such as grass or flower strips, and crop fields. These studies generally show fewer movements as distance to the managed area increases and higher levels of pest control at the field margins than in the centre, suggesting a limiting effect on natural enemy dispersal ability.

7.4.2 Landscape Structure and Natural Pest Control

Considerable research has focused on the effect of landscape structure on natural enemies, trophic interactions and natural pest control. The initial aim of these studies was to analyse the relationships between natural enemy abundance or diversity and landscape composition, which is most often characterized by proportions of land use types. Researchers then shifted their attention to the effects of landscape configuration by analysing how the spatial arrangement of habitats (e.g. functional connectivity) could affect natural enemy population dynamics and communities. A large majority of the studies exploring the effects of landscape structure on insect pest control have considered the effects of the proportion of semi-natural habitats in the landscape, because it generally correlates with other variables that indicate landscape heterogeneity. Additionally, and as mentioned above, semi-natural habitats are especially important in terms of resources for natural enemies and pests, which explains why many studies have focused on this issue at the landscape level.

For example, the abundance and number of natural enemy species in cultivated fields has been shown to rise as the proportion of semi-natural habitats in the surrounding landscape increases (Bianchi et al. 2006; Chaplin-Kramer et al. 2011).

Meanwhile, a review by Bianchi et al. (2006) indicated that in 74% of published cases, the abundance of natural enemies increased in tandem with the proportion of semi-natural habitats in the landscape, while no effect was detected in 21% of cases and 5% of studies indicated a decrease in natural enemy abundance. Maintaining habitats that provide key resources (e.g. overwintering and summering sites, food resources and alternative hosts) enables populations and communities to survive and even thrive in agricultural landscapes. The direct impact of landscape heterogeneity on the energy reserves and fecundity of some natural enemy populations can even be measured. For instance, omnivorous *Poecilus cupreus* ground beetles that live in more heterogenous landscapes are larger and have fecundity rates that are three times higher than individuals in simple landscapes (Bommarco 1998). However, the effects of landscape composition on natural enemies appear to be moderated by various parameters, and especially by the functional features (such as the degree of specialization or dispersal abilities) of the individuals or species under consideration. Thus, the positive effects of landscape heterogeneity on natural enemy abundance and diversity seem relatively marked for generalists (e.g. ground beetles or spiders), but not for specialists (e.g. parasitoids).

The positive effects of the proportion of semi-natural habitats on natural enemy abundance and diversity tend to result in greater natural pest control levels (via predation or parasitism) (Chaplin-Kramer et al. 2011; Rusch et al. 2016). This is explained by the processes of complementarity between species as described in Chap. 6. A recent study confirmed that an increase in cultivated land area in the landscape significantly decreases the potential for natural pest control (Rusch et al. 2016). In this case, natural aphid control was half as strong on average in homogeneous landscapes that were dominated by crops compared to more heterogeneous landscapes dominated by semi-natural habitats. Ongoing studies on other pests indicate significant variability in pest response to landscape heterogeneity, which again suggests that these effects are modulated by certain life history traits such as dispersal abilities, life cycle complexity or feeding behaviour diversity.

Other important landscape aspects can also affect insect pest population dynamics. For example, the diversity of crops or production systems (e.g. organic farming) at the landscape level can have a considerable structural impact on natural enemy communities and natural pest control. Muneret et al. (2018, 2019) demonstrated this in vineyard landscapes, where farming practices at the local and landscape scales strongly impacted natural enemy communities and natural pest control services. As more cropland was converted to organic, a higher average abundance of predatory spiders was found on the soil of organic fields but not in conventional fields, indicating local filter phenomena that modulate the positive effects on spider communities in organic vineyards. Landscape configuration, i.e. the spatial arrangement of landscape elements, can also influence natural enemy and pest population dynamics (Martin et al. 2019). For example, ground beetles in arable crop landscapes have been found to be much more affected by landscape configuration, and more specifically by a reduction in field size, which favours their functional diversity, than by the type of crop management or the proportion of semi-natural habitats (Gallé et al. 2019).

Very few studies have sought to characterize the effects of landscape structure changes on trophic interaction networks with regard to crop insect pests ("vertical approach", explained in Chap. 6). One study on the effects of host-parasitoid networks on cereal aphids showed that increased landscape heterogeneity resulted in simpler host-parasitoid networks and higher aphid parasitism rates, suggesting that at the space and time scales considered, the theoretical relationship between network complexity and functioning was not necessarily valid (Gagic et al. 2011). High-throughput sequencing technology, which has recently become much more accessible, can be used to analyse the structure of trophic interaction networks at large spatial scales. Various research programmes are currently exploring this issue but little evidence exists. These programmes will eventually be able to provide more mechanistic insights into network structures, and especially into the specific network patterns that explain the links between land-use change, trophic interactions and natural insect pest control.

7.5 Conclusion

Studies carried out to date reveal that the relative effects that different aspects of landscape structures have on natural enemies and biological control appear to depend on the landscapes themselves and the different taxa studied. We must now step back from the contextual dependencies emerging from all the experimental studies to see the bigger picture and identify information that can be extrapolated to other contexts. Functional and trophic network ecology approaches that integrate the functional traits of different species offer interesting perspectives that should soon lead to predictive tools on natural insect pest control. To date, very few studies have attempted to integrate the diversity of known factors from the plant scale to the landscape scale. However, it is vital that we do so if we are to truly understand the multiple interactions between agroecological drivers, synergies and even antagonistic forces and optimize natural pest control strategies. There are also very promising prospects for modelling trophic interaction networks.

Chapter 8
Biological Control for Weed Management

Sandrine Petit and Stéphane Cordeau

8.1 Introduction

Agroecosystems are home to hundreds of species of plants that grow spontaneously. For example, in France alone, some 1200 species of such unwanted plants have been identified (Jauzein 2001). These unwanted plants are known as weeds. Due to frequent disturbances related to farming activities (e.g. herbicide applications, tillage), weed flora is mainly composed of annual species producing large quantities of seeds. In agricultural landscapes, weeds are present in crop fields as well as along field margins, which today are home to many species in decline (Fried et al. 2009).

Weed management has received renewed attention over the past 15 years. This is partly due to the pressing need to reduce the reliance of cropping systems on herbicides and the lower environmental impacts offered by alternative techniques. But it is also because there is growing recognition that weed flora provides many important ecosystem services (even if poor control of certain weeds can significantly lower yields), such as trophic resources (flowers, seeds) and habitats that support entire sections of animal biodiversity in agroecosystems (Marshall et al. 2003). Weed flora can be managed effectively even with reduced chemical control by using multiple agronomic levers (Petit et al. 2015). Various studies show that low-input weed management must combine cultural techniques that disrupt weed growth at different points in the weed life cycle. Such techniques include diversifying crop rotations and seeding periods to reduce the growth of the weed seedbank, using false/

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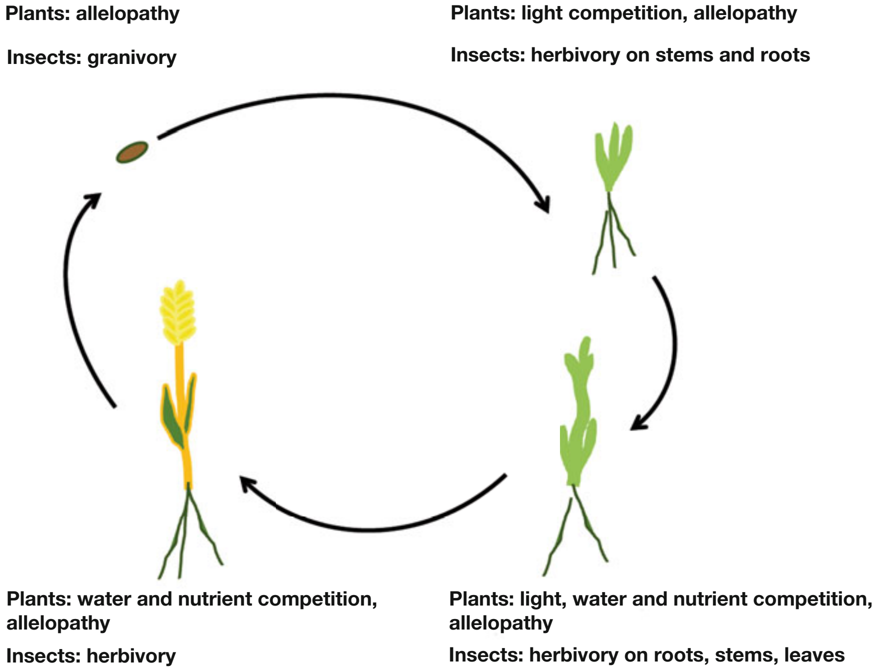

Fig. 8.1 The weed life cycle and processes that can occur during the plant's different phenological stages

stale seedbeds where weeds are allowed to germinate and then are mechanically destroyed, or delayed seeding after the weeds' preferential germination period.

A complementary approach to agronomic techniques consists in enhancing the many naturally occurring biotic interactions between various types of organisms and weeds, leading to natural weed control (Petit et al. 2018). These interactions take place at different times in the weed cycle: seed, seedling and plant at different phenological stages (Fig. 8.1). This chapter will deal exclusively with two types of biotic interactions. The first is based on a horizontal approach and concerns interactions between organisms at the same trophic level – weeds and sown plants (arable and cover crops) – and especially through competition processes between plants for local resources (see Chap. 6) and allelopathy. The second type of interaction involves a vertical approach between trophic levels, called herbivory, and more specifically granivory by seed-eating organisms.

8.2 Weed Control Using Sown Plants

Although using sown crops (arable and cover crops) for weed control is not considered a biological control method in a strict sense (Cordeau et al. 2016), it has been established for several decades that sown plants do play a role in weed management and is a welcome addition to extended biocontrol.

8.2.1 Competition and Allelopathy

The literature generally considers that above-ground competition has a stronger impact on plant growth than below-ground (root) competition, with sunlight being the main limiting factor for which plants compete. However, sometimes competition for nitrogen may be stronger. Phenology plays an important role in determining when plants coexist and compete during their life cycles. To date, few studies have focused on the intercropping period when sunlight availability (incident radiation) is at its highest and nutrient and water availability is often lower. However, root competition increases over the life cycles of weeds and cover crops, and is predominant when soil resources are low. Cover crops sown during the intercropping period and destroyed (although they may persist as mulch in the soil) before the next arable crop is planted can create shade and reduce the sunlight available to weeds. This shade can cause changes in growth or aerial morphology (especially leaf area, stem length) and root morphology, with consequences on seed production. For both weed and cover crop species, the ability of plants to compete for sunlight results from (i) their ability to affect the growth of plants with which they compete ("competitive effect") and (ii) their ability to withstand the effects of plants with which they compete ("competitive response"), which depends on their tolerance to shade.

Cover crops can reduce water and nutrient availability (including nitrogen), creating competition for weeds. They may also compete for nitrogen and water when the plants have a limited pool of resources that cannot meet all of their needs, which is common in cover cropping systems. For example, a cover crop may reduce water in the surface soil, thereby limiting weed growth through water competition (Cordeau et al. 2018). As with sunlight competition, competition for soil resources depends on the competitive effect of the species, which depends on (i) the root structure that determines the volume of soil explored, (ii) the dynamics of the plants' nitrogen and water needs, and (iii) the plants' preferred forms of nitrogen (ammonium, nitrate, organic nitrogen).

Allelopathy refers to several direct or indirect biochemical effects, which may be positive or negative, by one plant that affect another allomone. Various review articles describe the biological processes involved (e.g. Albuquerque et al. 2011). In cover cropping systems, this regulation process is leveraged based on the presence or absence of species with allelopathic effects in the mixture, including cereals, mixtures, Brassicaceae and legumes. However, it is difficult to characterize the specific allelopathic effects of a cover crop. The effects are generally inseparable from competition effects because, for a cover crop to have an allelopathic effect on weed flora, the roots of the cover crop and weeds must be near each other. Competition for sunlight between crops and weeds naturally arises in this case. Furthermore, no experimental attempts have ever been made to disentangle the allelopathic effect of decaying cover crop residues from their effect on weed germination by smothering.

8.2.2 Weed Control Using Sown Plants

8.2.2.1 Field Management

The effect of cover crops on weed control varies depending on the species used (most often in mixtures), and especially their biological characteristics (also called traits) and density. To compete with weeds, the cover crop must produce substantial biomass to reduce the resources (sunlight, water, nutrients) available to weeds. However, multi-species mixtures (high richness) are not always the best way to maximize biomass (Bybee-Finley et al. 2017) because there is not sufficient time to develop ecological niche complementarity and the relative species densities are not suited to the mixture created by the farmer. Cover crops are expected to have a high capacity to pre-empt environmental resources; this is why nitrophilic *Brassicaceae* are used as a nitrate trap, or why grasses with a high biomass are used to create shade. Nevertheless, the cover crop is usually planted in the summer between arable crop rotations, i.e. during the dry season, and sown over low post-harvest nitrogen residue (if the previous crop was properly fertilized). The autumn planting date is very important for cover crop growth potential, as the destruction date is often constrained by the planting date of the next crop. Thus, the intercropping period will determine whether there are enough degree days to maximize the accumulated biomass to achieve a sufficient level of weed control (Mirsky et al. 2017). If the sowing date is too late in the season to obtain sufficient cover biomass, this can be offset by sowing the cover crop more densely. However, there is a cut-off point beyond which the overall lack of biomass cannot be offset; the exact date will depend on the climate of the local area. Teasdale et al. (2007) summarized the mean percentage reduction of weed biomass using different cover crops (see Table 8.1). It is important to note, however, that these studies mainly concern annual weeds and that there are very few examples in the literature of perennial weed control via cover cropping (Bergkvist et al. 2010), and none with regard to cover cropping with short or long intercropping periods. Often, it is the cover crop destruction method (crimping, tilling, scalping) that helps manage perennials rather than the cover crop itself.

Table 8.1 Summary of the expected cover crop effects in reducing weed biomass

Growth period	Location	Cover crop species	Percentage of biomass reduction[a]	References
Summer fallow cover cropping	Nigeria	Velvet bean	85 (83–87)	Akobundu et al. (2000)
	Brazil savanna	Jack bean	72	Favero et al. (2001)
		Velvet bean	96	
		Lablab, pigeonpea	35 (22–48)	
	North Carolina (USA)	Cowpea, sesbania, soyabean, buckwheat	85	Creamer and Baldwin (2000)
		Soyabean, lablab	48	
		Sorghum-sudangrass, millet	94	
	Maryland (USA)	Hairy vetch	58 (52–70)	Teasdale and Mohler (1993)
	Japan	Hairy vetch	66	Araki and Ito (1999)
		Wheat	39	
	Alberta (Canada)	Yellow sweetclover	91 (77–99)	Blackshaw et al. (2001)
		Berseem clover	58 (51–70)	Ross et al. (2001)
		Alsike, balansa, crimson, Persian, red, white clover	35 (9–56)	
		Rye	64 (31–89)	
Summer intercrop	Brazil	Black mucuna, smooth rattlebox	97 (95–99)	Skora Neto (1993)
		Jack bean, pigeonpea	83 (71–90)	
		Cowpea	39 (29–48)	
	Mississippi (USA)	Hairy vetch	79	Reddy and Koger (2004)
	New York (USA)	Rye	61 (37–76)	Brainard and Bellinder (2004)
	Norway	Subterranean, white clover	48 (45–51)	Brandsæter et al. (1998)
Winter-surviving annuals	Oregon (USA)	Rye	97 (94–99)	Peachey et al. (2004)
		Oats	89 (81–96)	
		Barley	89 (78–99)	
	Italy	Rye	83 (54–99)	Barbari and Mazzoncini (2001)
		Subterranean, crimson clover	32 (0–67)	

(continued)

Table 8.1 (continued)

Growth period	Location	Cover crop species	Percentage of biomass reduction[a]	References
Winter-killed annuals	New York (USA)	Oilseed radish, mustard	94 (81–99)	Stivers-Young (1998)
		Oats	71 (19–95)	
	Michigan (USA)	Annual medics, berseem clover	54 (18–88)	Fisk et al. (2001)
	Illinois (USA)	Mustard	93	Grimmer and Masiunas (2004)
		Barley	94	
		Oats	76	

Based on Teasdale et al. (2007)

[a] Mean percentage reduction in weed biomass based on optimal cover crop growth conditions compared to a control without cover crop (bare soil). Data that accounts for more than one trial year are presented with the range shown in parentheses

8.2.2.2 Field Margins and Grass Strips

Weeds grow along the margins of crop fields, and many studies have investigated their potential to infest the adjacent crop field and to control them in these areas.

Weed species within the first five to ten metres of the crop field often come from field margins, which are outside the plot itself. But using grass strips in these adjacent metres – sometimes to create good agricultural and environmental conditions (GAEC) or buffer strips to protect watercourses – reduces the risk of weed dispersal in crop fields (Cordeau et al. 2012). This is especially true if the grass strip is mowed before seed set. But many farmers prefer sowing grass strips with mixtures, mainly grasses or grass-legume mixtures, rather than allowing the flora to regenerate spontaneously from the seedbank (natural seeding), out of fear that their fields will be infested.

Within field borders, multiple studies on establishment and maintenance methods have compared the (volunteer) weed flora present in strips (sown vs. unsown), as well as the effects of the type of mixture sown (grass vs. flower mixtures) on weed flora. Generally speaking, the plant community in grass strips often evolves more rapidly during vegetation succession if mowing residue is removed, which impoverishes the environment and increases plant diversity. Over time, the abundance of annual plants tends to decrease while perennial species abundance increases. Annual volunteer dicotyledons (known as dicots) are replaced by perennial volunteer monocotyledons (monocots). Harrowing and scalping turn over the surface soil, which can create disturbances while leaving the environment somewhat open to establishment. This can result in these two types of species coexisting; however, depending on when these practices are carried out, they may favour certain plants, such as thistles, which can sometimes be considered weeds.

Numerous studies have shown that grass strip maintenance is a major factor that determines how well new volunteer species are able to establish. The dense plant cover of grass strips usually reduces the ability of new species to establish, and grass strips sown with grassland species provide highly competitive conditions (for sunlight, space). The sown species remain dominant with very few volunteer weeds able to grow, thereby lowering the risk of weeds getting into cultivated plots.

8.2.2.3 Landscape Composition and Configuration

Weed species do not simply grow isolated in individual fields; strong seed and pollen flows link them at the landscape scale. For example, a significant proportion of herbicide-resistant blackgrass individuals are found in organic plots, suggesting high gene flow between these and conventional plots. A literature review on weed seed dispersal suggests that while natural processes are certainly at play, there also appears to be a strong link to farming activities, whether in terms of flows between the field and the field margin, or between different fields from farming equipment moving within or between farms (Petit et al. 2013). Consequently, the spatial distribution of farm fields within the territory modulates weed flows between different fields.

Various studies have analysed the extent to which the characteristics of the landscape surrounding a farm field affect its weed flora. Weed species richness tends to be greater in landscapes with more diverse habitats and a high proportion of organic crops (Petit et al. 2013). Few studies have looked at the effects of landscape on total weed abundance at field level, and those that have done so show no significant landscape effect, which suggests that local factors are more likely to determine abundance. These findings indicate that while the landscape can potentially increase the number of weed species arriving in a given field, species abundance will largely depend on field-based management. Species richness and abundance do not appear to be affected by the same factors, so it should be possible to develop coherent management strategies between the field and landscape levels to optimize weed species richness, which is important in maintaining many agroecological services without increasing overall infestation in fields.

8.3 Control by Seed-Eating Organisms

Weed flora mainly comprises annual species that produce large amounts of seeds that replenish the seedbank each year. These seeds are an important food resource in agroecosystems, and in fact many arthropods (e.g. ground beetles, crickets and ants), small mammals and birds consume significant quantities of weed seeds. Such seed-eating organisms are known as granivores or seed predators. In recent years, European and American research teams have been studying the factors determining weed seed predation by granivorous and omnivorous organisms naturally living in cultivated fields (Kulkarni et al. 2015). In temperate regions, beetles (Adephaga, Carabidae) are often the main invertebrates consuming weed seeds (Honěk et al. 2003) and most studies focus on the weed/beetle relationship.

8.3.1 Weed Seed Predation

Weed seed predation mostly occurs post-dispersal, i.e. at the end of seed set when seeds have fallen to the ground, and rarely when the seed is still on the plant. Seed-eating organisms thus consume some of the seed rain before it becomes part of the seedbank in the soil. Seed predation is often described as episodic: seed rains provide a sudden influx of food resources for consumers, which may gather and quickly consume the seeds. This episodic nature is interesting because the seeds can quickly become inaccessible to consumers by sinking into soil crevices. This is especially true for very small seeds or in the event of bad weather or farming practices.

In most studies, the identity of seed consumers is derived from the concomitant capture of potential seed predators and the estimation of seed predation rates on sentinel prey (seed cards on which weed seeds are glued and then placed in the field). The quantity of seeds consumed generally shows a positive correlation with the abundance or diversity of ground beetle species whose diet includes seeds (granivores, omnivores). Molecular biology tools have recently been developed to analyse the stomach contents of arthropods and enable the identification of the weed species consumed by a wide range of predators (Frei et al. 2019). Behavioural studies conducted in the laboratory make it possible to study predators' weed seed consumption strategies (Charalabidis et al. 2019). Cameras can also be used in the field to identify the guild of predators feeding on exposed seeds. These different methods show that the relationship between natural enemies and weed predation rates is quite complex. In particular, the specific natural enemies involved in predation and the intensity of predation change during the season, probably depending on the availability of alternative prey (Gray et al. 2021). Recent findings also suggest that the diets of groups that are considered to be relatively well-known, such as ground beetles, are full of surprises: for example, supposedly granivorous species consume a lot of animal prey in certain contexts, and weed seeds are consumed in larger quantities by a much wider spectrum of carabid species than previously thought (Frei et al. 2019).

8.3.2 Seed Predation and Weed Control

Studies quantifying the impact of seed predation on weed demography are rare, but they do suggest an impact on weeds, hence the importance of maintaining seed consumers in cultivated plots. However, this effect can be relatively small, which means that seed predation will never be a complete weed control method on its own.

It should be viewed as one weed management option among many in the agricultural and agroecological toolbox that can be used to limit weed infestations.

To date, there have been few experimental studies to demonstrate weed regulation by granivores. However, they have systematically demonstrated an impact: it may be relatively small, such as a 5–15% reduction in germination of *Abutilon theophrasti* Medik. and *Setaria faberi* Herrm., or much greater, such as a 40% reduction in germination of *Chenopodium album* seeds in different cover crops (Blubaugh and Kaplan 2016). Finally, a recent study carried out on 250 plots showed a negative correlation between ground beetle abundance and the seedbank renewal rate: the more ground beetles are observed in a field, the less seed is reintegrated into the soil seedbank from one year to the next (Bohan et al. 2011). These findings are robust and apply to various crops, under different crop management sequences and in all the regions studied.

Several models have quantified the impact of predation on weed demography. An initial model estimated the annual predation rate according to the time of seed exposure to predators from field data (Westerman et al. 2006). Based on around a dozen studies conducted in Europe and the United States that provide repeated field predation measurements, this model estimates that the annual rate of seed loss due to predation is about 40%, but can be quite variable (e.g. from 8% to 70%) depending on the agronomic context and weed species (Davis et al. 2011). Earlier modelling work suggested that an annual loss of 25–50% of weed seeds could be sufficient to affect weed population dynamics. Finally, weed seed predation is often considered preferential, i.e. some weeds are highly consumed while others are not, for various reasons such as seed size, seed coat thickness, toxicity and food value. One plausible consequence of these "preferences" is that the demography of some weeds will be more affected than others, resulting in a change in the existing weed community composition.

8.3.3 Controlling Weed Seed Predation

8.3.3.1 Field Management

Farming practices and crop type can significantly affect the presence and abundance of different weed seed predators such as ground beetles (Kromp 1999), and thus observed predation rates. Similarly, predation activity varies during the crop cycle and according to the crop type and rotation. Ground beetle abundance and weed predation are often supported by a dense cover of sown vegetation (Petit 2018), and using cover crops favours weed seed predation, both in the case of cover cropping between arable crop seasons (Davis and Liebman 2003) and more permanent cover crops (Blubaugh et al. 2016). Pesticide use appears to negatively impact weed predation (Ricci et al. 2019), and tillage practices can have a major impact on granivores. Cropping systems using little or no tillage are home to a higher diversity and abundance of ground beetles. This is likely because not disturbing the soil allows some species to breed in the fields. Meanwhile, it has been observed that no-till practices are more favourable to weed predation compared to tillage systems (Petit et al. 2017).

8.3.3.2 Field Margins

Establishing grass strips along field margins can have a positive impact on ground beetle abundance and predation intensity in the field by improving the continuity between semi-natural habitats and cultivated areas, and thus the possibilities of beetles penetrating and foraging in fields. The type of adjacent field can also explain predation intensity variations. Generally speaking, field margin habitats (hedgerows, grass strips, flower strips) can provide seed-eating organisms with additional food resources or shelter from disturbances related to farming practices. For example, grass or flower strips at the field margin have been shown to increase the fitness (or nutritional status) of ground beetles in the adjacent crop (Labruyere et al. 2018). Moreover, while some species complete their entire development within a crop, disturbances and winter conditions may be unsuitable for other species that spend only part of their time in the field during the crop's growth period. For these species, field margin habitats provide overwintering or oviposition sites (Wissinger 1997). In addition to field margins, grasslands around annual crops can provide additional food resources and refuges for certain species, including phytophagous ground beetles (Purtauf et al. 2005).

8.3.3.3 Landscape Composition and Configuration

Many organisms that feed on weed seeds are mobile in the agricultural landscape, and the landscape composition and configuration can affect their local abundance and weed control activities. The effects of landscape and predation intensity are still poorly described and may seem contradictory, which is not surprising as they will depend on the seed-eating species.

Various studies show that weed predation is higher in complex landscapes with many semi-natural habitats. The presence of meadows within a one-kilometre radius around annual crop fields increases seed predation rates in crops. In Germany, a similar positive effect was found in organic fields (Fischer et al. 2011). In conventional agriculture, the reverse has been found, with higher predation observed in simpler landscapes. Similarly, in Burgundy, France, a complex landscape was shown to favour weed predation in fields recently converted to no-till, whereas weed predation in no-till fields managed that way for more than four years is not affected by the landscape around the fields. This is probably because fields that have not been tilled for several years allow some ground beetles to complete their cycle, which means their coming to the field no longer depends on the surrounding landscape (Petit et al. 2017). The proportion of organic crops in the landscape also positively affects weed predation; this has been shown in both vineyards and in field crops. In the latter case, researchers demonstrated that a significant proportion of organic farming in the landscape not only increases ground beetle abundance, but that the individuals of those species are larger and thus show stronger weed predation (Diekötter et al. 2016). There are also cases where predation is high in simple landscapes dominated by arable crops; this is explained by the fact that weed predation is carried out by very generalist and hyper-abundant omnivorous ground beetle species (Jonason et al. 2013). In this case, seed predation depends only on the presence of one or two species, which could be risky as a strategy if these species were to decline.

8.4 Conclusion

The knowledge summarized in this chapter indicates that biotic interactions between weeds and other organisms that support biodiversity in the agroecosystem can significantly affect weed growth and demography. They also show that certain agricultural management methods, whether implemented in arable fields, in field margins or at the landscape level, can promote biological weed control. However, this knowledge may seem rather fragmented in some respects. For example, there are very few studies that track weed phenology in sown cover crops. Modern technological advances that now allow us to analyse organisms' feeding behaviours in agroecosystems will further advance our knowledge of granivory processes. This synthesis also shows that the levels of control to be expected from weed/natural enemy interactions are likely to be less intense or less stable than achieved by chemical means. It is therefore important to leverage a combination of several types of control interactions. Researchers must analyse possible antagonisms and synergies between regulatory processes and identify practices and structures in the landscape that make it possible to modulate these positive or negative interactions between natural pest control services.

Part III
Microorganisms and Biological Control

Matthieu Barret

The previous sections of this book discussed the different biological control strategies using *macroorganisms*. This section will focus on *microorganism*-based biological control solutions. Although the use of microorganisms as biological control agents has been documented since the end of the nineteenth century, this subject has received renewed attention since the late 2000s. This growing interest is linked not only to efforts to reduce chemical pesticides worldwide, but also to the research on microbial complexes associated with plants, which are known as microbiota. Many studies have indeed correlated changes in microbiota composition and the expression of soil-borne and leaf diseases. Although it is now recognized that the plant microbiota impacts plant health, the modes of action and mechanisms involved have yet to be explored.

This section is divided into four chapters. Chapter 9 reviews current knowledge on the plant microbiota, detailing its taxonomic structure, the ecological processes involved in its assembly and its impact on host fitness. Chapter 10 then suggests several means of action to modify the microbiota composition for crop protection. This type of conservation biological control, which supports agroecosystems with high microbial diversity, is not yet operational. As a result, biocontrol companies selling microbial products are currently targeting conventional approaches to classical and even augmentative biological control. Chapter 11 presents the main microbial biological control agents and their modes of action. Factors impacting the in situ effectiveness of these biological control solutions are also discussed. Chapter 12 explains the value of microbial secondary metabolites in protecting plants against certain pathogens.

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Chapter 9
Plant Microbiota: Diversity, Transmission and Function

Matthieu Barret, Marc Buée, Christophe Mougel, and Corinne Vacher

9.1 Introduction

Plants host a wide array of viruses and microorganisms (eubacteria, archaea, filamentous fungi, oomycetes, protists and nematodes) inside or on the surface of their various organ tissues (Leach et al. 2017). These microorganisms form the plant microbiota. The interactions between a plant and its microbiota can impact the plant's development and its adaptation to abiotic and biotic constraints. Understanding the biological and ecological processes involved in microbiota assembly and dynamics during plant development is vital to implementing strategies to control which microorganisms develop.

9.2 Microbial Diversity According to Habitats

The bacterial and fungal taxa present in the plant microbiota have been estimated in many annual and perennial plant species (Muller et al. 2016). These studies revealed the presence of four major bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. In lower taxonomic levels, such as family or genus, differences

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in microbial communities have been observed depending on the plant organ, species and environment. For example, differences were observed for fungal communities that are dependent on tree leaves or roots (e.g. Coince et al. 2014). Such differences have also been observed between rhizosphere- and phyllosphere-associated bacterial communities (e.g. Wagner et al. 2016). While some bacterial taxa are preferentially associated with the aboveground or belowground plant components, others colonize both habitats indiscriminately and can therefore be considered generalists (Bai et al. 2015).

Historically, the soil has been considered the major source of plant microorganisms; as such, the rhizosphere has been the most studied plant compartment due to its important relationship with the surrounding soil. Plant root exudation of carbon-based compounds will induce the development of large bacterial and fungal populations (10^8–10^{10} bacteria and 10^5–10^6 fungi per gram of rhizosphere) with a high level of taxonomic diversity (Egamberdieva et al. 2008; Bulgarelli et al. 2013). Some of these microorganism populations will interact more closely with the plant when they are on the surface (epiphytes) or inside the roots (endophytes). Root endophytes colonize healthy plant roots, but do not form complex structures or specialized interfaces with the host to exchange nutrients, unlike what is observed in mycorrhizal fungi (Brundrett 2006). Endophytes are widely found in plant roots, but knowledge about their diversity and ecological functions is lacking. Since nearly 90% of plant species are colonized by mycorrhizal fungi, endophytes commonly coexist in the roots with mycorrhizal fungi as well as with other microbial groups, potentially improving plant fitness (Porras-Alfaro and Bayman 2011). Dark septate endophytes (DSE) – highly melanized fungi – have been observed in roots colonized by arbuscular and ectomycorrhizal mycorrhizal fungi (Bonito et al. 2016). Under controlled conditions, dark septate endophytes can increase root biomass.

The aerial parts of plants are also colonized by numerous microorganisms. The phyllosphere is thought to be home to around 10^6–10^7 bacteria per square centimetre of leaf area (Vorholt 2012). Epiphytic microorganisms living on the leaf surface come from several inoculum sources such as aerosols, precipitation, insects, surrounding vegetation and the soil (Vacher et al. 2016). Unlike in the rhizosphere, nutrient resources on the leaf surface are limited. For example, some bacteria associated with the genus *Methylobacterium* can use the methane or methanol produced by the leaves as a main source of carbon (Vorholt 2012). Additionally, the phyllosphere microbial populations are also subjected to intense UV radiation and develop pigment systems for protection (Ibid.). While the influence of the leaf epiphytic microbiota in the host physiology needs to be clarified, its role in protecting plants against leaf diseases and the mechanisms involved are increasingly understood (Hacquard et al. 2017).

Initial work on the plant microbiota taxa mainly focused on the phyllosphere and rhizosphere microbial communities during the vegetative phase of plant growth. More recently, other habitats associated with the reproductive organs of plants that reproduce sexually, such as flowers (anthosphere), fruits (carposphere) and dry or germinated seeds (in this latter case, the spermosphere), have also been analysed (Nelson 2018; Shade et al. 2017). While these habitats generally have less microbial diversity than the phyllosphere and rhizosphere, the main bacterial and fungal phyla are found within these compartments. Thus, it is likely that part of the plant microbiota is inherited via vertical transmission. While the percentage of taxa transmitted vertically is undoubtedly smaller than that transmitted horizontally by the local habitat, the seed microbiota is the primary inoculum source for the plant microbiota and can thus contribute to the early stages of its development, especially during germination (Shade et al. 2017).

9.3 Microbiota Assembly and Transmission Processes

The ecological processes involved in microbial community assembly can be grouped into four distinct categories (Fig. 9.1): (i) selection, (ii) diversification, (iii) dispersal and (iv) ecological drift (Nemergut et al. 2013).

Selection is generally considered to be the main ecological process that modulates microbial community assembly and is therefore the most studied process. It refers to changes in microbiota composition caused by a difference in fitness between microorganisms. The fittest microorganisms on a given host plant and in a given environment are those that are favoured by selection and increase proportionally in the microbiota. Differences in fitness may result from interactions between the host and its microorganisms or be related to environmental parameters. For example, soil type, farming practices and climate are essential components of the rhizosphere microbiota composition (Philippot et al. 2013). Other studies have shown a significant effect of the host genotype in both the rhizosphere microbiota (Walters et al. 2018) and phyllosphere composition (Horton et al. 2014). This impact of the host

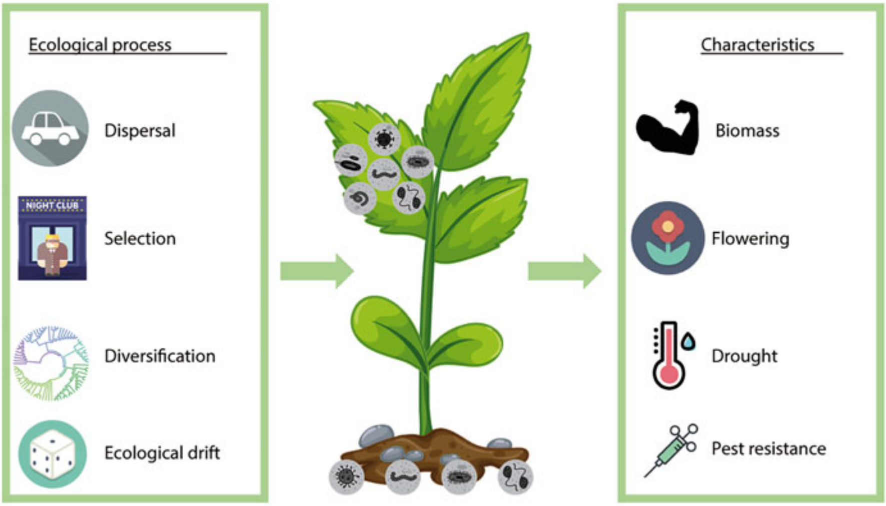

Fig. 9.1 Assembly of the plant's microbiota and impact on host fitness

genotype on the selection of certain microbial taxa can be explained in part by the quality and quantity of carbon-based compounds exuded by the roots or by the plant's immune response (Hacquard et al. 2017).

New genetic variations by mutation, homologous recombination or horizontal gene transfer is similar to evolutionary diversification. Depending on the selective pressures exerted by the host and the environment on the microbial assemblage, this diversification can lead to the emergence of new species. This process could have a major impact on microbial community assembly because, unlike with macroorganisms, microorganisms have a short generation time and can therefore evolve rapidly. However, the impact of diversification within microbial communities remains difficult to assess. Evolution experiments, consisting of repeated successive inoculations of microorganisms on plants over several generations, have nevertheless made it possible to identify adaptive mutations in certain plant-associated bacteria (Guidot et al. 2014).

Through the combination of evolutionary diversification and selection, each microbial species acquires various traits that help it adapt to a particular habitat and occupy a unique ecological niche. In contrast to the ecological niche theory, the neutral theory of biodiversity postulates that all members of a microbial community, whatever their traits, have the same fitness in the habitat being considered. According to this theory, the distribution of community members is mainly due to dispersal and ecological drift. Microbial dispersal refers to the movement of microorganisms from an original habitat to a new habitat. Unlike for macroorganisms, the dispersal capacity of microorganisms has not been widely studied. This lack of data on microorganisms can be explained by the simple fact that it is difficult to quantify their dispersal due to their size, rapid generation time and high number of individuals. Thus, in microorganisms, spatial distribution is used to estimate dispersal. For example, differences in the abundance of microbial taxa in the phyllosphere can be partly explained by dispersal (Maignien et al. 2014). Similarly, the fungal taxa of the seed microbiota also appears to be impacted by dispersal (Rezki et al. 2018). Microbial dispersal is a predominantly passive process, mediated by phenomena such as spores being carried by wind or air. The influence of propagule dispersal has been studied more comprehensively in mycorrhizal fungi macromycetes, which form spore-bearing fruiting bodies called sporophores. In these microorganisms, the number of spores produced per species is one of the key factors involved in the composition of plant-associated communities (Peay et al. 2012).

The last of the four ecological processes mentioned, ecological drift, refers to stochastic, i.e. random, changes in relative species abundance. Ecological drift can even lead some organisms within a community to become extinct over time (Nemergut et al. 2013). This process plays a major role in community assembly when selection is weak and species richness, i.e. the number of species, is low. Microbial taxa with low relative abundance are more sensitive to the stochastic effects of ecological drift, which can drive them to extinction. It seems that the composition of the bacterial communities of seeds (Rezki et al. 2018) and the phyllosphere (Maignien et al. 2014) are partly influenced by ecological drift.

9.4 Impact of the Plant Microbiota on Host Fitness

Groups of plant-associated microorganisms can modify many of a host's phenotypic traits by stimulating the plant's growth or influencing its adaptation to abiotic and biotic constraints.

It is accepted that plant growth is strongly related to the biotic characteristics of the soil in which they grow. For example, differences in the biomass of the aerial parts of *Arabidopsis thaliana* have been associated with changes in the composition of soil bacterial communities (Sugiyama et al. 2013). However, it is difficult to separate the effects due to microbial activity from those related to the soil's physicochemical properties in this phenotypic modification. Another trait impacted by plant-microorganism interactions concerns the transport of minerals in the soil. One strategy that plants use to increase their capacity to absorb minerals (e.g. nitrogen or phosphate) is to establish beneficial relationships with soil microorganisms such as nitrogen-fixing bacteria in the Rhizobiales order or mycorrhizal fungi. These bipartite plant-microorganism symbiotic interactions modulate and can be modulated by other members of the plant microbiota (Zgadzaj et al. 2016). Thus, microbial-interactions have a direct impact on mineral accumulation in plant tissue.

Soil microbial communities can also affect plant flower development. For example, differences in the soil microbiota can lead to delayed flowering in *A. thaliana* (Panke-Buisse et al. 2015). Although the microbial taxa and molecular determinants involved in modulating flowering are still unknown, the use of microbial inoculum to modulate the flowering time offers interesting possibilities, such as to prevent flowering too early.

Fluctuations in environmental conditions will impact a plant's physiology, which can in turn induce changes in its microbiota. For example, a drought episode can cause a water deficit in plants. In response to this abiotic stress, a plant will modulate its root architecture as well as the quantity and quality of the exudates produced. These changes in plant physiology will affect the root-associated microbiota (Naylor and Coleman-Derr 2018). During repeated drought episodes, changes in rhizosphere microbiota can improve the host plant's ability to withstand water stress. In a study conducted on *Brassica rapa*, the plants showed greater drought resistance when they were grown in previously dry soils (Lau and Lennon 2012). When these plants were grown in dry soils over several generations, they had a higher bacterial abundance and diversity around their roots compared to plants cultivated in irrigated soils, suggesting these microorganisms had an influence on their water stress tolerance.

Finally, the expression of certain soil-borne or leaf diseases can be modulated by the plant's microbiota. One of the best documented cases concerns suppressive soils. In these soils, disease development is limited by certain microbial assemblages near roots. Examples of pathogens suppressed by such soils include (i) take-all in wheat caused by the fungus *Gaeumannomyces graminis* var. *tritici*, (ii) black root rot in tobacco caused by *Thielaviopsis basicola*, and (iii) damping off of sugar beet seedlings caused by *Rhizoctonia solani* (Weller et al. 2002). The decrease in symptoms shown in these suppressive soils is due to the gradual selection of certain rhizobacterial populations. Rhizobacteria can inhibit the growth of the pathogen by producing different antimicrobial compounds such as rhamnolipids, lipopeptides or polyketides (see Chap. 12).

Plants can also be protected against plant pathogens indirectly by stimulating the plant immune system by members of root-associated microbial communities, which is known as induced systemic resistance (ISR). ISR will limit or inhibit the penetration of plant pathogens into the plant tissue via a hypersensitive response that results in rapid cell death at the point of infection, thus reducing disease severity. ISR can reduce various leaf and root diseases caused by a range of bacterial and fungal plant pathogens (Pieterse et al. 2014).

The protective activity of plant-associated microbial communities is not limited to the rhizosphere. Indeed, correlations between resistance to biotic stresses and certain changes in the microbiota composition have been observed in the phyllosphere (Ritpitakphong et al. 2016). Resistance to plant pathogens can be induced by a microbial assemblage or a specific microbial population. For example, bacteria related to *Sphingomonas*, a bacterial genus found abundantly in the phyllosphere of several plant species, can significantly reduce the degree of colonization of the phyllosphere of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato*. While the exact nature of this protective effect observed in *A. thaliana* is still unknown, several mechanisms have been put forward, such as competition for resources between these bacterial populations or the induction of defence reactions in the plant via salicylic or jasmonic acid-dependent pathways (Innerebner et al. 2011).

9.5 Leveraging the Microbiota to Improve Plant Growth and Health

For several decades, assessing the plant microbiota composition according to phenotypic changes in the plant has been one of the first steps in finding associations between certain microbial taxa and traits of interest.

Exploiting the plant microbiota to promote the growth and health of its host is one alternative to using plant protection products. To date, several bacterial and fungal strains are already being sold commercially and used as natural control agents capable of reducing some of the effects of plant diseases (see Chap. 11). This is the case of certain fungal strains of the genus *Trichoderma*, which can inhibit plant pathogens in the soil through their antagonistic activities (Hermosa et al. 2012), or arbuscular mycorrhizal fungi, such as *Rhizophagus irregularis*, which promote plant growth in soils with low fertility (Khasa et al. 2009). Although the activity of these commercial strains is satisfactory under controlled or semi-controlled conditions, their effectiveness in situ may prove disappointing. Such variations in effectiveness can be explained by several factors such as environmental fluctuations or farming practices, which may impact the colonization, survival or activity of the inoculated strain (see Chap. 11). It is also likely that interactions between members of the plant microbiota and the introduced biological control agents are also involved in the successful colonization of the latter.

The impact of the inoculated or selected microorganism on the ecosystem is a fundamental issue in classical biological control (see Chap. 3). One of the best-known examples is the case of a cultivar of tall fescue (*Festuca arundinacea* Schreb). The cultivar, Kentucky 31, was initially selected for its ability to grow in nutrient-poor soils and tolerance to water stress. An endophytic fungus (*Epichloë coenophiala*, Morgan-Jones & W. Gams), transmitted vertically by the plant's seeds, was responsible for the grass being able to adapt to these abiotic stresses (Schardl et al. 2004). Unfortunately, following the mass commercialization of the Kentucky 31 variety, significant ecological and economic damage was caused by this grass-endophyte partnership. The fungus produces several alkaloids that are toxic to animals, and the livestock that fed on the fescue were plagued by various pathologies (Hoveland 1993). This example highlights both the value of identifying the components of the plant microbiota with a view to managed co-selection, as well as the need to control and better understand the underlying effects of this biotic partnership and anticipate undesirable effects.

Chapter 10
Agroecological Protection to Support Plant Health: Where the Microbiota Fits In

Claudia Bartoli, Jean-Noël Aubertot, Isabelle Litrico, and Christophe Mougel

10.1 Introduction

One of the major challenges for modern agriculture is to ensure food security while mitigating the adverse effects of chemical inputs on the environment. The agronomic transition process aims to reduce the use of plant protection products while continuing to ensure sufficient crop production. It is supported by agroecology, which seeks to transpose natural ecosystem processes into agroecosystems (Ferguson and Lovell 2013). In this context, the beneficial potential of plant microbiota for the growth and health of their hosts (see Chap. 9) can make agricultural systems more productive. Farming practices can indeed influence the plant microbiota; for example, recent studies have highlighted the positive effect of organic farming on microbiota diversity and composition (Hartman et al. 2015, 2018). Improving our knowledge about the impacts that farming practices have on plant health and supporting agroecosystem biodiversity, combined with a deeper understanding of the underlying mechanisms of plant-microbiota interactions, will make it possible to develop agroecological crop protection methods.

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10.2 Agricultural Production and Pest Management

Agriculture in the twentieth century was characterized by a high degree of mechanization and production specialization, which led to more standardized agricultural landscapes. This first "green revolution" was accompanied by a greater use of inputs (fertilizers and plant protection products) and high water consumption along with the use of high-yield varieties. The potential of these varieties is a result of genetic improvement and is conditioned by farming practices that aim to adapt and optimize the environment to the needs of the varieties.

There is a general consensus that an agroecological transition towards more sustainable farming practices is crucial to avoid irreversible damage for future generations and the depletion of non-renewable natural resources (De Schutter and Vanloqueren 2011). Focusing on ecology means identifying new drivers to maintain production levels, both in terms of quantity and quality, while seeking to minimize the environmental impact of agriculture. Ecological ideas and methods, which are under-used in agriculture, must be applied to agroecosystems, while ecology itself must benefit from agronomists' ability to analyse and explain the effect of farming operations on the structure and functioning of ecosystems modified by human activities. Agroecological principles are based on natural ecosystem functioning, where biodiversity plays a central role with regard to the ecosystem services provided (Millennium Ecosystem Assessment 2004). The aim is to introduce, manage and optimize functional biodiversity in agroecosystems at different scales, both spatial (from field to landscape) and temporal (from crop to rotation), in order to best leverage biodiversity (Reboud and Malézieux 2015) and thus help crops adapt to abiotic and biotic stresses. Ecological processes that can support crop adaptation to biotic stresses include biological interactions, such as those between microorganisms and pests (e.g. competition, predation or parasitism), and interactions between microorganisms, plants and pests that modulate plant immunity. Directly managing organisms or environments to facilitate biological regulation is therefore one agroecological lever to protect crops.

Agroecological crop protection capitalizes on biodiversity's many aspects to benefit from the interactions between pests and other organisms (e.g. an insect pest and its natural enemies). These processes underpin conservation biological control (see Chap. 7), but what about fungal or bacterial diseases? Soil-borne diseases and suppressive soils are an emblematic case of pathogen-microbiota interactions that can explain how some soils are able to reduce the infectious potential of certain diseases such as seedling damping off, root necrosis or coffee wilt disease. Such examples remain limited, but better understanding the processes, which involve antibiosis and/or competition for resources, could open up avenues for practical application. For instance, the decline of take-all in wheat, a soil-borne disease caused by the fungus *Gaeumannomyces graminis* var. *tritici*, is a well-documented phenomenon. The ability of some soils to reduce the incidence of this disease is largely explained by the selection of bacterial strains of *Pseudomonas* spp. that produce antibiotics such as 2,4-diacetylphloroglucinol or phenazines (Imperiali et al. 2017).

Soil suppression of vascular wilt diseases occurs via various complementary mechanisms, such as competition for iron between the pathogen and certain strains of *Pseudomonas fluorescens*, or competition for carbon between non-pathogenic strains of *Fusarium* sp. (Lemanceau and Alabouvette 1993). These ecological interactions must be coupled with microorganisms' ability to modulate plant resistance mechanisms to pests, i.e. plant immunity, and thus increase phenotypic resistance (Hacquard et al. 2017). Modulating plant immunity by the microbiota or some of its components requires better taking account of the plant in plant-microbiota-pest interactions.

10.3 New Levers to Explore: Plant-Microbiota Interactions and Their Role in Agricultural Ecosystems

The impact of microbiota on crop health and yield has been demonstrated in recent studies describing the relationships between microbiota diversity and various plant phenotypic traits, such as disease suppression or resistance and growth promotion (Trivedi et al. 2017). Moreover, microbiota can expand the host plant's genotypic and metabolic capacity by providing a set of essential functions such as nutrient acquisition, immune system modulation, and biotic and abiotic stress tolerance. Observations on the effects of microorganisms on their hosts, both in the animal and plant kingdoms, have resulted in a new holistic perspective from which all living organisms are considered polygenomic entities. This holobiont theory assumes that the variation in the hologenome of the different entities – the genomes of the host and the metagenome of associated microbiota – leads to phenotypic variation in the host, on which natural selection and genetic drift act (Bordenstein and Theis 2015). The hologenome has undoubtedly shifted Darwin's concept of the "extended *phenotype*" to the "extended *genotype*", in which living organisms include the extended effect of genetic modifications of the microbiota on their phenotype.

From this holistic perspective, identifying the factors that influence the composition of the plant microbiota seems essential in order to develop crop management strategies based on improving or maintaining beneficial plant-microbiota interactions. In particular, soil properties are important determinants for supporting the taxonomic and functional diversity of the root microbiota and maintaining a minimum number of functional microbial groups in the soil. For example, soil must have populations of efficient symbionts (rhizobia, mycorrhizal fungi, antagonistic microorganisms, etc.) for agroecosystems to function well.

Farming practices can have major consequences on soil and root microbiota diversity. Understanding how these practices influence crop microbiota can aid in choosing strategies to select for microbiota that can modify certain plant traits related to primary production or abiotic/biotic stress adaptation (Fig. 10.1). More specifically, from a microbiota management standpoint, it is important to understand which microbiota are sensitive to various farming practices and whether those practices

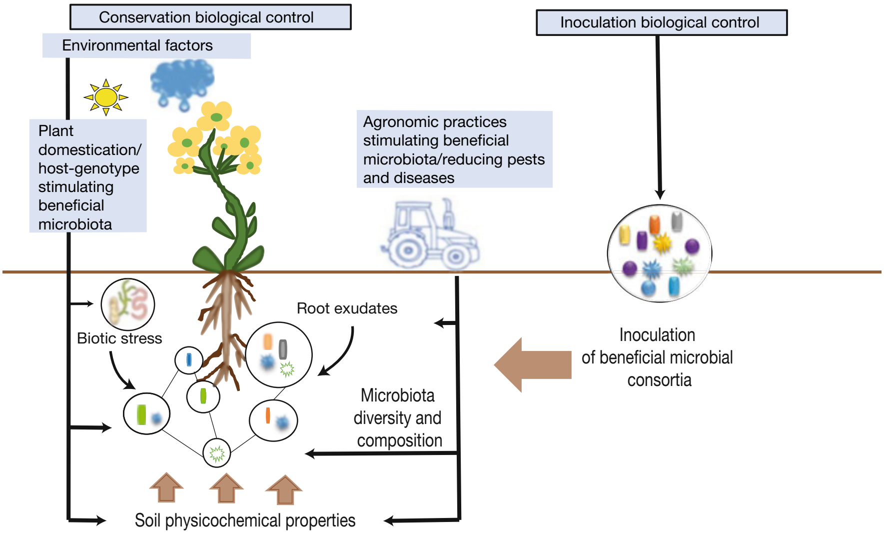

Fig. 10.1 The plant microbiota is able to modulate and reduce the negative effects of pathogens and pests on crops. Soil microbiota can be managed using a conservation biological control approach (*left*). This approach enables practitioners to select beneficial microbiota using genotypes or varietal mixtures coupled with specific farming practices. These practices can have a direct impact on microbiota diversity and composition, or an indirect effect via changes in root exudate composition or soil physicochemical properties. Inoculation biological control (*right*) could restore the diversity of soil microbial communities in severely disrupted agricultural systems by directly introducing certain microbial consortia, which would have positive consequences for pest and disease control

impact interactions between microbial species. Because microbial keystone taxa can play a key role in microbiota functioning and dynamics, farming practices must strive to support those species that are vital to ecosystem functioning. A study on winter wheat showed that ploughing changes the composition of microbial and fungal communities in roots and soil (Hartman et al. 2018). The authors also found that certain root microbiota keystone taxa were not only sensitive to ploughing but also, more generally, to production methods.

Similar results were obtained in wheat by comparing fields from conventional, organic and no-till production systems (Banerjee et al. 2019). The authors showed that the abundance of some mycorrhizal fungi keystone taxa was higher in organic than in conventional fields. Moreover, a negative correlation between agricultural intensification and network connectivity in root fungal species of wheat was also demonstrated (Banerjee et al. 2019). Similarly, Longa et al. (2017) showed that different management practices in viticulture (biodynamic practices with or without organic fertilizers) alter the abundance of certain microorganisms. These studies have improved our understanding of the impact of farming practices on microbial ecosystem dynamics. However, the combined effects of crop management sequences and other environmental factors on the plant microbiota still need to be better understood.

The plant genotype is also a very important factor to consider when developing practices that support beneficial microbiota recruitment. Different plant genotypes can recruit extremely different microbiota, which in turn can confer tolerance to abiotic and biotic stresses or contribute to plant growth and nutrition.

While conventional production practices have radically changed soil physico-chemical properties, plant domestication has also eroded the diversity of the crop microbiota. As such, selection methods should consider not only the host genotype but its associated microbiota as well. However, this type of approach is currently limited by an insufficient understanding of how the microbiota functions and its interaction mechanisms with the host. For example, we have very limited knowledge of plant genes associated with microbiota diversity parameters. Recent studies on the model plant *Arabidopsis thaliana* have shown a very strong link between the genetic variability of the plant and root microbiota composition. More specifically, the authors identified a gene from the flavin-containing monooxygenase family associated with the diversity of bacterial and fungal communities (Bergelson et al. 2019). A study on the foliar microbiota of *A. thaliana* also identified certain genetic determinants of the host involved in the response to bacterial and fungal communities (Horton et al. 2014). The genes governing plant-microbiota interactions in crops have yet to be described, although the variation in microbiota diversity has been explained in part by the plant genotype for rice (Santos-Medellin et al. 2017) and maize (Gomes et al. 2018). In maize, the relative abundances of root-associated bacterial taxa are partly explained by genetic differences between the lines studied (Walters et al. 2018).

The role of varietal associations (combinations of different genotypes within the same species) and multi-species associations is another aspect to consider when developing approaches to select for microbiota that are beneficial to crops. Indeed, plant associations in agricultural systems can significantly alter soil microbiota abundance and composition, with a beneficial effect on crop growth and yield. For example, a positive effect of mycorrhizal fungi associated with certain nurse plants (i.e. plants that can promote the growth of neighbouring plants) has been demonstrated (Carrillo-Garcia et al. 1999). Recent studies have shown that nurse plants can modify the composition of soil bacterial communities and select more efficient microbiota for nutrient mineralization and plant growth (Rodríguez-Echeverría et al. 2016). Theoretical ecology has shown that varietal mixtures can be designed to improve crop productivity and limit pathogen development (Litrico and Violle 2015). A recent study on natural populations of *A. thaliana* showed that the genes involved in plant-plant interactions belong to families of genes linked to pathogen resistance (Frachon et al. 2019). The findings of this study suggest that plant-plant interactions can modulate plant resistance traits by directly inducing immune responses or by indirectly modulating immunity via the microbiota of neighbouring plants. However, the impact of species or variety associations on the structuring of the microbiota remains largely unexplored.

10.4 Connecting Microbiota and Agroecological Practices

Managing microbiota in order to improve agricultural production can be envisaged through two approaches that are not mutually exclusive. The first approach is similar to conservation biological control (see Chap. 7) and consists in modulating the soil microbiota by leveraging different plant associations integrated into different production practices. The second approach, which can be likened to inoculation biological control (see Chap. 6), involves directly introducing a microbial strain or consortium with properties that are beneficial to plant growth and health into crops.

Microorganisms with biological control activity have generally been identified by screening for strains under controlled conditions (see Chap. 11). This type of screening process has produced encouraging results in identifying candidate biocontrol agents. However, applying the findings obtained under controlled conditions directly to agroecosystems remains a major operational challenge, which might be explained by various and sometimes overlapping theories (see Chap. 11). To overcome these limitations and develop robust biological control agent implementation measures, understanding the ecology of biological control agents and pathogens is key. An emerging approach to circumvent the obstacles of moving from the laboratory to the field is to use mixtures of strains with complementary modes of action. Recent studies have shown that combinations of bacteria that have moderate or no effect on plant growth when inoculated alone can have significant effects when used in a consortium of strains (Durán et al. 2018). For example, a combination of eight strains of *Pseudomonas* can reduce invasion by the pathogenic bacterium *Ralstonia solanacearum* in tomato (Hu et al. 2016). Similar results were obtained for kiwifruit bacterial canker disease, caused by *P. syringae* pv. *actinidiae*, by combining *P. syringae* strains from non-agricultural environments that were closely related to the pathogen (Bartoli et al. 2015).

The above-mentioned studies produced fundamental knowledge about the ecological processes that prevent pathogens from gaining a foothold. However, more “naturalistic” approaches that take into account the ecology of the habitat where plants, microbiota and pathobiota coexist are needed to develop solutions to suppress the spread of pests and pathogens in natura. For instance, an in situ study conducted on the root and leaf microbiota of natural populations of the model species *Arabidopsis thaliana* revealed a negative relationship between microbiota diversity and pathobiota diversity in these two plant compartments (Bartoli et al. 2018). These results suggest that the more diverse the microbiota, the less likely the plant is to be attacked by pathogens. These findings must now be applied to agriculture to determine whether an increase in soil microbial diversity can limit pathogen attacks in agricultural systems.

Studying the mechanisms involved in microbial consortia establishment and dynamics is a crucial step for future biological control approaches. For this purpose, the first scientific “transition” will consist in conducting field studies on microbiota on crops grown across a large number of sites with different agricultural practices and pedoclimatic conditions. To identify microbial consortia with biological control potential, metabarcoding and metagenomic approaches will have to be combined with culturomic approaches in order to allow the broadest possible characterization and identification of microbial strains. Recent studies have shown that most bacterial strains can be isolated from the microbiota using different media and culture conditions (Martiny 2018). For example, by using a combination of more than 70 culture conditions, researchers were able to isolate 70% of the identified bacterial species from the human gut microbiota (La Scola 2015). Moreover, this study shows that certain growth media can enrich the minority functional classes that are underestimated by metagenomic approaches.

Isolating microbial strains with culturomic methods and designing microbial consortia using synthetic community methods can provide a functional characterization of the plant microbiota, which is essential for reconstructing artificial consortia that can reduce plant diseases and improve crop health and growth. To reconstruct microbial consortia with biocontrol activity, the metabolic potential of each strain must be characterized to avoid the establishment of ecological niche competition processes that could negatively impact host plant colonization by the consortium. This characterization would make it possible to choose microbial combinations that could use different resources, and therefore able to “cohabitate”, by maximizing the ecological niches available in the host plant. Saturation of these niches would be important, not only to avoid invasion by pathogenic species or pests, but also to control, through competitive processes, any harmful agents already present in the habitat (Yang et al. 2017).

A detailed characterization of the interaction mechanisms between the beneficial microorganisms and the host plant would also make it possible to better identify the microbial molecules underlying the biocontrol activity. This knowledge is necessary to develop and obtain marketing authorization for biocontrol products.

Chapter 11
Microorganisms as Biocontrol Products

Marc Bardin and Philippe C. Nicot

11.1 Introduction

Biological control has been extensively studied in recent decades. Many microorganisms have been identified as potential biological control agents against plant pests and diseases, but only very few of them are now sold commercially. Developing and marketing these products requires considerable scientific and industrial investment as well as a thorough understanding of the factors that make them effective. The efficacy of microbial biocontrol agents against plant pests on a commercial scale depends on complex factors related to their modes of action and ecological competence. Environmental factors, the biological control agent's properties and product quality, as well as factors related to farming practices and the target pest may influence the survival, establishment and activity of microbial biocontrol agents. In this chapter, we will discuss the different types of microbial biocontrol agents and their modes of action, as well as the factors that determine their efficacy.

11.2 Biological Control: An Array of Microorganisms Described

Using entomopathogenic microorganisms to regulate pest populations was first proposed in the late nineteenth century by several scientists, among whom was Louis Pasteur. One documented example is the use of the pathogenic fungus *Metarhizium anisopliae* by Ilya Mechnikov in Russia to control beetles in different

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crops in the 1880s. A wide range of microorganisms (bacteria, viruses, fungi, protozoa) have since been identified as potential biological control candidates against insect pests.

With regard to controlling plant pathogens (viruses, bacteria, fungi, oomycetes, nematodes), the first documented work, dating from the 1910s and carried out by Carl F. von Tubeuf in Germany, dealt with the use of the fungus *Tuberculina maxima* as a parasite of pine rust. Further work was then carried out in the 1920s on introducing microorganisms to control soil-borne pathogens. Since then, microorganism-based biological control against plant pathogens has been extensively investigated and numerous microbial biocontrol agents have been identified. For example, according to Nicot et al. (2011a), 157 species of microorganisms have been described as effective against five major aerial fungal diseases (*Botrytis*-incited diseases, powdery mildew, rust, downy mildew, late blight and brown rot).

While interest in the use of microorganisms as weed biocontrol agents dates back more than 200 years, the first microorganism-based herbicide was developed in China in the 1960s. It involved a suspension of spores of the fungus *Colletotrichum gloeosporioides* f. sp. *cuscutae* to control dodder. Biocontrol microorganisms can limit weed populations by causing disease or inhibiting seed germination and plant growth (Radhakrishnan et al. 2018). A complication of biocontrol against weeds is that they must be targeted in a way that does not harm crops or other plants.

After identifying a potential biocontrol benefit against a pest under laboratory conditions, developing a microbial biocontrol agent requires studying its biology, creating a production method that meets the needs of the experiments (both in the lab and under natural conditions), and validating the lab experiments through tests under growing conditions (greenhouse or open field).

11.3 Commercial Products

According to van Lenteren et al. (2018), 209 strains of microorganisms (bacteriophages, viruses, bacteria, fungi, oomycetes) are approved worldwide as biocontrol agents against plant pests, but this figure is probably underestimated due to the lack of accurate data for some countries such as India or China. In the European Union, microorganisms subject to a marketing authorization in an EU Member State are listed in the EU Pesticides Database (European Commission 2021). As of April 2021, 69 strains of microorganisms are registered in the European database (37 fungal strains, one oomycete strain, 23 bacterial strains and eight viral strains) and ten strains are awaiting approval. The 69 strains are approved for the development of biocontrol products such as fungicides (37 strains), insecticides (26 strains), bactericides (four strains), elicitors (four strains) and nematicides (three strains). No microbial strains are currently registered in the European Union as herbicides.

11.4 Mode of Action of Microbial Biocontrol Agents

The mechanisms behind the protective activity of a given microbial biocontrol agent are often only partially understood. However, the data available to date suggest that plant protection against pests using microbial biocontrol agents is based on mechanisms related to direct and/or indirect interactions with the pest (Fig. 11.1). These modes of action do not always act alone and a combination of mechanisms has been identified for some of them.

11.4.1 Antibiosis

In the case of antibiosis, the antagonistic microorganism produces secondary metabolites that are toxic to the target pest. One of the best documented examples is the bacterium *Bacillus thuringiensis*, which produces proteins (Cry and Cyt proteins belonging to the class of pore-forming toxins) that form crystals that are toxic to insects. When an insect ingests these proteins, pores form in the intestinal cell walls and destroy the midgut. The cell content spreads and is used by the bacteria to multiply, causing septicaemia and eventually death.

Metabolites produced at low concentrations can also inhibit spore germination, mycelial growth and sporulation in fungal plant pathogens. Many bacteria and fungi produce antimicrobial compounds, including *Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces* sp. and *Trichoderma* sp. Researchers have characterized substances responsible for antibiosis in strains belonging to various microbial species, and have also identified genes involved in the synthesis of some of these substances (Raaijmakers et al. 2002; see Chap. 12). Although it is easy to show the antibiosis mechanism against plant pathogens in the lab (via dual culture assays on nutrient agar), the ability of biocontrol agents to synthesize these substances under field conditions on crops has rarely been demonstrated. Many environmental factors, including the water potential and pH, the temperature or the type of available nutrients, may all have an impact on the production of antibiotic compounds (Whipps 1987).

In some cases, the mode of action of microorganisms used for weed control may be similar to antibiosis. For example, the fungus *Fusarium oxysporum* f. sp. *strigae* excretes the amino acids L-leucine and L-tyrosine, which are harmless to maize but toxic to the parasitic plant *Striga hermonthica* (Nzioki et al. 2016).

This mode of action is similar to that of chemical pesticides. It can therefore be very effective in inhibiting pest development, but may raise concerns about the safety of the substances produced with regard to the environment, users and consumers, as well as about the emergence of pest-resistant strains (see Chap. 23).

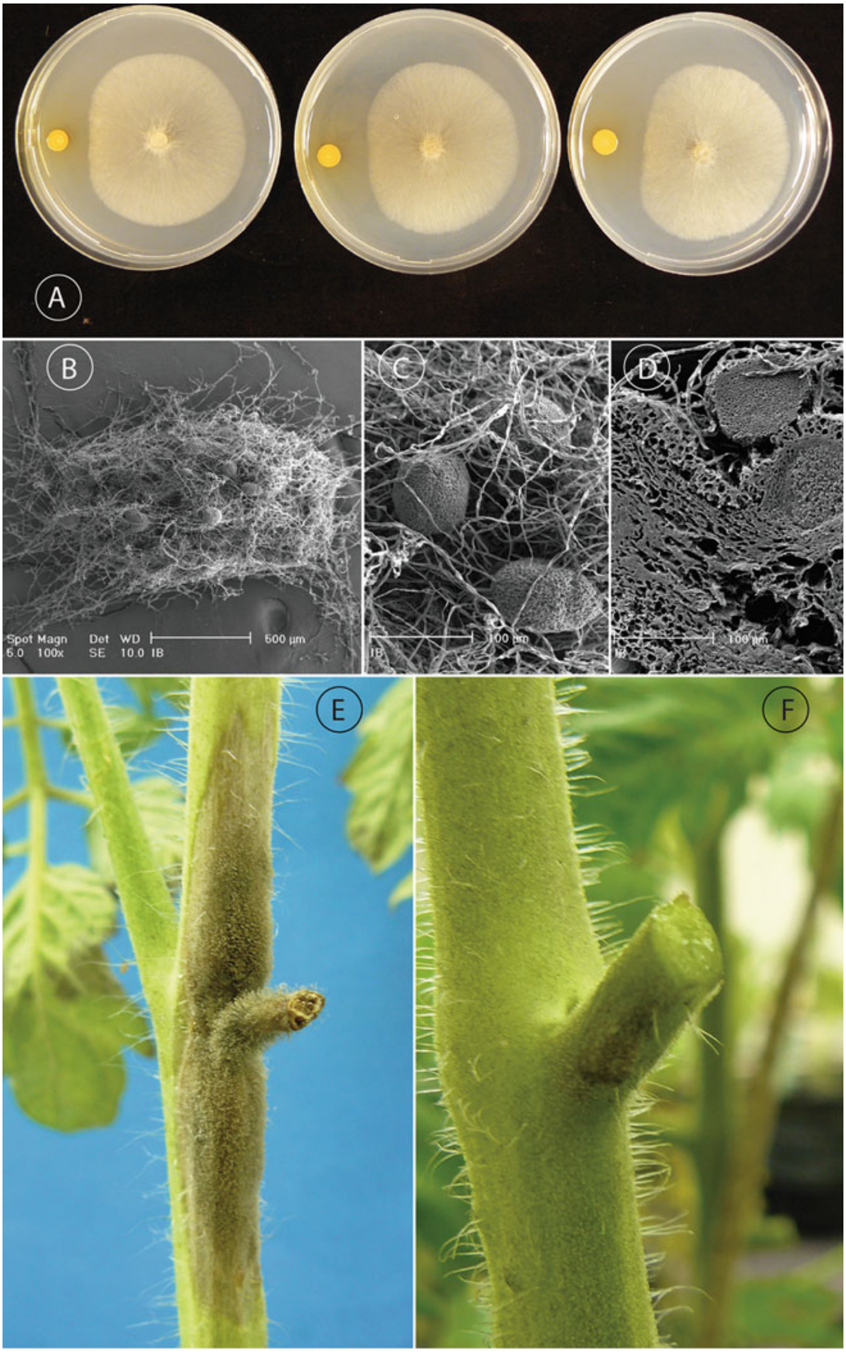

Fig. 11.1 (**a**) Dual culture on nutrient agar medium to reveal the antibiosis properties of various bacterial strains against *Botrytis cinerea*. (Partially based on Bardin and Pugliese 2020). (**b**, **c**, **d**) Hyperparasitism of sclerotia of *Sclerotinia sclerotiorum* by *Coniothyrium* (*Paraphaeosphaeria*)

11.4.2 Hyperparasitism

In the case of hyperparasitism, the antagonistic microorganism is a parasite of the pest. It specifically recognizes its target, penetrates its cells and kills it by colonizing its organs.

Most entomopathogenic fungi are hyperparasites. They enter their host through natural openings or by breaking down the insect cuticle with enzymes. For example, chitinases are necessary for the fungus *Beauveria bassiana* to parasitize insect pests. Entomopathogenic viruses (especially baculoviruses) replicate in the host cell nucleus after being ingested by the target insect pest. Some microorganisms are capable of parasitizing other microorganisms. For example, *Coniothyrium (Paraphaeosphaeria) minitans* is a hyperparasitic fungus of the plant pathogenic fungus *Sclerotinia* sp. and is capable of producing enzymes that break down the pathogen's cell walls (Whipps and Gerlagh 1992). The fungus *Ampelomyces quisqualis* specifically parasitizes the fungi responsible for powdery mildew (Kiss et al. 2011). Viruses called bacteriophages are also biocontrol agents that can target bacterial pathogens. Lytic phages proliferate and destroy the host bacterial cell, whereas lysogenic phages will integrate their genome into the genome of the bacterial host and replicate without destroying the bacterial cell.

Microorganism action on weeds is usually based on complex interactions between a pathogen and a plant, and can therefore be considered as parasitism. Microbial biocontrol agents act by secreting toxic metabolites or by altering the cellular functions of plants. The most studied candidate microorganisms are the fungi *Colletotrichum*, *Phoma* and *Sclerotinia*, the bacteria *Xanthomonas* and *Pseudomonas*, as well as various viruses (Harding and Raizada 2015).

The use of hyperparasites in biological control comes with certain constraints, such as the need for direct contact with the pest and properly applying the treatment to ensure the pest is controlled before it causes too much damage to the crop to be protected.

11.4.3 Competing for Nutrients and Space

Some microorganisms can inhibit or reduce the growth of other microorganisms because of their ability to rapidly absorb nutrients in the environment (nutrient competition) and occupy available space (spatial competition). This mode of action is particularly effective against plant pathogenic fungi whose spores need a source of nutrients to initiate germination (e.g. *B. cinerea*). In this case, the reduced nutrient levels in the environment lead to less germinative capacity of spores from the

Fig. 11.1 (continued) minitans. (**e**, **f**) Protection of tomato stems against *B. cinerea* by an antagonistic fungus: (**e**) plant attacked by the pathogen, (**f**) plant protected by the biocontrol agent pathogen and slower mycelial growth, thus reducing the number of infections and the spread of lesions (Blakeman and Fokkema 1982). Some microorganisms (e.g. bacteria, yeast, filamentous fungi) can inhibit conidial germination in plant pathogens by competing for nutrients such as nitrogen, carbon or macro- or micronutrients. Competition for nutrients or space has been demonstrated in different yeasts applied to apples in post-harvest treatments against *B. cinerea*, such as *Candida oleophila* (Mercier and Wilson 1994). The antagonistic fungus *Trichoderma harzianum* strain T-39 also inhibits germination of *B. cinerea* conidia by competing for nutrients at early stages of interaction (Zimand et al. 1996). Nonpathogenic biocontrol strains of *F. oxysporum* have been shown to compete for carbon with pathogenic strains of *F. oxysporum* on plants (Alabouvette et al. 2009).

11.4.4 Interference with Pathogenicity

During the first phase of host-pathogen interaction, plant pathogens – and particularly fungi – synthesize hydrolytic enzymes (cutinases, pectinases, etc.), which is crucial to the infection process. Biocontrol agents can interfere with pathogenicity factors in fungi by inhibiting or breaking down specific hydrolytic enzymes (Duffy et al. 2003). Pathogenicity interference has been described, for example, in *T. harzianum* strain T-39, where the activity of different pectinolytic enzymes produced by *B. cinerea* is reduced in the presence of the antagonistic fungus (Kapat et al. 1998). As a result, the penetration rate of *B. cinerea* into host tissues and the impact of the disease are significantly reduced. Some microorganisms can have an indirect effect on pathogenesis development by modifying the pH of the medium. *B. cinerea* shows optimal pectinolytic activity at acidic pH levels (Manteau et al. 2003), so alkaline pH levels make the enzymes that break down tissues less efficient. Some antagonistic microorganisms, such as *Bacillus pumilus* and *P. fluorescens*, alter the pH of the medium (Swadling and Jeffries 1998). These bacteria thus indirectly interfere with the pathogenicity of *B. cinerea*. Finally, microorganisms that break down oxalic acid, a compound produced by *S. sclerotiorum* or *B. cinerea* when they interact with plants, can protect plants against attacks by these pathogens (Schoonbeek et al. 2007).

11.4.5 Modifying Plant Leaf Surface Properties

Some bacteria can change plant leaf surface characteristics by synthesizing biosurfactants such as surfactins or other lipopeptides (Ongena and Jacques 2008; see Chap. 12). This hampers pest attachment and growth processes on the leaves. For example, microorganisms such as *Pseudomonas* spp. can modify leaf wettability and thus interfere with pathogen development (Bunster et al. 1989). The spread and drying of water droplets on leaves leads to a decrease in moisture and so prevents favourable conditions for pathogen development.

11.4.6 Induction of Host Plant Resistance

Some microorganisms can stimulate plant defences, making them more resistant to biotic stresses. Many microorganisms are able to induce defence mechanisms in plants following a signal recognition phase (Pieterse et al. 2014). These signal molecules (called elicitors) that lead microorganisms to induce resistance are called microbe-associated molecular patterns. They include oligosaccharides, lipopolysaccharides, peptides and proteins, various enzymes, biosurfactants and siderophores. When a plant recognizes an elicitor, a cascade of events is triggered that may eventually induce systemic resistance. Three plant hormones are responsible for intercellular communication in plants: salicylic acid, jasmonic acid and ethylene. Resistance can result from various modifications induced in the plant:

- thickening of the parietal structures, which reinforces their role as a physical barrier;
- stimulation of secondary metabolic pathways for the synthesis of antimicrobial substances (such as phytoalexins);
- accumulation of defence proteins, aka pathogenesis-related (PR) proteins.

Much research is currently devoted to this subject.

11.4.7 Combined Modes of Action

In some cases, the combination of several modes of action is possible for a single microbial biocontrol agent. The most studied case concerns the fungus *Trichoderma*, for which different modes of action have been demonstrated for the same strain, for example by combining hyperparasitism and antibiosis (Lorito et al. 1993). When a biocontrol agent shows combined modes of action, the precise role and importance of each in controlling the pest is generally not known.

11.5 Factors Affecting the Efficacy of Microbial Biocontrol Agents

While examples of biological control agents with consistent efficacy have been described, the use of microorganisms is often still considered unreliable under crop growing conditions. This is generally attributed to changing environmental conditions and insufficient product quality. But other factors may explain such inconsistent performance.

11.5.1 Local Environmental Context

In field crops, where environmental conditions fluctuate and are generally difficult to predict, biocontrol agents are often reported as being unreliably effective (Paulitz and Belanger 2001). Changing microclimate conditions (e.g. temperature, relative humidity, radiation) are generally identified as one of the key factors in inconsistent efficacy of biocontrol agents. These microclimatic conditions can affect the survival, establishment and activity of microbial agents introduced on plants or in the soil. Under commercial growing conditions, in addition to fluctuations in temperature and relative humidity, biocontrol agents are subject to variations in nutrient availability that can affect their efficacy on the leaf surface. The chemical exudates present on the plant surface change frequently; these changes can directly affect the introduced microbial biocontrol agent or have an indirect impact, such as by altering the metabolic state, morphology and chemistry of the leaf surface and modifying the microbiota already present on leaves (see Chap. 9).

11.5.2 Farming Practices

The protective efficacy of a microbial biocontrol agent can vary depending on the variety of the plant that is grown, fertilizing practices and phytosanitary treatments. For example, *Trichoderma atroviride* and *T. harzianum* offer different levels of protection against *B. cinerea* in tomato depending on the tomato accession used (Tucci et al. 2011). High nitrogen fertilization is associated with improved efficacy of two antagonistic fungi (*Trichoderma atroviride* and *Microdochium dimerum*) to protect pruning wounds in tomato against *B. cinerea* (Abro et al. 2014). With regard to plant protection products, good compatibility between three biocontrol agents has been demonstrated to protect the crop against two fungal pathogens and an insect pest in greenhouse tomatoes (Bardin et al. 2008) but further research is necessary to explore the compatibility of microbial biocontrol agents with all phytosanitary products, including those for biocontrol.

11.5.3 Biocontrol Product Quality and Method of Application

Microbial biocontrol agents provide different degrees of protection depending on factors related to the product itself and its mode of application, persistence, ecological competence and mode of action. Microbial products do share similarities with chemicals with regard to these factors, but there are some important differences. They must contain a sufficient quantity of living cells not only by the end of the manufacturing process, but above all during their use; this depends on the microorganism's survival ability, the production method and the formulation. Transport and storage conditions of the biocontrol product can also have an impact on how effective it is. For example, exposure to excessive temperatures, even temporary, can seriously degrade products. Aside from the number of living cells, biocontrol agent efficacy also depends on the microorganisms' physiological state and the speed with which they resume activity once applied. Product purity is an additional important factor in ensuring consistent performance over time, as is the proper distribution of antagonistic microorganism on the plant and its ability to survive and even multiply. These factors depend on the microorganisms' specific characteristics, which can be modified and improved by the product formulation. As with a chemical product, the application of an active microorganism by contact will be more complicated than if it acts at a distance. One important benefit of microbial biocontrol agents is that they can multiply and colonize the surfaces to be protected, thus offsetting a slightly imprecise application. But the ecological competence of these microorganisms and their resilience towards environmental changes are often only partially understood.

Performance is largely related to the mechanisms involved in the biological activity of the microorganism. The different modes of action determined above will influence how it is used in practice. For instance, induced resistance in plants only occurs after a delay between product application and the establishment of resistance in the plant.

11.5.4 Variability of Target Pests

The characteristics of the pests to be controlled can also impact the efficacy of a biocontrol agent. For example, diseases that evolve quickly are harder to contain than slow-spreading monocyclic diseases. The amount of pathogen inoculum is also a factor that determines how effective a microbial biocontrol agent will be. Finally, pest populations generally present high phenotypic and genetic diversity. Recent studies have shown variability in efficacy of microbial biocontrol agents depending on the strains of pests (Bardin et al. 2015; Siegwart et al. 2015). Thus, the range of susceptibility of pests to biocontrol agents and their ability to develop resistance must be taken into account to ensure effective and durable biological control (see Chap. 23).

11.6 Conclusion

The current socio-economic and political context is especially favourable to the development of alternative crop protection methods to replace chemical plant protection products. This has led to a rising need for microbial biocontrol agents. Available products do not yet cover all plant diseases and pests, which means that further research is necessary to develop new products, especially for arable crops. In particular, high-throughput screening tests should be developed on large collections of thousands of isolates to assess the efficacy of these microorganisms against target pests. A challenge for research will be to select better performing microorganisms by more fully taking into account their ecological competence and resilience to fluctuating environmental conditions, as well as the stability and durability of their efficacy. Existing products also need to be assessed to determine the scope of their efficacy and ensure they are properly integrated into complex crop management schemes (such as by creating decision support tools) so that farmers are best able to optimize their use.

Chapter 12
The Role of Microbial Metabolites in Biological Control

Valérie Leclère, Christophe Clément, Stéphan Dorey, and Claire Prigent-Combaret

12.1 Introduction

Bacteria belonging to the genera *Bacillus* and *Pseudomonas* are widely used as biocontrol agents (see Chap. 11). The secondary metabolites they produce play a major role in their plant protection activities. Depending on the metabolites, the mode of action may be (i) direct, by inhibiting the growth of the targeted plant pathogen, or (ii) indirect, by inducing plant defence mechanisms. Therefore, some bacterial metabolites, called elicitors, can induce local and systemic plant resistances. This process of plant immunization could be compared to vaccination in animals to some extent. Secondary metabolites involved in these activities include amphiphilic compounds such as rhamnolipids or lipopeptides, as well as polyketides. These compounds have a significant structural diversity related to their mode of biosynthesis. For instance, lipopeptides and many polyketides are produced by multienzyme complexes or modular megaenzymes that function as assembly lines. A deeper understanding of the biosynthetic pathways and mechanisms of action of these secondary metabolites as well as their role in plant protection should allow researchers to identify other microorganisms that could be used for extended biocontrol.

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12.2 Rhamnolipids: Multi-faceted Compounds

Rhamnolipids are produced by bacteria of the genus *Pseudomonas* or *Burkholderia*, some strains of which are known for their biocontrol activity against fungi and plant pathogenic oomycetes (Perneel et al. 2008). These glycolipids are composed of a fatty acid chain coupled with one or two rhamnoses in their carboxy-terminus. Rhamnolipids are synthesized from two precursors, R-3-hydroxy fatty acids and L-rhamnose, by the actions of three enzymes: RhlA, RhlB and RhlC. RhlA dimerizes R-3-hydroxy fatty acids forming congeners of R-3-((R-3-hydroxyalkanoyl)oxy) alkanoic acids (HAAs). The rhamnosyl transferases RhlB and RhlC catalyse the sequential addition of the first and second rhamnose to HAAs, forming monorhamnolipids and dirhamnolipids, respectively (Fig. 12.1). Rhamnolipids play an essential role in motility and biofilm development, as well as in the absorption and assimilation of poorly soluble substrates by increasing bacterial cell surface hydrophobicity (Abdel-Mawgoud et al. 2010). As surfactants, rhamnolipids are used in many industrial applications, especially in pharmaceuticals, cosmetics, bioremediation (e.g. remediating hydrocarbon-contaminated soil) and the agrifood industry (e.g. as food additives). Although rhamnolipids are most often purified from *Pseudomonas aeruginosa*, they are non-toxic to humans, have a low ecotoxicological impact (Monnier et al. 2018) and are biodegradable (Mohan et al. 2006). They are very stable, especially at high temperatures and strong pH fluctuations. Additionally, rhamnolipids have proven antimicrobial action against a wide range of microorganisms including fungi and oomycetes (Vatsa et al. 2010; Crouzet et al. 2020). The antimicrobial activity of rhamnolipids has been studied on several plant pathogens belonging to the genera *Botrytis*, *Rhizoctonia*, *Pythium*, *Phytophthora* and *Plasmopara*. The antifungal and antioomycetal activities of rhamnolipids involve direct lytic action on spores by insertion into their plasma membrane. Rhamnolipids also inhibit mycelial growth of *Botrytis cinerea* and *Pythium myriotylum*. The antimicrobial properties of rhamnolipids against oomycetes has been confirmed in various plant species such as tomato and cucumber (Vatsa et al. 2010; Delaunois et al. 2014).

Rhamnolipids can be perceived by grapevine, rapeseed and *Arabidopsis* cells, and they can induce an immune response in these plants (Schellenberger et al. 2019). They activate early signalling, including calcium influx and the production of reactive oxygen species. They also stimulate the production of defence enzymes and antimicrobial compounds such as phytoalexins by the plants. Under controlled conditions, rhamnolipids are particularly effective in inducing local resistance against the necrotrophic fungus *B. cinerea* in several plants such as grapevine and rapeseed. They also trigger an immune response that is effective against hemibiotrophic bacteria and biotrophic oomycetes in *Arabidopsis*. This response involves different signalling pathways depending on the type of pathogen, and salicylic acid, an essential plant hormone, is a key component in rhamnolipid-triggered immunity (Sanchez et al. 2012). Rhamnolipids also potentiate the elicitor effect of other substances such as chitosan. This property can be particularly

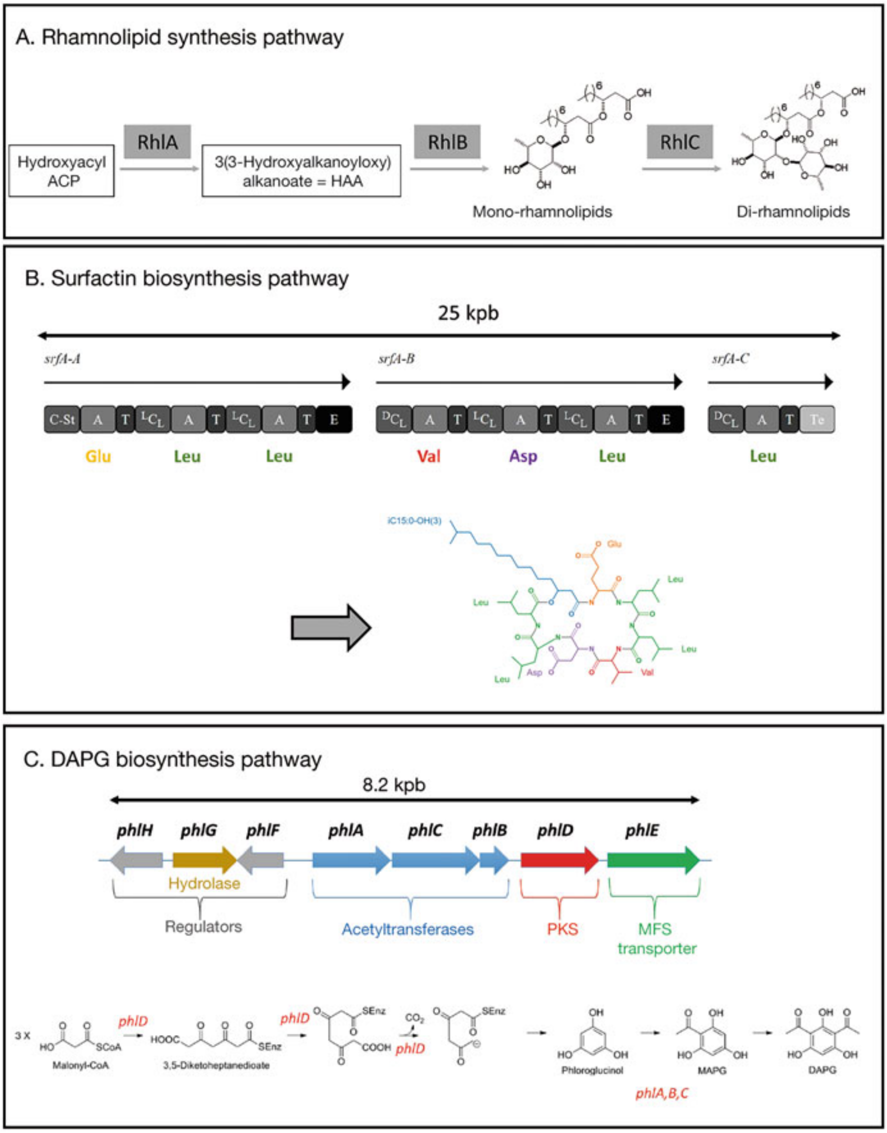

Fig. 12.1 Biosynthesis pathways of metabolites involved in extended biocontrol. (**a**) Rhamnolipids; (**b**) Surfactin synthesis performed by enzymatic domains: A (adenylation), T (thiolation), $^{L}C_{L}$ and $^{D}C_{L}$ (condensation), C-st (C-starter coupling the fatty acid onto the first amino acid), Te (Thioesterase); (**c**) DAPG

interesting when several elicitors are used together. Rhamnolipids have no deleterious effects on plants when used at reasonable (micromolar) concentrations. For example, spraying rapeseed leaves with rhamnolipids does not affect photosynthesis or plant growth (Monnier et al. 2018). Researchers still do not have a good understanding of how plants perceive rhamnolipids. Due to their amphiphilic properties, it has been suggested that rhamnolipids may insert into plant membranes, thereby activating the immune response. In line with this hypothesis, biophysical methods were recently used to demonstrate that rhamnolipids can penetrate and interact with lipid bilayers in model membranes (Schellenberger et al. 2019).

Several rhamnolipid patents, with some referring to crop protection, attest to the application potential of these compounds. A mixture of *P. aeruginosa* rhamnolipids is sold as a biofungicide under the name Zonix™ for the preventive treatment of turf and ornamental plants. Another American company, Rhamnolipid Companies, also sells rhamnolipids from *P. aeruginosa*, mainly as a wetting agent to facilitate application of crop pesticides and as an antifungal agent to target oomycete zoospores. Rhamnolipids offer a lot of promise for crop protection. They are active under controlled conditions and in the open field on various plant species (including tomato, tobacco, wheat and grapevine), often conferring very significant protective effects (Delaunois et al. 2014).

12.3 Lipopeptides: Structural and Activity Biodiversity

Cyclic lipopeptides (CLPs) are another class of amphiphilic metabolites produced by microorganisms, including bacteria of the genera *Bacillus* and *Pseudomonas*. They are composed of a hydrophobic fatty acid chain linked to a hydrophilic peptide sequence, which is synthesized by an original mechanism, thanks to modular multienzymatic complexes called nonribosomal peptide synthetases (NRPSs) (Finking and Marahiel 2004). NRPSs function as assembly lines that recruit amino acids and bind them together step by step into the peptide (Fig. 12.1). Incorporating each amino acid requires a succession of enzyme activities carried out by several domains: the adenylation domains (A) select an amino acid that is then transferred to the thiolation domain (T); the formation of peptide bonds is carried out by condensation domains (C). At the end of the assembly line is the final thioesterase domain (Te), which allows the release of the neoformed peptide and its cyclization. In lipopeptide synthesis, the NRPS usually starts with a special condensation domain called C-starter, which will condense the lipid on the first amino acid of the peptide chain.

Compared to classical peptide synthesis (translation process), nonribosomal synthesis via NRPS will generate structural biodiversity in lipopeptides. First, the C-starter domain can attach linear or branched fatty acid chains of different lengths and with different degrees of saturation, some of which can be hydroxylated. Second, adenylation domains can recruit amino acids that are not among the twenty amino acids involved in protein and peptide synthesis. Thus, dehydroaminobutyric acid (Dab) is a frequent component of many CLPs secreted by *Pseudomonas*, such as sessilin, which is produced by the biocontrol strain *Pseudomonas* sp. CMR12a. Sessilin, which displays antifungal activity via direct antagonism on *R. solani,* contains two Dab monomers among the 18 residues that are part of the peptide sequence.

Table 12.1 Structural diversity of CLPs produced by *Bacillus* and *Pseudomonas*

Group/family	Genus	Number of amino acids	Amino acids involved in the ring	Fatty acid length	3 (OH)[a]
Surfactins	*Bacillus*	7	AA1 → AA7	C13 → C15	
Iturins	*Bacillus*	7	AA1 → AA7	C14 → C17	
Kurstakins	*Bacillus*	7	AA4 → AA7	C11 → C14	
Locillomycins	*Bacillus*	9	AA1 → AA9	C12 → C14	
Fengycins	*Bacillus*	10	AA3 → AA10	C14 → C17	
Bananamides	*Pseudomonas*	8	AA3 → AA8	C10 → C12	×
Pseudofactins	*Pseudomonas*	8	AA3 → AA8	C16 → C18	
Syringomycins	*Pseudomonas*	9	AA1 → AA9	C12 → C16	×
Viscosins	*Pseudomonas*	9	AA3 → AA9	C10 → C16	×
Orfamides	*Pseudomonas*	10	AA3 → AA10	C10 → C13	×
Amphisins	*Pseudomonas*	11	AA3 → AA11	C8 → C12	×
Putisolvins	*Pseudomonas*	12	AA9 → AA12	C6	
Entolysins	*Pseudomonas*	14	AA10 → AA14	C10	×
Xantholysins	*Pseudomonas*	14	AA7 → AA14	C10 → C12	×
Tolaasins	*Pseudomonas*	18	AA14 → AA18	C18	×
Fuscopeptins	*Pseudomonas*	19	AA15 → AA19	C10	×
Corpeptins	*Pseudomonas*	22	AA18 → AA22	C10 → C12	×
Syringopeptin 22	*Pseudomonas*	22	AA15 → AA22	C10 → C14	×
Syringopeptin 55	*Pseudomonas*	25	AA18 → AA25	C10 → C12	×
Syringafactins	*Pseudomonas*	8	Linear	C10 → C12	×

[a] Presence of a hydroxyl group on the third carbon atom

Given the diversity of CLPs produced by several strains of *Pseudomonas* and *Bacillus*, they have been classified into families or groups according to the number of amino acids, the position of amino acids involved in the ring and their fatty acid chain length (Table 12.1; Roongsawang et al. 2011; Guedens and Martins 2018). This structural biodiversity generates biological activity biodiversity, and while some CLPs clearly have a negative impact on plants when produced by pathogens, others show activity that can be useful for extended biocontrol applications. Some are antibacterial (syringopeptins), antiviral (viscosin), insecticidal (orfamide) or antiprotozoal (viscosin), while some may induce the formation of biofilms (iturin) (Geudens and Martins 2018). However, currently there is no obvious and known relationship between CLP structure and activities, since variants of the same family may have different activities. CLPs increase root colonization efficiency by lowering surface tension, which modifies the physicochemical properties of the microbial habitat. The most studied activities of CLPs for the development of biocontrol strains are antifungal activities against plant pathogenic fungi and oomycetes, which can result from direct antagonism or an indirect effect via the induction of a systemic defence response. The antagonism mode of action has been demonstrated for several antifungal CLPs, such as iturin and fengycin, produced by *Bacillus* strains, or viscosinamide or tensin produced by *Pseudomonas*. The presence of these CLPs leads to morphological changes in the target fungi with increased branching, hyphal swelling, vacuolization and metabolite leakage related to the formation of pores in the plasma membrane following the insertion of CLPs into the lipid bilayer (Gotze and Stallforth 2019). The induction of systemic resistance by surfactin and fengycin is explained by their effect on signalling pathways, mainly those for jasmonic acid/ ethylene, involved in plant defence (Fan et al. 2018).

Up to three CLPs belonging to three different families can be co-produced by the same bacterial strain. For example, *B. amyloliquefaciens* FZB42 produces a surfactin involved in biofilm formation and induces systemic resistance, as well as bacillomycin D and fengycin, two lipopeptides with antagonistic and systemic resistance-inducing properties (Radhakrishnan et al. 2017). This production of different CLPs by a single strain helps guarantee the effectiveness of commercial extended biocontrol products such as Cedomon® and Cerall®, which contain *Pseudomonas* strains, or Serenade®, Quantum-400® and Kodiak®, which contain *Bacillus* strains.

12.4 Polyketides Synthesized by Biological Control Agents of the Genus *Pseudomonas*

In *Pseudomonas* species, aside from rhamnolipids and lipopeptides, several antimicrobial compounds with a role in extended biocontrol have been identified, such as phloroglucinols (the best known and most studied being 2,4-diacetylphloroglucinol or DAPG), pyoluteorin, pyrrolnitrin, phenazines, hydrogen cyanide and butyrolactone (Couillerot et al. 2009).

Some of these antimicrobial compounds belong to the class of polyketides. Polyketides are a very important class of natural substances produced by a wide variety of micro- and macroorganisms. They account for about one-third of the chemically and biologically characterized secondary metabolites. These compounds are produced by polyketide synthases (PKSs). PKSs (types I to III) are enzyme complexes that may or may not be multimodular, with the modules themselves composed of several domains with specific enzyme activities. These enzyme complexes catalyse the condensation of acyl units (such as acetyl-CoA or malonyl-CoA) to produce polymer chains that can be cyclized and substituted by different types of groups (Shen 2003). PKSs, like NRPSs, thus enable the production of a very high structural diversity of secondary metabolites with extremely varied biological activities. In agriculture, polyketides have been widely associated with extended biocontrol because they show antibacterial, antifungal and antihelmintic activities or can be used to induce systemic plant responses.

Two aromatic polyketides produced by *Pseudomonas*, DAPG and pyoluteorin, have been studied in greater detail. DAPG can inhibit the growth of a wide range of bacterial and fungal pathogens, as well as oomycetes, nematodes, protozoa and viruses (Weller 2007; Weller et al. 2007; Couillerot et al. 2009; Thomashow et al. 2019). Pyoluteorin is an aromatic polyketide that is effective against bacteria, fungi and oomycetes (Howell and Stipanovic 1980). The biological activity of DAPG or pyoluteorin has been mainly characterized via the use of bacterial mutants impaired in the production of these compounds in in vitro growth inhibition assays against plant pathogens or in plant protection assays. More recently, the mode of action of DAPG on eukaryotic cells was clarified by comparing the impact of this polyketide on a bank of mutants of *Saccharomyces cerevisiae* compared to its impact on the wild strain. DAPG affects key cellular functions, such as membrane biosynthesis or mitochondrial function, and triggers overproduction of reactive oxygen species (Kwak et al. 2011). When the DAPG acetyl groups are chemically substituted with more lipophilic groups, its antifungal activity increases significantly (Gong et al. 2016). As for pyoluteorin, its mode of action at a cellular level has not been precisely characterized.

Pseudomonas protegens CHA0, a biocontrol strain model, can produce both DAPG and pyoluteorin. *P. protegens* CHA0 protects tobacco against black root rot caused by *Thielaviopsis basicola*, and wheat against seedling damping off and take-all caused by *Gaeumannomyces graminis* var. *tritici*. Through loss-of-function mutations, DAPG has been shown to be the main antifungal agent involved in resistance to seedling damping off in wheat and black root rot in tobacco (Keel et al. 1992), while pyoluteorin is involved in suppressing seedling damping off in cucumber caused by *Pythium ultimum* (Maurhofer et al. 1992).

These biocontrol strains were identified after being isolated from naturally disease suppressive soils. These soils were characterized for their resistance to take-all in wheat (Weller 2007; Weller et al. 2007) and black root rot in tobacco (Almario et al. 2014). Microbial communities in these soils have been shown to differ from those in nearby non-suppressive soils, with DAPG-producing *Pseudomonas* populations being larger and/or producing more DAPG in suppressive soils.

In addition to its effect against various pathogens, DAPG also has phytostimulation effects on plants at low concentrations, which is of interest when applying biocontrol agents that produce this compound (Vacheron et al. 2018). It increases root hair density and secondary root growth of tomato seedlings by modulating auxin-dependent plant hormonal pathways (Brazelton et al. 2008) or by stimulating auxin production in other plant growth-promoting rhizobacteria (PGPR) of the genus *Azospirillum* when co-inoculated into the rhizosphere of the same plant (Combes-Meynet et al. 2011). It is also a powerful inducer of systemic resistance in *Arabidopsis thaliana* (Weller et al. 2012).

DAPG produced in situ by *P. protegens* CHA0 induces the expression of genes encoding the proteins involved in the biosynthesis of this antibiotic in *Pseudomonas* spp. cells present in the rhizosphere (Maurhofer et al. 2004). The same applies to pyoluteorin produced by *P. protegens* Pf-5 cells on pyoluteorin-producing *Pseudomonas* spp. cells (Brodhagen et al. 2004). Adding pyoluteorin to bacterial cultures of

CHA0 suppresses the expression of genes involved in DAPG biosynthesis and reduces DAPG production (Baehler et al. 2005). As a result, beyond their antibiotic roles, DAPG and pyoluteorin act as messengers that facilitate intercellular communication, which can positively affect the biocontrol activity of all rhizosphere populations producing these polyketides. The presence of the amoeba *Acanthamoeba castellanii* has been shown to increase DAPG production by strains of *P. protegens* (Jousset and Bonkowski 2010). Since there is a metabolic cost to DAPG biosynthesis, the amount produced by *Pseudomonas* strains is adjusted to the density of amoebic populations and the associated level of predation (Ibid.). Thus, as with other antimicrobial substances, DAPG plays a major role in the ecology of the rhizobacteria that produce those compounds (Besset-Manzoni et al. 2018).

The polyketide-producing strains of *Pseudomonas*, which are ubiquitous in agricultural soils, have many interesting traits that make them good biological control agents:

- they produce bioactive metabolites of a much wider range than the polyketide family alone,
- they efficiently colonize the rhizosphere by efficiently using root exudates,
- they have high metabolic versatility,
- they show strong competitiveness compared to other rhizospheric microorganisms (Weller et al. 2007).

However, because these microorganisms do not sporulate, developing formulas that guarantee their survival and effectiveness in the field remains one of the great challenges yet to address. Thus, the number of extended biocontrol products involving polyketide-producing strains of *Pseudomonas* is still limited (Thomashow et al. 2019).

12.5 Conclusion

The effectiveness of microorganisms used in extended biocontrol is often linked to their potential to produce metabolites with varied structures and activities. It seems most promising to search for strains capable of producing various metabolites (rhamnolipids, lipopeptides and other nonribosomal antibiotics and polyketides) in order to identify effective biocontrol agents that combine several activities. Screening of microorganisms likely to secrete such compounds is an interesting way to have more biocontrol agents available. High-throughput screening can be facilitated by in silico methods applied to a growing number of sequenced genomes for which it is now possible to identify genes for the synthesis of active metabolites, and thus to predict their production. This bioinformatics approach requires the development of tools to identify gene clusters enabling the biosynthesis of secondary metabolites, such as antiSMASH (Blin et al. 2017), and databases, such as Norine, dedicated to nonribosomal peptides (Pupin et al. 2018). However, being able to systematically determine the biological activities associated with structural diversity remains a major challenge today. Meanwhile, the direct use of substances stimulating plant defence potentially produced by these biocontrol organisms is another major challenge. Indeed, these compounds can be used in combination with other alternatives, but also with synthetic substances, in order to significantly reduce their application in the field. Elicitors are simpler to use and should benefit from easier approval as biostimulants in the future.

Part IV
Botanical Biopesticides

Marc Bardin

While plants cannot run away from a threat, they do have ways to defend themselves. To cope with abiotic or biotic stresses, plants have developed both physical and chemical defence mechanisms. The most effective strategies have been selected over time as plants and pests have co-evolved. The chemical defence compounds in plants can be divided into two main categories: defence proteins and secondary metabolites. Since most botanical biopesticides are based on secondary metabolites, Chaps. 13 and 14 will focus specifically on this category. Unlike primary metabolism, which produces the universal substances plants need to develop and grow, secondary metabolism produces various substances that play a role in reproduction and defence.

This wide range of diverse substances thus allows for an array of possible defence responses, some of which could be specific to a particular pest. Some compounds used for defence are constantly produced by plants while others are induced by pest attacks. All plant organs can produce these toxic compounds. Because these toxins can be harmful to the plants themselves, some plants have developed special means of storage. The various plant organs can also emit volatile molecules that are toxic or likely to modify the behaviour of certain pests.

Plant secondary metabolites are produced from universal precursors and are classified according to their biosynthetic pathways. These metabolites are grouped into four main classes: nitrogen-containing compounds, phenolics, terpenoids and polyacetates. Pesticides can be found within all of these classes, except perhaps polyacetates. They are mostly toxic to crop pests, but they can also have sublethal effects. In herbivorous insects these compounds act either on behaviour (repellent or antifeedant effects, scent masking) or on the physiology of individuals (inhibition of development, growth or reproduction).

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Since the dawn of agriculture, humans have naturally been inspired by these chemical interactions between plants and their pests to develop botanical biopesticides. Aromatic plant extracts and decoctions were used for thousands of years before being replaced by synthetic pesticides in the nineteenth century. With synthetic pesticides now reaching their limits – namely because of their high environmental persistence and their impact on biodiversity – botanical biopesticides are receiving renewed attention. Some of these compounds have demonstrated their efficacy in the field. They also have various modes of action that could limit the development of resistant pests (see also Chap. 23). Their supposed biodegradability means they are assumed to be more environmentally friendly. All these characteristics make them particularly interesting for crop pest management. Botanical biopesticides do fall outside the narrow definition of biological control, but just like other products of living organisms (in this case, plants) they are a relevant contribution to extended biocontrol.

Chapter 13 describes the recent history of botanical biopesticides, the characteristics of the regulations on which they depend, and their current use. Chapter 14 delves into the conceptual and technical obstacles that must be lifted to develop their use to make agriculture more environmentally friendly.

Chapter 13
Botanical Pesticides as Biocontrol Products

Myriam Siegwart and Anne-Violette Lavoir

13.1 Introduction

Our historical knowledge of plant extracts being used as pesticides is fragmented. We do know that in Europe people relied on this practice over 3000 years ago to fight ectoparasites and to protect stored foods (Pavela 2016). *Chrysanthemum* flowers and aromatic plants were the main sources of such extracts. Later, with the development of agriculture, a greater variety of botanical pesticides were used to control crop pests. The first commercial botanical product was nicotine, which was used against plum beetles from the seventeenth century. Rotenone, a substance extracted from many Fabaceae species, was also widely used as an insecticide in agriculture from 1850 onwards. However, these practices became anecdotal by the Second World War with the discovery of synthetic chemical pesticides. Since then, side effects of this generation of pesticides have led us to take a fresh look at ancestral practices and draw inspiration from them to develop new methods and products.

Botanical biopesticides can be divided into two categories:

- those that are not commercially available, i.e. extracts made directly by farmers from local plants. It is difficult to evaluate their frequency of use, but some ethnobotanical studies have mapped cultural practices using local flora for protection against insects (Belmain and Stevenson 2001).

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- industrially produced extracts sold as biopesticides. There are many different products, but most are produced by combining plant-based active ingredients with co-formulants.

At the beginning of the twenty-first century, despite the proven undesirable effects of synthetic pesticides and strong research interest in botanical biopesticides, these products represented only 5% of the biopesticide market, which itself accounted for only 8% of the global pesticide market in 2014 (GeoData.gouv 2021). Although many predicted a renaissance of this type of pesticides, it has not yet materialized, despite slightly higher sales in recent years. This modest growth can be explained by a change in strategy by agrochemical companies following the withdrawal from the market of the old families of synthetic pesticides (organochlorine, organophosphate and carbamate compounds) with unacceptable toxicological and ecotoxicological profiles. These companies replaced them with a new generation of synthetic pesticides (neonicotinoid, spinozine, oxadiazine, diamide compounds) that are considered less persistent and more specific, and thus as having a lesser impact on human health and the environment. This also explains why, on a global scale, the use of chemical pesticides has not fallen.

In Europe, the inertia regarding botanical pesticides can also be attributed to very strict regulations on placing plant protection products on the market. Botanical products must follow the same cumbersome and costly registration process as synthetic compounds. Only recently has a more streamlined evaluation procedure been approved for “low-risk active substances” in Europe (Regulation (EC) No 1107/2009). In France, biocontrol products benefit from accelerated evaluation procedures to obtain marketing authorization as well as reduced fees for these procedures.

Other difficulties specific to botanical biopesticides also limit their market growth, such as the supply of plant matter, the extraction process and yield, and the development of very specific and complex formulations. These conceptual and technical obstacles must be overcome to optimize and ensure environmentally friendly use of botanical biopesticides (see Chap. 14). In this chapter, we will look at France as a case study along with French and European regulations on botanical pesticides before going into more detail on these active substances.

13.2 Botanical Biopesticides and Organic Agriculture

Botanical biopesticides are based on plant extracts, which means they are natural products. But simply being natural products does not mean they can be systematically used in organic agriculture. Nicotine, for example, was banned as an insecticide because of its toxic side effects, despite its natural plant origin.

The regulatory framework for biopesticides varies considerably between countries and is often poorly understood. The basic concept is simple: non-synthetic (natural) substances can be used in organic agriculture, as long as they are not specifically prohibited and catalogued. Synthetic substances are automatically prohibited unless explicitly authorized on a national or EU-level list. The terms "synthetic" and "non-synthetic" are not clearly defined and have been subject to debate. However, the general principle is more or less followed by most countries. For example, in Japan, synthetic products may be used in organic agriculture if there is an imminent or serious threat to the crop or when alternative measures are ineffective. In Europe, biopesticides must have a marketing authorization, and conditions of use are specific to each EU country. Botanical products are considered plant protection products and are therefore subject to the same regulations, with a few exceptions.

The International Federation of Organic Agriculture Movements (IFOAM) is an international non-governmental organization that centralizes discussions between organic farming organizations from more than 172 countries. It has developed a common system of standards, verification and commercial identity. In Europe, active substances are evaluated at the EU level based on toxicity for humans (proven or presumed effects, including endocrine-disrupting effects) and environmental pollution (persistent, bioaccumulative and toxic pollutants). The initial marketing approval of an active substance is valid for 10 years. Commercial products composed of active substances to which various adjuvants are added may only be used or placed on the market if they have been authorized in and by the relevant Member State.

In the following paragraphs, we will take a closer look at French regulations as a case study to illustrate these points. In France, the Rural and Maritime Fisheries Code defines biocontrol products as agents and products using natural mechanisms within the scope of integrated pest management. It differentiates among macroorganisms and microorganisms, semiochemicals and natural substances of plant, animal, microbial or mineral origin. Aside from macroorganisms, the other three categories are subject to the same regulations as plant protection products.

These products may then be added to the list of biocontrol products drawn up and published by the French Ministry of Agriculture in its official bulletin. This list is updated regularly (French Ministry of Agriculture 2021). The products on the list benefit from several advantages, including authorization for commercial advertising, fewer use restrictions, authorization for sale and use in home gardens, parks, forests, roads and walking areas accessible or open to the public, and a reduction in the sales tax rate earmarked for the phytopharmacovigilance scheme.

Extended biocontrol products must meet several criteria to be included on the list. First, they must have a valid marketing authorization. The "active substances" are then classified according to the following categories: insect traps, microorganisms, semiochemicals, or natural substances of plant, animal, microbial or mineral origin. A natural substance is defined as any compound, molecule or mixture of the two existing in nature. These substances can come directly from a natural source (plants, microorganisms) or be chemically synthesized if the molecule produced is strictly identical to its natural counterpart. Finally, the last criterion for inclusion on this list is based on the assessment of the risk to human health and the environment. While the active substance of these products must be of natural origin, the various added adjuvants are not held to this standard. However, the entire formulated product is evaluated based on toxicity and ecotoxicity criteria.

Not all biocontrol products can be used in organic farming; to be authorized for such use, a product must be included in Annex 2 of European Commission Regulation (EC) No 889/2008 (amended by Regulation (EU) 2016/673). This additional, more restrictive step is nevertheless necessary. This verifies the absence of genetically modified organisms in the production chain of these substances, as well as the use of pheromones, which must be limited to traps and diffusers, thereby excluding kairomones and allomones (Acta Biocontrole 2018).

13.3 Description of Botanical Biopesticides Currently Used as Biocontrol Products in France

The number of active substances (or mixtures of active substances) of plant origin authorized for extended biocontrol in agriculture is low compared to other biopesticide categories in the world, as the French case study illustrates (Table 13.1). Certain substances such as azadirachtin (Text Box 13.1) have only provisional authorizations for sale in some countries (including France). Others, such as certain pyrethrum-based formulations, have been removed from the list of biocontrol products because one of the adjuvants has an ecotoxicological profile that does not meet the necessary criteria.

13.3.1 Pyrethrins

The active substances known as pyrethrins that are used as insecticides are synthesized from flowers of the Asteraceae family, with the main species belonging to the *Tanacetum* genus (synonym *Chrysanthemum*). *Tanacetum cinerariifolium* (also known as Dalmatian pellitory and pyrethrum), *T. coccineum* and *T. pinnatum* have been specifically described as having high levels of these compounds (Dajoz 1969). The term pyrethrum refers to a mixture of six esters (Table 13.2) produced by esterification of two acids and three alcohols with similar structures.

These compounds are neurotoxins. They act on the sodium channel, a protein in the cytoplasmic membrane of the axon in neurons involved in action potential propagation along these cells, and thus nerve signal transmission (see Chap. 16). Pyrethrins settle on these canals and slow down their closure. At the individual scale,

Table 13.1 List of various biocontrol plant protection products based on botanical substances authorized on the French market in 2021

	Active ingredient	Use	Date placed on the market	Formulated product (commercial)	Company
Insecticide	Pyrethrins (+ abamectin[a])	**Ornamental trees and shrubs and flower crops**: mites, whiteflies, scale insects, caterpillars, aphids, thrips	2009	Fazilo	Compo France SAS
	Rapeseed oil	**Vegetable, fruit and ornamental crops:** whiteflies, mites, aphids, scale insects, psyllids, true bugs	2017 2011	Nativert Naturen	Compo France SAS Scotts France SAS
	Rapeseed oil + pyrethrins	**Ornamental crops:** mites, aphids, leafhoppers	2009	Spruzit	Neudorff GMBH KG/Compo France SAS
	Sweet orange essential oil	**Field crops; vegetable, fruit, ornamental, tropical and grapevine crops:** whiteflies, thrips, mites, froghoppers, scale insects, psyllids, leafhoppers, true bugs	2017 2009	Prev-Am Plus Essen'ciel, Limocide, Prev-Am	Oro Agri/ Nufarm SAS Vivagro
	Maltodextrin	**All vegetable and ornamental crops:** aphids, mites, whiteflies	2016 2016	Eradicoat Blanmoscate	Certis Europe M. Cazorla, S.L.
	Terpenoid mixture	**Vegetable, fruit and ornamental crops:** whiteflies, mites, aphids, scale insects, psyllids, true bugs	2019	Requiem prime	Bayer SAS
Fungicide	Eugenol + geraniol + thymol	**Grapevine:** grey rot	2017 2017	Cagenoleta Mevalone	M. Cazorla, S.L. Sumi Agro France SAS
	Sweet orange essential oil	**Field crops, vegetable and fruit crops, grapevine:** powdery mildew, blight, rust, blister mites	2017 2009	Prev-Am Plus Essen'ciel, Limocide, Prev-Am	Oro Agri/ Nufarm SAS Vivagro
	Clove essential oil	**Fruit crops:** conservation diseases	2011	Bioxeda	Xeda International SA
Nematicide	Garlic extract	**Vegetable crops**: nematodes	2016 2017	Nemguard Namoteli	

(continued)

Table 13.1 (continued)

	Active ingredient	Use	Date placed on the market	Formulated product (commercial)	Company
					Certis M. Cazorla, S.L.
Herbicide	Pelargonic acid	**General treatments; vegetable, fruit, ornamental, tropical crops; viticulture; non-agricultural areas; fragrant, aromatic and medicinal plants:** mosses, dicotyledons and grasses	2014 2017 2017	Natur'net, Herbistop Kalipe Beloukha, Herbatak, Bromory, Starnet, Devatol, Finalsan	Compo France SAS Bayer Belchim/ Compo France SAS Protecta, SBM, Scotts, Start
	Acetic acid	**General treatments, ornamental crops, non-agricultural areas**	2013	Naturen Express, Cito fast, Cito max	Evergreen garden care SAS Aroma SAS Aedes protecta SAS
	Caprylic acid	**General treatments, ornamental crops, non-agricultural areas**	2018	Desherb'nat	SBM development
Other	Spearmint essential oil	**Potato:** inhibition or suppression of germs	2010	BioX-M	Xeda International SA

Source: Acta Biocontrole (2018) and French Ministry of Agriculture (2021). This list is updated regularly

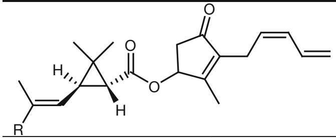
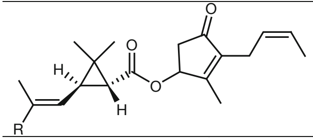
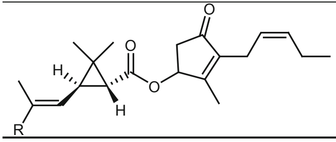
[a] Abamectin is an avermectin, i.e. a macrocyclic organic compound of bacterial origin

poisoning with these active ingredients results in hyperactivity followed by convulsion. Because these products are broken down in the gut, they act through direct contact and inhalation rather than ingestion.

These products are not only biologically effective, but they also have low environmental persistence: the molecules are unstable in light, air and water. They have a broad spectrum of action within the arthropoda phylum. They will kill not only all species of pests but all arthropods that come into contact with the product, including natural enemies. However, they are not very toxic to mammals (lethal dose estimated at 50–100 g for humans; Lauwerys 1990).

People have used the insecticidal properties of these flower extracts since the nineteenth century (Ware 1991). In the 1920s, their use was limited because of their high cost and fleeting effect. During the 1930s, the massive production of these extracts in Japan caused prices to fall, resulting in increased use (Regnault-Roger et al. 2008). However, because the active ingredients are so unstable, researchers sought out more persistent derivatives, which gave rise to a large family of synthetic

Table 13.2 List of the six major components of pyrethrum and their proportion in *T. cinerariifolium*

Structure	Common name	Proportion in an extract of *T. cinerariifolium*	Relative toxicity
	Pyrethrin I	35%	100%
	Pyrethrin II	32%	23%
	Cinerin I	10%	71%
	Cinerin II	14%	18%
	Jasmolin I	5%	Unknown
	Jasmolin II	4%	Unknown

R = CH_3 (pyrethric acid): series I
R = CO_2CH_3 (chrysanthemic acid): series II

insecticides (pyrethroids), the first of which began being used in the 1960s. Pyrethrins then fell into disuse. After 40 years of using synthetic pyrethroids, growing awareness of their side effects meant a return to the natural substances in the 2000s. Today, using natural pyrethrins is controversial. For example, in France, they are authorized as a biocontrol product, when combined with abamectin or rapeseed oil, on ornamental trees and shrubs, flower crops and house plants to control mites, whiteflies, herbivorous caterpillars, scale insects, aphids and thrips (Table 13.1). However, when added to mixtures with piperonyl butoxide, they are not considered biocontrol products. Pyrethrins can be used in conventional agriculture to control pests of cereals and tobacco, as well as to disinfect premises used for food storage (Acta Phytosanitary 2019). In 2016, 1774 kg of natural pyrethrins were sold, accounting for 17% of microbiological and botanical insecticides (Agreste 2021).

13.3.2 Vegetable Oils: The Example of Rapeseed Oil

Non-volatile vegetable oils are fatty and viscous extracts from plants, composed of fatty acid esters with a high molecular weight (Regnault-Roger et al. 2008), unlike essential oils, which are mainly composed of volatile molecules (described below). Vegetable oils are used as contact insecticides or as adjuvants for fat-soluble active ingredients. They work by forming an impermeable film that cuts off the air supply of the insect (or its eggs), causing asphyxiation, and by deeply penetrating the cuticle due to the amphibolic nature of some of their compounds. They are therefore only effective if the arthropods are present at the time of treatment. They may also affect insect pest behaviour by limiting oviposition; the application of vegetable oils is thought to limit the emission of volatile molecules pests use to recognize their host plant (demonstrated by Mensah et al. 2005 with mineral oils; suggested by Nicetic et al. 2011). Because their mode of action is more related to physical rather than chemical properties (Nicetic et al. 2011), vegetable oils are essentially non-selective and will have this insecticidal effect on both pests and non-target beneficial organisms (see Chap. 14).

Rapeseed oil is marketed worldwide as an insecticide to control pests in maize, orchards, vegetable crops, and ornamental or indoor plants. It is also sold as an adjuvant in the manufacture of fungicidal, herbicidal or insecticidal mixtures. There is little data in the scientific literature on its effectiveness as an insecticide or fungicide. In one review, Sams and Deyton (2002) reported three studies describing the fungicidal effects of rapeseed oil on powdery mildew, including an anti-sporulation effect. Cloyd et al. (2009) compared different botanical biopesticides and showed that rapeseed oil-based products varied in their effectiveness depending on the amount of oil used and the target pest. Although this biopesticide has a very broad spectrum of action, the quantity used must be adapted to the pest and the crop. Finally, Nicetic et al. (2011) compared the insecticidal effect of rapeseed oil versus mineral oils. The findings showed that rapeseed oil does increase pest mortality and limit oviposition compared to the control plot, but less effectively than the mineral oils tested.

Text Box 13.1: Azadirachtin and Neem Oil: To Be Or Not to Be a Biocontrol Product, That Is the Question

The insecticidal activity of neem oil, derived from the seeds of an Indian tree called *Azadirachta indica* A. Juss. (Meliaceae), is mainly due to limonoid compounds, which are modified triterpenoids. Neem oil also contains salanin and nimbin, antifeedant substances. Andiroba oil is also extracted from the seeds of another Meliaceae species, *Carapa guianensis*, for its limonoid content and insecticidal bioactivity.

The main limonoid in neem oil is azadirachtin, which inhibits feeding and imaginal moulting, and is a chemical sterilizing agent (Veitch et al. 2008). It is especially effective on young larval stages as its main mechanism of action alters the release of a growth hormone from the insect, causing morphological abnormalities at moulting (Dwivedi 2008). This active substance is absorbed by crop plant tissue, which ensures systemic action. It can therefore be used in many ways: classic sprinkling and by root absorption in hydroponic conditions or by injection in the stems.

(continued)

Text Box 13.1 (continued)

This substance is probably the most emblematic product among insecticidal compounds based on plant extracts. Its effectiveness has been demonstrated on over 400 species of arthropods (Flamini 2003; Ntalli and Menkissoglu-Spiroudi 2011), which has led to the plant from which it originates being called “the tree for solving global problems” (National Research Council 1992).

Azadirachtin is the only botanical pesticide authorized on the US market in the last 20 years[1] (Miresmailli and Isman 2014). NeemAzal®-T/S sold by Trifolio-M® is the most widely sold product in some European countries and has a declared azadirachtin A content of 10,000 ppm (Pavela et al. 2009). In France, two products that use azadirachtin A – NeemAzal®-T/S and Azatin® – have been authorized for sale and used since 2018, but only for crops grown under cover. NeemAzal®-T/S has also received provisional marketing authorization for 3 months each year since 2015 to control aphids in apple and pear crops. This restriction is in place because the active ingredient is a suspected endocrine disruptor (French National Assembly 2014). Its low toxicity to mammals and the environment is relative. For example, compared to glyphosate, it is more harmful to the environment and has a higher acute toxicity (Agritox 2021).

13.3.3 Essential Oils from Aromatic Plants

Essential oils are considered a very promising option for the development and production of botanical biopesticides (Isman 2000; Tripathi et al. 2009; Isman et al. 2011; Regnault-Roger et al. 2012; Pavela and Benelli 2016). They are produced using an extraction method set out in the AFNOR T-75-006 standard, which specifies how they must be obtained via steam distillation, dry distillation or mechanical processes. They are a complex mixture of secondary metabolites; they have a low molecular weight and are therefore volatile. Their bioactivity is due to several compounds, mainly terpenoids, including monoterpenes and sesquiterpenes, and to a lesser extent phenylpropanoids. Some compounds present in lower amounts may also impact their effectiveness or cause synergistic effects between compounds.

More than 3000 species of aromatic plants are known to date and about 10% are already sold commercially as raw materials in food, cosmetics, perfume, aromatherapy and alternative medicine (Bakkali et al. 2008). Aromatic plants yield around 0.5–2% essential oils, making them highly concentrated plant extracts compared to other plant sources (Isman 2017). Such yield levels are considered relatively good. Extraction and analysis methods are not very complex and their low costs are

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[1] Products formulated with essential oils are subject to other regulations.

positive arguments for their use as biopesticides. Another major interest of essential oils is also their low persistence in the environment due to their high instability: they are rapidly degraded by light or high temperatures (Turek and Stintzing 2013). All these arguments explain why essential oil-based biopesticides are exempt from regulation by the United States Environmental Protection Agency.

Several botanical insecticides using essential oils (rosemary, mint, cinnamon, thyme, black pepper, clove) are available on the North American market and have uses ranging from farming to stored food protection, park management and disease vector control (Cloyd et al. 2009; Isman et al. 2011; Pavela 2016). Sweet orange essential oil is sold commercially around the world as an insecticide, especially against whiteflies, and as a fungicide, mainly against powdery mildew (Table 13.1). As insecticides, essential oils can affect pest behaviour (attraction, repulsion, inhibition of feeding and reproduction), metabolism (via deregulation of the endocrine balance), or nervous system activity (Regnault-Roger 1997; Isman 2006; Rattan 2010; Mossa 2016; Jankowska et al. 2017). Treatment is based on contact or fumigation; both types of application work by inhalation (Ikbal and Pavela 2019). Not all pests are sensitive to all essential oils and their relative specificity of action could guarantee an ecotoxicological profile that is considered safer for the environment. However, developing effective control strategies requires many tests to identify functional pest-essential oil pairs while assessing plant toxicity risks on crops (Cloyd et al. 2009; Ikbal and Pavela 2019).

13.3.4 Fatty Acids: The Example of Pelargonic Acid

The herbicidal activity of fatty acids has been known for many years (Poignant 1954). Fatty acids with medium aliphatic chains, such as caprylic acid (C8, octanoic acid) and pelargonic acid (C9), are the most effective (Coleman and Penner 2006). Pelargonic acid is one biological herbicide that is available in a range of formulations. For example, there are 24 commercial products containing pelargonic acid in France, ten of which are authorized for extended biocontrol and marketed as non-selective herbicides (Table 13.1). Commercial products are formulated with aliphatic fatty acids of different lengths that are mixed with vinegar or acetic acid and emulsifiers. These herbicidal oil solutions act very quickly by destabilizing plant cell membranes, leading to a rapid loss of cell functions. They have no selectivity and will kill all vegetation. However, sufficiently developed weeds or species with particularly developed underground organs (thistle, weed grasses, etc.) tend to grow back due to the lack of residual activity after the initial burning effect of the application.

Pelargonic acid is therefore a broad-spectrum commercial herbicide that is non-selective on crops (except for perennial crops) that works on contact and mainly acts against annual plants and mosses. It is considered an herbicide with low toxicity for humans with a transient environmental impact because it has no residual activity. Adding organic acids, such as succinic, lactic or glycolic acids, makes pelargonic acid formulations more effective (Coleman and Penner 2008). Oleic acid is usually a major component of these mixtures, although the exact compositions of these commercial products are trade secrets.

13.3.5 Sulphur Compounds in the Brassicaceae Family and **Allium** *Genus*

Some plants in the genus *Allium* (including garlic) or in the Brassicaceae family are rich in sulphur compounds and can be used directly to control the proliferation of soil organisms harmful to crops (insects, mites, weeds, nematodes, fungi and pathogenic bacteria). The sulphur compounds in these plants are the active ingredients of interest and can be classified into two groups: non-protein sulphur amino acids in *Allium* and glucosinolates in Brassicaceae. Non-protein sulphur amino acids are all cysteine derivatives and are precursors of volatile substances with biocidal activity. Similarly, glucosinolates can be degraded into unstable compounds that will produce different volatile compounds depending on the environmental conditions: thiocyanates, nitriles and isothiocyanates. The latter are the most abundant and widely studied.

These two plant families have proved useful in controlling Diptera species thanks to the repulsive action of their sulphur compounds: the smell of onion repels cabbage flies (*Delia radium*) while garlic extract repels mammalian parasites (*Simulium indicum* and *Culex quinquefasciatus*). These compounds may also inhibit feeding, which has been described, for example in the green peach aphid *Myzus persicae*. Finally, the toxic effect of these compounds has been demonstrated in many species of coleopterans, lepidopterans, hymenopterans, hemipterans, orthopterans and dipterans (Auger et al. 2002). *Allium* sulphur compounds have been shown to be effective against the nematode *Meloidogyne incognita* and Brassicaceae are often recommended for control of root-knot or cyst nematodes by biofumigation (Potter et al. 1998). These compounds have only a marginal acaricide effect, but their herbicidal effect is well known: farmers must wait to replant crops after biofumigation so the germination of the seeds planted is not inhibited. Finally, bactericidal and fungicidal effects are mainly known in human pathogens, although a few cases seem promising for possible application in agriculture. For example, *Pectobacterium carotovorum* and *Rhizobium radiobacter* are sensitive to three *Allium* extracts (Grainge and Ahmed 1988) and *Botrytis allii* is sensitive to thiols and sulphides (Kadota and Ishida 1972).

Biofumigation is the most widespread process for using these natural substances. This involves crushing and burying a brassicaceous plant (mustard, radish) or an *Allium* in the soil, which then generates biocidal gases. One limitation of this method is its lesser effectiveness on insect pests that have adapted to these compounds through co-evolution (case of *Acrolepiopsis assectella* larvae).

13.3.6 Maltodextrin

Maltodextrin is a substance of plant origin (more precisely, from maize *Zea mays*) composed of glucose polymers and obtained by hydrolysing starch. It is widely used in food processing, cosmetics and pharmacology, but its use as a biopesticide is relatively recent (European Food Safety Authority 2013) and very few scientific studies have been published on the subject. Cahenzli et al. (2018) showed that maltodextrin was toxic to *Drosophila suzukii*, albeit with lower efficacy compared to other botanical pesticides tested.

The insecticidal mode of action is quite similar to that of vegetable oils, i.e. it acts via suffocation by sealing the respiratory openings and limiting movement by causing the legs and wings to stick. The cross-linking of glucose polymers by rapid drying becomes impermeable to air. Maltodextrin-based products sold as biopesticides mainly target small pests such as aphids, mites and whiteflies on all vegetable and ornamental crops grown in greenhouses. Products are currently under development for future use in the field, particularly in arboriculture. Maltodextrin is also often used as a formulant in preparing essential oils for pesticide purposes (Luiz de Oliveira et al. 2018). Perhaps there could be a dual benefit to using this component itself as an insecticide formulant.

13.4 Conclusion

Botanical biopesticides contain an array of active substances with biological activity that varies in terms of specificity against a target. Currently, the use of such products is limited and few are authorized for sale. However, public and private research actors around the globe are now working to develop new solutions to meet the environmental challenges of modern agriculture and better support human health and the environment. Several challenges must be tackled, including identifying new botanical substances, understanding their mode of action and specificity, optimizing their production and assessing the risks generated by their use. These challenges are outlined in Chap. 14.

Chapter 14
Challenges in Developing Botanical Biopesticides for Pest Control

Anne-Violette Lavoir, Thomas Michel, Jean-Luc Poëssel, and Myriam Siegwart

14.1 Introduction

Experts predict significant growth in the sales of botanical biopesticides in the coming years, especially due to their growing popularity with consumers (Isman 2015; Pavela 2016). Research must adapt to this renewed interest, both in terms of intensity and means, to drive the discovery and development of new botanical pesticides (Isman 2017). Multidisciplinary research efforts will be required to (i) identify new active botanicals,[1] (ii) characterize their modes of action and (iii) assess the risks associated with their use (Fig. 14.1).

The main academic challenges in developing new botanical biopesticides are:

- creating innovative research methods for new substances to identify effective and specific biopesticide-pest-crop combinations;
- improving extraction methods to increase the yield of active ingredients for economically viable production;
- adapting their formulations for better and guaranteed product effectiveness and consistent quality across production batches;

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[1] In this chapter, both single compounds (e.g. dicaffeoylquinic acid, azadirachtin) and complex blends (e.g. essential oils) extracted from plants are considered active botanicals.

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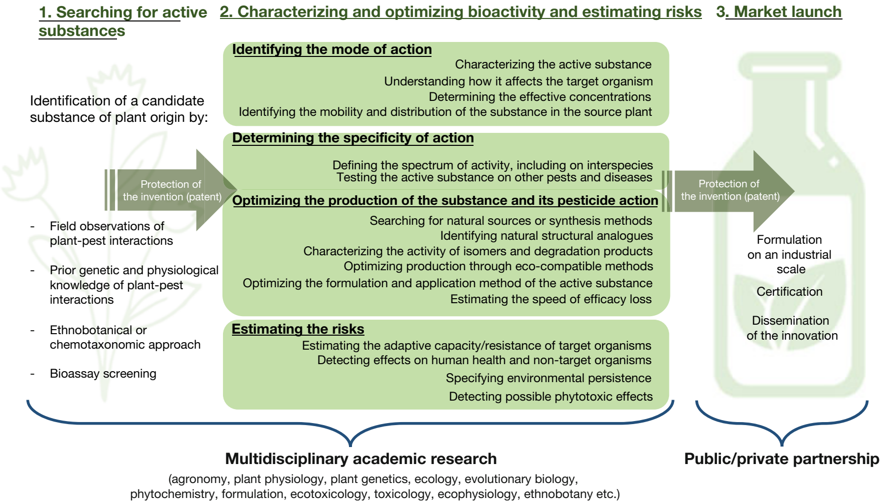

Fig. 14.1 Theoretical steps in developing an active botanical for use as a biopesticide

- identifying non-target effects so they can be minimized;
- informing users about the optimal use of these products, which are more technical than synthetic pesticides.

In this chapter, we will outline the theoretical steps required to develop an active botanical as a biopesticide (Fig. 14.1), based on the multidisciplinary approach adopted by two of the authors of this chapter and their team members at INRAE in Avignon to develop aphicidal and fungicidal products using phenolic compounds. We will then present several scientific and technical obstacles that must be overcome in order to optimize the botanical biopesticide development stages. Finally, we will discuss human health and environmental safety issues related to botanical biopesticides.

14.2 Applied Research Process for Compounds Based on Plant-Pest Interactions

Over the last 20 years or so, our knowledge of the functional relationships between plants and pests has increased considerably, mainly due to the development of the "omics" approaches: genomics, transcriptomics, proteomics and metabolomics. Metabolomic approaches in particular have shed light on many secondary metabolites, some of which are involved in plant-pathogen or plant-herbivore interactions (Aliferis and Chrysayi-Tokousbalides 2011). These substances, which have repellent, antifeedant and/or toxic effects of varying specificity on pests, can be constitutive (e.g. phytoanticipins) or induced by the action of the parasite or pest on the plant (e.g. phytoalexins).

Increasing the levels of these compounds in a plant species via breeding is one way to use this knowledge to create cultivars that are resistant to a given pest. One example is maize lines created with high levels of maysin, a flavonoid that is toxic to the corn earworm *Helicoverpa zea* (Meyer et al. 2007). Another way to exploit these natural defence compounds is to use them as biopesticides. This strategy differs from the use of complex natural extracts by targeting a single purified molecule whose content can be fully controlled in the commercial product. This has the advantage of making it easier to estimate not only its effectiveness on the target organism, but also its toxicity on mammals and other non-target organisms, unlike complex extracts where the proportion of each compound depends on many factors, such as the physiological state and genotype of the plant from which they are derived or the extraction method used. However, using a purified compound cannot leverage the synergistic effects that are often observed in natural extracts, which may contain several dozen different compounds (Tak et al. 2017). The risks of adaptation by target pests may also increase due to the simple composition. As is the case with synthetic pesticides, these purified compounds must be used while minimizing the risk of developing resistance (see the Sect. 14.4.1 on "Pest resistance to natural plant extracts" and Chap. 23).

Using such compounds as biopesticides based on research on plant-pest interactions requires a long-term multidisciplinary approach where plant physiologists and geneticists, chemists, entomologists/pathologists and ecologists all work hand in hand with professional partners with the necessary skills to develop an approved commercial product.

Various issues must be addressed when developing botanicals for use as biopesticides. These issues are summarized in Fig. 14.1 and are illustrated below through the example of an ongoing process led by INRAE's Avignon centre for the use of phenolic compounds as aphicidal and fungicidal substances.

This research stems from a programme on the genetic resistance of peaches to the green peach aphid, *Myzus persicae*. The aim was to understand the genetic determinism of this resistance and the underlying physiological mechanisms with a view to creating peach cultivars with high-quality fruit and sustainable resistance to this pest. *Myzus persicae* is a highly polyphagous sap-feeding insect that is a vector of multiple viruses. It poses a threat to many crops due to the direct and indirect damage it causes. The insect has developed resistance to synthetic insecticides, which are most commonly used for aphid control. Moreover, some neonicotinoids – including imidacloprid, the most effective aphicide – were recently banned from sale in Europe because of their harmful effects on bees. Controlling aphid populations with biocontrol solutions as an alternative to synthetic insecticides is therefore a key priority.

The metabolomic approach used to study the resistance mechanisms in peach trees to aphids revealed the involvement of a phenolic compound, 3,5-dicaffeoylquinic acid (diCQA) (Poëssel et al. 2006). The study demonstrated the repulsive[2] action and high toxicity of purified diCQA on green peach aphid. This compound, derived from caffeic acid, and its isomers are present in many foods, sometimes in high concentrations (coffee, yerba mate, artichoke, sweet potato, etc.). It is a strong antioxidant that adds to the health benefits of fruits and vegetables. It also has many interesting medicinal properties (Clifford et al. 2017). The absence of harmful effects described in the literature is a key element that led us to consider its use as a biopesticide. Safety for human health is indeed an essential condition to replace synthetic pesticides with biopesticides. Significant failures have been observed when using botanicals as biopesticides due to their harmful impacts on human health (e.g. rotenone; see Sect. 14.5 "Are botanical biopesticides safe for humans?").

Another decisive element in initiating the development of diCQA as a biopesticide was the discovery of a very promising plant source for its production: the nontuberized roots of sweet potato (*Ipomoea batatas*). Under certain growing conditions, the underground organ of this plant can accumulate a considerable amount of diCQA, representing 5–10% of its dry matter content. This substance is thus the overwhelming secondary compound in the root. It is therefore easy to purify, and eco-extraction or exudation methods are available, making it an interesting source. As with many secondary compounds, chemical synthesis is also possible but complex (Raheem et al. 2012). The production of diCQA from sweet potato root is now a patented process (Poëssel et al. 2009), which is currently being industrially exploited for non-agricultural applications.

In terms of its development as a pesticide, diCQA has a particularly interesting spectrum of action. We showed that this substance was harmful to all generalist and specialist aphid species tested thus far and did not seem to have any effect on other insect orders (Hymenoptera, Lepidoptera, Coleoptera, etc.). This aphid-specific spectrum of action is of particular interest because we hope it will reduce the risk of negatively affecting non-target organisms such as bees and other beneficial fauna (see the discussion on this aspect in the Sect. 14.4.2 "Environmental impact of these products").

Moreover, although diCQA acts specifically on aphids, it shows pesticidal activity on other phylogenetically different organisms. For example, in a larger study on diCQA's pesticidal properties, we demonstrated its inhibitory activity on certain plant pathogenic fungi. As a result, diCQA is currently also being developed as a fungicide.

The mode of action of diCQA against aphids remains unknown, but it likely has both repellent and toxic effects. Understanding the compound's mechanism of action will be a crucial step in guiding the formulation parameters for the active product and enhancing its effectiveness. By analysing the compound's structural-functional relationships, we discovered other natural substances similar to diCQA whose aphicidal action has been patented (Poëssel et al. 2015). As part of a collaborative project, INSA and INRAE in Lyon also obtained new aphicidal compounds similar

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[2] Substance limiting insects' food intake after tasting a plant (unpalatable taste).

to diCQA via chemical synthesis (Li et al. 2016). While not natural substances, they will provide insight into their mode of action with regard to aphids as well as knowledge on their chemical properties, which will be essential for controlling their stability. Our collaboration with the Micronutrients: Reactivity & Digestion team of the INRAE/Avignon University Joint Research Unit for the Safety and Quality of Plant Products has also made it possible to characterize the phenomena of diCQA degradation and isomerization according to light, temperature and pH, which are essential for formulation.

Lasting biopesticide effectiveness is a critical factor that must be achieved. The adaptation of pathogenic and herbivorous organisms to the secondary substances of the plants on which they feed is part of the co-evolution of these pathosystems (Ehrlich and Raven 1964). This phenomenon must be considered in order to estimate the abilities of target organisms to develop resistance to treatments based on natural compounds or their derivatives. Thus, it has been shown that a nicotine detoxification mechanism present in a subspecies of *Myzus persicae* that had adapted to tobacco made it better able to resist neonicotinoids, chemical insecticides derived from this natural toxin (Bass et al. 2013). Similarly, the existence of cross-resistance to synthetic pesticides and biopesticides could quickly compromise their effectiveness and should be investigated. Finally, there is also a possibility that a pest's resistance to botanicals used as biopesticides may lead it to adapt to plants containing them, and thus to changes in its host range. We therefore used different strategies to conduct an *ex-ante* study of the adaptive capacities of *Myzus persicae* to diCQA on clones bred in the laboratory and in natural populations with or without resistance to synthetic pesticides.

The findings allowed us to establish a joint project with a private partner from the phytopharmaceutical industry that develops diCQA-based formulations. These formulations are designed to facilitate, with the help of co-formulants of natural or synthetic origin, the conservation, application and adhesion to the plant and the spreading, penetration and persistence of the compound. Field efficacy testing of the diCQA-based product is now under way. Demonstration of the formula's efficacy will be a critical step in using diCQA as a biopesticide. Bringing a diCQA-based pesticide product to market will validate the innovative approach to extended biocontrol built on the resistance mechanisms of plants to their pests. It will make it possible to broaden the limited range of plant-based biopesticides for agriculture.

14.3 Technical Obstacles to Overcome

14.3.1 Difficulties in Standardizing Plant Extract Production

Unlike synthetic pesticides, which are often formulated with a single active ingredient, plant extracts generally contain several bioactive compounds (Miresmailli and Isman 2014). This means that interactions between substances may have synergistic or antagonistic effects. Only one or two major compounds are generally characterized for these extracts, but the presence and quantity of other compounds in the mixture can significantly influence the extract's effectiveness. A deeper understanding of each constituent's bioactivity would allow producers to create more effective mixtures with relatively consistent effectiveness (Akhtar and Isman 2012). It is also possible to fortify a mixture with certain compounds with well-known bioactivity. But can such fortified products be considered natural mixtures? This is an open debate.

Plant extracts are rarely available in large enough quantities and their quality is often difficult to standardize (Turek and Stintzing 2013; Velasques et al. 2017; Luiz de Oliveira et al. 2018). The quantity and composition of active substances in plant extracts vary considerably. Many factors are responsible for this variability in the chemical composition of botanical active substances (Figuereido et al. 2008; Isman et al. 2008): geographical origin, genetic variability, environmental conditions, seasonal variations, plant age, harvesting period and extraction techniques. In fact, the effectiveness of a plant-based product is not necessarily consistent. Growing, sampling and extraction techniques – which are currently costly and complex to implement – must be improved to ensure a high-quality commercial product (Pavela and Benelli 2016).

14.3.2 Optimizing the Formulation for Effective and Durable Botanical Biopesticides

Despite the many different secondary metabolites with insecticidal activity, only a few are sold commercially and used today (see Chap. 13). Compared to synthetic pesticides, botanical insecticides are unstable and degrade rapidly when exposed to light, air or high temperatures (Misra et al. 1996; Miresmailli and Isman 2014). Thus, their short duration of action and variable effectiveness limit the natural substances that can be placed on the plant protection product market. To offset the instability of these botanical insecticides and optimize the release of active ingredients, various formulation and encapsulation methods have been developed.

The chief objective of formulation is to enhance the biopesticide's biological activity, such as by controlling its release over time, limiting its spectrum of action, protecting it against biotic and abiotic degradation, or reducing its phytotoxicity. The formulation stage also aims to create a safe and easy-to-use product. Depending on the physicochemical properties of the active ingredient in question, various formulation strategies can be adopted. In agricultural chemistry, biopesticides are formulated like pesticides, in the form of granules, wettable powders, concentrated solutions, emulsions or capsules, enabling farmers to use them without changing their equipment (Knowles 2008; Luiz de Oliveira et al. 2018). There are three main types of encapsulation techniques for biopesticides:

- chemical encapsulation, e.g. microencapsulation via interfacial polymerization or molecular inclusion;

- physicochemical encapsulation, e.g. coacervation or liposome encapsulation;
- physical encapsulation, e.g. spray drying, spray chilling, co-crystallization or extrusion.

These technologies allow control over active ingredient concentrations, and thus can optimize the release and prevent breakdown of the compounds in the environment (Miresmailli and Isman 2014).

Formulation techniques must also meet the low hazard classification requirements with regard to humans and the environment in the same way as the active substances used in extended biocontrol, which is not always the case. For example, the formulation of Prev-Am Plus© (see Chap. 13) contains surfactant compounds such as alcohol ethoxylates, which are known to be harmful to human health and the environment. In addition, Cloyd et al. (2009) observed phytotoxic effects in bioassays conducted with biopesticide products containing sodium lauryl sulphate as an emulsifier. However, this compound is considered to be an “organic material”, and therefore approved for organic farming in North America. *Tween 80*®, often used as an emulsifier in nanoemulsions of essential oils, also shows phytotoxic effects. Accordingly, products and formulation processes that are non-polluting and safe for human health and the environment must be identified so that biopesticides can be considered truly low risk (Pavela and Benelli 2016).

New formulation methods that mimic the transport and storage of these active ingredients in crops are currently emerging. Mouden et al. (2017) put forward a “green” alternative that uses plant-based solvents called natural deep eutectic solvents, or NADES. These solvents are obtained by mixing two solids of natural origin. NADES have many benefits, such as low toxicity, low vapour pressure and a wide solubility range. This last point is extremely interesting with respect to natural insecticides, because NADES can solubilize both hydrophilic and hydrophobic molecules. They can therefore limit the use of organic solvents that are harmful to the environment. Additionally, the hydrogen bonds that are created between NADES and insecticides help stabilize the metabolite under different temperature and light conditions, as well as support its gradual release over time.

14.4 Side Effects to Be Considered

14.4.1 Pest Resistance to Natural Plant Extracts

Rising pesticide resistance in crop pests is a phenomenon that developed with their massive use after the Second World War. It is generally accepted that biocontrol products are immune to such evolutions. But is this really true? The development of pest resistance to botanical biopesticides should be considered in the same way as for synthetic pesticides.

To answer this question, we need to understand this adaptive phenomenon and observe what actually happens in fields where biocontrol products are regularly used in substantial quantities (see Chap. 23).

How quickly resistance emerges depends on many factors, some of which are linked to the use of these products themselves. The two main factors are:

- induced selection pressure, which depends on a combination of elements: number of treatments × treatment effectiveness × product persistence × surface area treated × dose used;
- the complexity of the product's mode of action: single target protein (unisite) versus multiple molecular targets (multisite).

Resistance management programmes can reduce selection pressure if the products' modes of action are well understood. However, such knowledge is still lacking for many plant extracts that have new or complex modes of action. Further research is therefore needed to ensure the durability of these products.

As for mode of action complexity, this is a factor inherent to the product itself. The belief that biocontrol products do not induce resistance is often fuelled by the fact that they can be comprised of a mixture of active substances, as is the case with essential oils. However, the most common biopesticides (see Table 13.1 for products used in France) have an active ingredient consisting of a single purified substance for reasons that may be toxicological or ecotoxicological, or to make production and quality certification easier.

Neem oil is a good illustration of this complexity (see Chap. 13). It comprises around a hundred potentially insecticidal and repellent molecules belonging to the terpenoid family. Its major active ingredient is azadirachtin (Akhila and Rani 1999). The oil can be used without being purified with its entire cocktail of substances, or after being purified to retain only azadirachtin. Very few studies have demonstrated resistance to neem oil, given the oil's many substances and therefore modes of action. But the same is generally true for purified azadirachtin, because this substance alone has many modes of action. However, one study did show that resistance to purified azadirachtin was possible in green peach aphid populations (Feng and Isman 1995). After 40 generations, the authors did not observe resistance in aphids treated with neem oil, while those treated with azadirachtin alone showed a ninefold increase in resistance compared to aphids not treated at all. Under field conditions, this would be the equivalent of resistance reached in four to five years, since these aphids produce up to 12 generations per year.

14.4.2 Environmental Impact of These Products

Within an agricultural context, the arthropod community can be roughly divided into two: pests and beneficial organisms. The latter provide ecosystem services and include pollinators as well as parasitoids and predators that act as natural enemies providing biological control. But can botanical biopesticides be used for pest control without negatively impacting these beneficial organisms?

Spraying pesticides, even when targeted, can potentially affect non-target natural enemies. According to some authors, the two pest control methods – biopesticides and biological control – are compatible when used as part of an integrated pest management (IPM) approach. Some biopesticides have not shown negative effects on non-target organisms (Charleston et al. 2005; Asogwa et al. 2010; Issakul et al. 2011; Pavela 2014). Meanwhile, other authors have found a strong negative impact of botanical insecticides on beneficial arthropods, with both lethal and sublethal effects observed (see review by Ndakidemi et al. 2016). Parasitoids often prove to be more susceptible than the target pests (Simmonds et al. 2002; Tunca et al. 2012), which then compromises their ability to participate in pest control.

The ecosystem service of pollination not only supports natural biodiversity, but it also substantially boosts economic returns for agricultural production (Leonhardt et al. 2013). The effect of botanical insecticides on pollinators, including the western honey bee *Apis mellifera*, needs to be assessed. In one bioassay-based study, Xavier et al. (2015) observed lethal and sublethal effects on both adult bees and larvae from direct contact or ingestion of seven different botanical biopesticides. To protect pollinator populations, their exposure to botanical insecticides must be reduced, such as by following the principles of ecological selectivity (Bacci et al. 2009). Biopesticides could, for example, be applied outside of the pollination period (Joshi and Joshi 2010) or in conjunction with additional pollen sources near the hives when biopesticides are applied to flowers to limit the pollinator foraging distance during this period (Riedl et al. 2006).

Similar approaches, based on the principle of ecological selectivity, could also be adopted to reduce the exposure of natural enemies used as biological control agents to botanical insecticides. In theory, the more selective the insecticide, the lower the risk of negatively affecting non-target organisms. However, tests comparing bioinsecticide-natural enemy pairs on a case-by-case basis that look at both lethal and sublethal effects are highly recommended to determine a sustainable and effective long-term IPM strategy (Desneux et al. 2007; Gentz et al. 2010). These studies could even reveal synergistic effects (Kraiss and Cullen 2008). The formulation of bioinsecticides, their responsible application in terms of quantity and period of use, and the method of application are all important in being able to fully leverage biological control and biopesticides within an IPM approach.

14.5 Are Botanical Biopesticides Safe for Humans?

Among the first generation of botanical insecticides, two of them showed sufficient toxicity in mammals to warrant their withdrawal from the European market. The first was nicotine, a stable alkaloid extracted from tobacco, which had been widely used as an aqueous extract since 1690. Although highly active against insects, this neurotoxin was proven to be non-targeted, with an oral lethal dose for humans of 50–60 mg (Lauwerys 1990).

Rotenone is a flavonoid isolated from several South American species from the Fabaceae family. It has also been widely used to control crop pests since the seventeenth century. It blocks electron transfer in the mitochondrial respiratory chain. It is less toxic than nicotine in humans: it has a lethal dose ranging from 60 to 1000 mg/kg. However, accidents where NADH oxidase – rotenone's molecular target – was inhibited have been described in humans, and research suggests its involvement in Parkinson's disease (Uversky 2004). This, coupled with high toxicity in cold-blooded animals, led to it being withdrawn from the French market in 2011.

The main active substances in essential oils are monoterpenes, which act on the octopaminergic system. However, vertebrates have very few octopamine receptors, which suggests that essential oils would be harmless to mammals. However, high doses can have neurotoxic, cytotoxic and genotoxic effects and influence oestrogen production (Regnault-Roger et al. 2002).

Regardless, the large-scale use of active ingredients, even if they are of natural origin, increases the risk of undesirable effects and these risks must be clearly assessed (Fig. 14.1). Precautions must be taken when using botanical biopesticides, just as with any synthetic pesticide.

Part V
Semiochemicals and Pest Control

Emmanuelle Jacquin-Joly

The term "chemical ecology" may sound like an oxymoron to a neophyte. But chemistry is natural before it becomes synthetic. Chemical ecology is thus a discipline of ecology that is interested in all interactions between organisms mediated by natural substances that enable them to communicate with each other in their environment. The discipline developed in the late nineteenth century with the observations of French entomologist Jean-Henri Fabre, who relates in *Fabre's Book of Insects* the attraction of hundreds of male giant peacock moths to the smell of a captured female. The discipline earned its name with the 1970 publication of the book *Chemical Ecology*, directed by scientists Ernest Sondheimer and John B. Simeone (1970).

Chemical communication involves an infinite number of different substances called semiochemicals. Semiochemicals are present everywhere, on land as well as the ocean floor, and are used by all living beings, from fungi to bacteria, plants, mammals, birds and insects. Their study combines chemistry, biochemistry, ethology, ecology as well as all the modern "omics".

Deciphering and even mastering this chemical language presents a challenge in finding different biobased applications in many fields, such as agri-food, cosmetics, medicine, ecological engineering, bioconservation and, of course, livestock and crop farming.

The three chapters in this section explore chemical communication in insects, and in particular crop pests, within the context of crop protection. Chapter 15 describes the different types of semiochemicals insects use to communicate with each other and with their environment, as well as the main methods used to identify and study

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these substances and their effects on insects. Chapter 16 outlines current knowledge of the neurobiological mechanisms used by insects to detect and interpret chemical cues and signals. Finally, Chap. 17 identifies current semiochemical-based pest control methods and explores new avenues of research that could eventually expand the range of biocontrol methods.

Chapter 15
Semiochemicals and Communication in Insects

Nicolas Montagné, Jérémy Gévar, and Philippe Lucas

15.1 Introduction

All living beings emit a large number of substances into their environment. When these substances are perceived by other organisms, they constitute chemical cues and signals, aka semiochemicals, which play a key role in the interactions between plants, animals and microorganisms. Although this mode of communication is universal, it is particularly developed in insects, which rely heavily on their sense of taste and smell to detect food sources, sexual partners and predators. The study of chemical communication in insects has long attracted researchers' interest. The extensive knowledge acquired – from the exact nature of semiochemicals to the way insects perceive them – forms the basis of biocontrol solutions for certain pest species, particularly crop insect pests.

15.2 Semiochemicals Regulate Many Insect Behaviours

15.2.1 Intraspecific Signals

Chemical signals used to communicate between individuals of the same species are called pheromones. The first pheromone was identified more than 50 years ago in

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females of the moth *Bombyx mori* (Butenandt et al. 1959), which emit very small amounts of this substance into the air to attract males of their species and initiate copulation. Volatile and long-distance sex pheromones used to attract mates have since been identified in many other moth species, as well as in other insect orders, including cockroaches, aphids and beetles (El-Sayed 2011). There are also non-volatile sex pheromones in insects that are detected via contact between individuals and play an essential role in mating, such as in flies (Ferveur 2005).

Aside from reproduction, pheromonal communication is involved in many other aspects of insect life (Yew and Chung 2015). For example, there are aggregation pheromones, which attract individuals of both sexes to a food site, and alarm pheromones, as in aphids and bees, which warn fellow species members of immediate danger. Social insects are largely able to recognize colony members due to non-volatile compounds present on their cuticle. Ants and termites also secrete trail pheromones, which mark the path to a food source. Finally, maintaining a social hierarchy in a colony also depends on pheromones emitted by the queen, such as the queen mandibular pheromone in honeybees, which induces physiological changes in the worker bees.

15.2.2 Interspecific Signals and Cues

The semiochemicals involved in communication between different species are called allelochemicals. They are notably involved in many interactions between insects and plants. There are three types of allelochemical compounds, depending on the effects induced in the organism emitting and receiving the signal.

When the signal is only advantageous for the emitter, it is called an allomone. For example, many orchids are known to lure pollinating insects by emitting what are known as deceptive pheromones, which mimic the insects' sex pheromones (Brodmann et al. 2009). By attempting to mate with the flower, the insect provides pollination but does not benefit from it. Conversely, when the cue is for the benefit of the receiving organism alone, it is called kairomone. For example, when searching for a host plant for feeding and egg-laying, herbivorous insects rely primarily on the detection of kairomones emitted by plant roots, leaves or flowers (Bruce and Pickett 2011). Signals that benefit both the emitter and the recipient are called synomones. The most common are the odorant molecules emitted by flowers to attract pollinating insects, which allow the insects to feed on nectar while promoting pollination (Raguso 2008). Another example of synomones are the chemical signals that plants release in the event of attack by herbivorous insects and which can then attract natural enemies of these herbivores, such as predatory or parasitic insects (Turlings et al. 1990).

15.3 An Extraordinary Diversity of Semiochemicals

Chemical signals play an important role in the life of insects. These signals are extremely varied in terms of their chemical make-up, whether emitted by animals, plants or microorganisms.

15.3.1 Volatile Substances

Olfaction is classically defined as the perception of semiochemicals at a distance from their source of emission. It is important to note that olfactory signals are almost always perceived by insects as complex mixtures containing a large number of compounds (Bruce and Pickett 2011). The term odour thus does not refer to a single substance, but the mixture that is perceived. Whatever their function (pheromone or allelochemical compound), organic substances with physicochemical characteristics that allow them to be volatilized and carried by the air are referred to generically as volatile organic compounds (VOCs). The most abundant VOCs in the atmosphere are those emitted by plants and microorganisms. VOCs are also emitted in the soil, particularly through the plant root system (Rasmann et al. 2005). VOCs are small compounds that can be biosynthesized via several metabolic pathways, thus making it possible to distinguish three main classes: terpenoids, benzenoids/phenylpropanoids and fatty acid derivatives (Knudsen et al. 2006).

Terpenoids are synthesized from precursors containing five carbon atoms and therefore always contain carbon atoms in multiples of five. This VOC class has the greatest diversity of compounds, and they are often emitted in large quantities by plants. This is especially the case of isoprene, emitted by tree leaves, and certain monoterpenes such as limonene, pinene or linalool, emitted by many flowers and widely used in the cosmetics industry. Benzenoids and phenylpropanoids are also commonly called aromatic compounds because they are synthesized from an aromatic amino acid, phenylalanine. The most common benzenoids are those emitted by flowers, such as benzaldehyde or benzyl acetate (the main constituent in the scent of jasmine). Finally, fatty acid derivatives are carbon chains of variable length and may contain many different functional groups. The leaves of flowering plants emit many small alcohols (so-called green odour), such as hexanol, which is reminiscent of the smell of freshly cut grass.

Like plant odours, volatile pheromones synthesized by insects can belong to any of the above-mentioned VOC families (Yew and Chung 2015). Moth sex pheromones, for example, are derivatives of long-chain fatty acids (usually 12–16 carbon atoms). The size of the chain, the functional groups present, and the number and position of double bonds result in an incredible variety of molecules, allowing for a diverse range of signals between species. In aphids, the most common alarm pheromone is a terpenoid, (E)-β-Farnesene, while in the honey bee the queen mandibular pheromone contains several aromatic compounds.

15.3.2 Non-volatile Substances

Although VOCs have been more widely studied, many of the semiochemicals perceived by insects are not volatile and are detected when their sensory structures come in direct contact with the emission source. These substances are known as tastants, because they provoke a taste sensation. Detection of tastants by the gustatory system allows insects to assess the quality of a food source or an oviposition site, or recognize a sexual partner or fellow colony member. Olfaction and taste often act in synergy in these processes. Many tastants are water-soluble compounds, but the insect gustatory system can also detect non-solubilized molecules such as those present on the leaf surface, which contribute to the choice of host plant in herbivores (Thiéry et al. 2013).

While olfaction allows insects to differentiate between a very large number of scents, taste only offers a more limited discrimination between flavours. These include sweet, salty, bitter, sour and umami flavours, which are common to both insects and mammals (Liman et al. 2014). Perception of saltiness is produced by ions. The main ion is sodium, but other ions such as potassium and chloride also produce the salty taste in insects. Saline solutions at very low concentrations (and therefore with low osmolarity) can be interpreted as water sources for insects. Sugars are the main source of energy for insects in which the perception of mono- and disaccharides, such as glucose, fructose or saccharose, is especially well developed. In particular, flower nectar contains a high concentration of these sugars, which play an important role in attracting pollinating insects. Finally, bitter compounds have very diverse chemical structures. Many secondary metabolites synthesized by plants, such as alkaloids, are perceived as bitter by insects. Among the best known alkaloids are caffeine and quinine.

In addition to the previously described flavours, the insect gustatory system also has the particularity of being able to detect various stimuli such as calcium, carbonation, amino acids and fatty acids (Liman et al. 2014). Being able to detect fatty acids enables them to find energy-rich food sources. Sweet flavours, salts in low concentrations and amino acids are generally palatable substances for insects, while salts in high concentrations and secondary plant metabolites have an antifeedant effect. Thus, host plant selection in herbivorous insects depends on the balance between these two types of compounds (Chapman 2003).

Finally, many insects use non-volatile pheromones that are also detected by the gustatory system. Most often, these are cuticular hydrocarbons derived from fatty acids with very long carbon chains (generally 20–30 carbon atoms), which mainly act as waterproofing agents to prevent insect desiccation. This has been very well described in fruit flies, which use this type of signals as sex pheromones, and thus "taste" their partner when mating (Ferveur 2005). This is also the case in social insects, which often use these substances as a chemical signature that is specific to a colony.

15.4 Methods for Identifying Chemical Signals and Analysing Their Perception

In the early 1950s, Adolf Butenandt studied the “smell of females” of the silkworm moth *Bombyx mori*. With the help of his team, and after several years of research and the dissection of 500,000 females, he identified the very first pheromone in 1959: bombykol (Butenandt et al. 1959). Since that time, analytical chemistry has made great progress and researchers now have many tools at their disposal to make it easier to identify the molecules underlying chemical communication in insects. The main steps involved in identifying a semiochemical relevant to insects are described below and illustrated in Fig. 15.1.

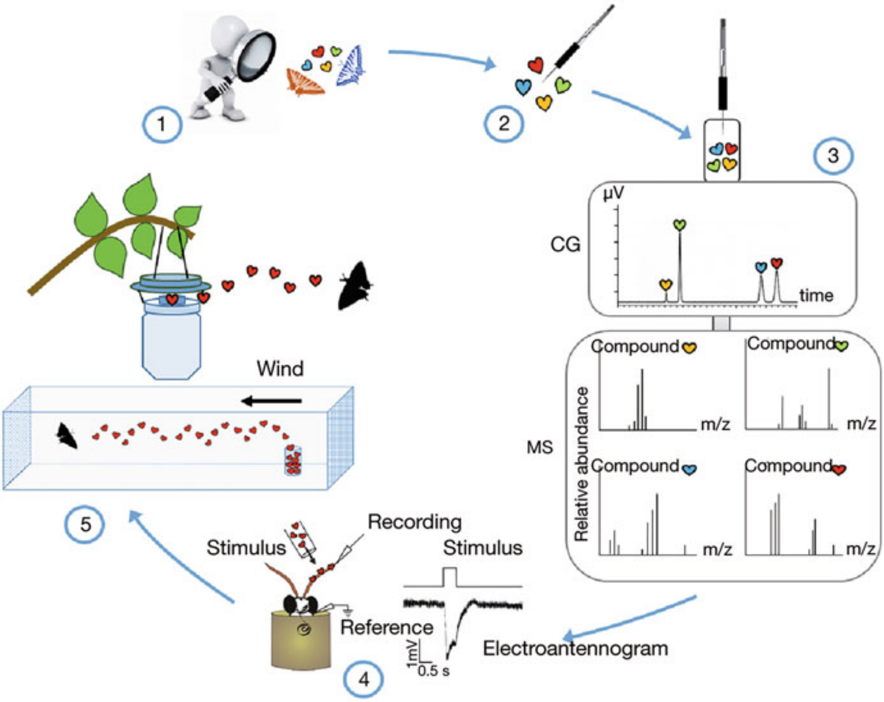

Fig. 15.1 Main methodological approaches required to identify the type and role of semiochemicals in insects. (1) Observation (e.g. courtship behaviour in moths). (2) Collection of semiochemicals (e.g. solid-phase microextraction). Molecules of interest are adsorbed on a fibre. (3) Separation of mixture constituents. Molecules injected into the gas chromatograph (GC) are separated according to their affinity with the stationary phase within the column. They are then ionized by the mass spectrometer (MS). They are identified via the analysis of mass spectra. (4) Study of physiological mechanisms (e.g. electroantennography). This electrophysiology technique is used to identify which compounds are detected by an insect’s antennae. (5) Study of behavioural responses. The effect of the molecules is tested by olfactometry in the laboratory (e.g. wind tunnel) and then in the field (e.g. trapping)

15.4.1 Collecting Chemical Signals

Collecting semiochemicals in the environment is a key step that is rendered complex by their very low concentration in the air, as well as by the very great diversity of the chemical families studied. However, there are very effective sampling methods that can detect trace compounds. The three most commonly used methods are solid-liquid extraction, solid-phase microextraction and stir bar sorptive extraction.

Solid-liquid extraction consists in extracting molecules on the surface or in a solid matrix using a solvent. Extraction can be carried out with a cold solvent (called maceration) or hot solvent (called digestion). This technique has been widely applied to study hydrocarbons on the insect cuticle, as well as to extract pheromones in specific glands.

Solid-phase microextraction (SPME) was invented in 1990 and allows the extraction of solid, liquid and gaseous samples. It involves adsorbing the molecules to be analysed (also called analytes) on a fused silica fibre coated with a polymer. This polymer constitutes a stationary phase which, according to its physicochemical properties and especially its polarity, determines the adsorption capacity. To extract the analytes, the fibre is placed in an odorized air environment (e.g. within a field plot), immersed in a solution or rubbed on a solid. The compounds are then adsorbed by the fibre, with an equilibrium time between the stationary phase covering the fibre and the gas or liquid phase. The compounds then undergo thermal desorption in a gas chromatograph before being analysed.

Stir bar sorptive extraction using a polymer-coated magnetic bar is a method based on the same principle as SPME, but it allows the extraction of a larger quantity of analytes and therefore offers greater sensitivity. The bar can be directly immersed in a solution, making it a tool of choice to extract semiochemicals in a liquid medium. It can also be used to extract VOCs from the air.

15.4.2 Mixture Separation and Determination of Molecular Structure

Various techniques are used to separate the mixture constituents, such as gas chromatography, high-performance liquid chromatography or capillary electrophoresis. These separation techniques are then combined with mass spectrometry, which can determine molecular structure from just ten nanograms of a substance.

Gas chromatography, the most common VOC analysis technique, consists of an injection system that allows for the introduction and volatilization of the sample, and an oven fitted with a column. Today, capillary columns – hollow tubes with a diameter of less than 1 mm – are the most common type. A stationary phase (polymers derived from polydimethylsiloxane) is grafted onto the inner wall of the column, giving it a specific polarity. The molecules in the mixture are pushed by a carrier gas (which is the mobile phase) and move through the column. They exit it one after the other after a certain amount of time (known as the retention time), which depends on the affinity of the stationary phase with the molecules being analysed.

Once the constituents have been separated, they are transferred one by one into the mass spectrometer. The molecules are ionized by an ion source. The most common ionization method used in conjunction with gas chromatography is electron ionization: electrons are emitted in the source and interact with the molecules being analysed, transforming them into radical ions, which are then separated by an analyser according to their mass-to-charge ratio (m/z). The transformation of ions into an electric current, amplified by a detector, generates a mass spectrum. The analytes are identified on the basis of comparison of their spectrum with commercial databases or libraries that list tens of thousands of mass spectra.

15.4.3 Study of the Biological Effect of Semiochemicals

15.4.3.1 Electrophysiology

Before semiochemicals can induce an effect on an insect's behaviour or physiology, they must be detected by sensory neurons located in sensory organs called sensilla (see Chap. 16). Electrophysiology is used to study the types of molecules detected and the underlying physiological mechanisms of the detection process. Electroantennography (EAG) records the overall electrical activity of insects' antennae – their main olfactory organ – in response to olfactory stimuli. The EAG response amplitude is correlated to the number and sensitivity of neurons responding to each of the odorants tested. This technique can quickly establish the olfactory sensitivity profile of an insect species. It can also be used in the field for VOC concentration measurements (van der Pers and Minks 1998).

Single sensillum recording (SSR) consists of measuring only the activity of the few olfactory receptor neurons that innervate an olfactory sensillum. SSR is a more complicated technique, but it offers more detailed results than EAG. For example, it can detect compounds that are biologically important for an insect, but which cannot be detected by EAG due to the low number of neurons sensitive to these compounds in the antenna. EAG and SSR can be combined with gas chromatography: after constituents are separated in the column, they are sent both to the chromatograph's detector and to an insect antenna which acts as a second detector. This coupling thus makes it possible to identify which VOCs within a complex mixture are actually detected by the insect.

Measuring the detection of tastants is similar to SSR on olfactory sensilla, and consists in covering the gustatory sensillum with a recording electrode. This electrode is filled with an electrolyte that conducts the current, and so allows the electrical activity of the gustatory receptor neurons to be recorded. The electrode also contains the stimulus to be tested, which is diffused towards the pore located at its end (see Chap. 16). The electrode thus plays the dual role of stimulating and recording responses to stimuli. Because it is difficult to solubilize lipophilic compounds in the saline solution used as an electrolyte, studies on the detection of these compounds have been limited.

Electrophysiology only determines whether the compounds studied are detected by the insect; it does not indicate whether they will lead to a certain behaviour. This is why this technique is often associated with behavioural studies.

15.4.3.2 Studying Insect Behavioural Responses to Chemical Stimuli

Various experimental devices are used to analyse the effects of odorants on insect behaviour (attraction, repulsion, oviposition stimulation, etc.). Orientation behaviour in response to an odour can be observed using olfactometers, which are devices of various shapes and sizes into which an odorized air flow is introduced. Depending on their size, they can measure how olfactory stimuli modify the insect's locomotor behaviour when walking (small olfactometers) or in flight (wind tunnel for 3D trajectory analysis). These instruments can also force the insect to choose between several odours in order to evaluate its preferences (two-way Y olfactometer, four-way olfactometer, etc.). In these devices, insect movement is limited in space by the olfactometer's size, which ranges from a few centimetres up to about five metres for the largest wind tunnels.

The locomotion compensator, developed on bees and subsequently used on other insects, remedies the issue of limited movement. An insect moves freely on a sphere and its filmed movement is compensated by rotating the sphere to keep the insect on the sphere's apex. The sphere movement is then translated into a 2D trajectory. In a variant of the locomotion compensator, the insect is held in a fixed position by a pin glued to the top of its thorax. The insect can rotate 360° and walk. The movement of its legs when walking causes a polystyrene sphere to rotate, which floats on compressed air and whose rotation is translated into a 2D trajectory. Measurements of locomotor behaviour on a sphere are advantageous in that the insect is constantly kept in the odorized air flow, which allows these devices to be coupled with gas chromatography, as for EAG.

On a larger scale, field trapping tests can be used to validate in natural conditions the conclusions obtained during laboratory experiments, as well as to analyse crop colonization and migration processes.

Several tests are used to evaluate insect gustatory performance. A simple and effective choice test developed for fruit flies consists of offering them two food substrates coloured with tasteless red or blue dye. Since the abdomen of a fruit fly is translucent, the observation of its colour at the end of the test reveals the flies' food preferences. Observation of the proboscis extension in response to gustatory stimuli (which indicates feeding behaviour in bees or butterflies, for example) is a more conclusive technique: the sensilla can be stimulated using tastants linked to this feeding behaviour, which can provide insight on the role of specific gustatory receptor neurons. Palatable compounds such as sugars induce proboscis extension, while the addition of antifeedant compounds reduces the frequency of this response.

15.5 Conclusion

The use of semiochemicals emerged quite early in the story of evolution, and many organisms – from bacteria to humans – rely on them to communicate. This mode of communication is especially vital to insects: they use semiochemicals in feeding, reproduction and social relationships, to name but a few key functions. Generally speaking, insects' possibilities for effective long-distance communication by mechanical and visual means are limited by their size, which may have contributed to their general dependence on chemical senses (Greenfield 2002). However, this mode of communication has definite advantages over other sensory modalities, starting with how specific it is. Insects can emit a wide variety of chemical signals over long distances, day and night, without any environmental barriers aside from wind direction. Although the information conveyed by these signals is limited (presence/absence), one benefit of semiochemicals is that they are more durable than visual or auditory signals. Finally, the metabolic cost of these emissions is low: the extreme sophistication of insect chemosensory systems (see Chap. 16) means that only small quantities are required. The elucidation of the nature and role of semiochemicals is therefore a fundamental issue with regard to their use in biocontrol of insect pests (see Chap. 17).

Chapter 16
Anatomy and Functioning of the Insect Chemosensory System

Philippe Lucas, Nicolas Montagné, and Emmanuelle Jacquin-Joly

16.1 Introduction

As the previous chapter explained, semiochemicals play a major role in insect ecology. Understanding how these extremely varied signals and cues are detected, how they are coded and interpreted by the brain, and how this translates into a given behaviour in an insect are questions that have piqued many researchers' interest. Progress in neuroanatomy, functional imaging, electrophysiology, genetics and bioinformatics techniques has provided a particularly advanced understanding of the anatomy and functioning of the olfactory and gustatory systems in many insects. In some ways, this knowledge is more extensive than our understanding of mammalian chemosensory systems. This chapter provides an overview of this knowledge, enabling readers to understand how insects perceive their chemical environment, from the detection of semiochemicals in the sensory organs to the integration of nervous messages in the brain, and the plasticity of the chemosensory systems according to insects' living conditions.

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16.2 Neurophysiological Organization of Chemosensory Systems

Like any sensory system, the insect chemosensory system is organized as a peripheral system, made up of sensory organs that detect chemical stimuli, and a central system, which processes the neural input produced by these organs to trigger an appropriate behavioural or physiological response.

16.2.1 Chemoreceptor Organs and Sensilla

Insects have an exoskeleton, formed by a rigid cuticle, which acts as a protective barrier against the surrounding environment. Specialized structures called sensilla (Fig. 16.1a, b) are found across the cuticle and allow insects to detect an array of stimuli: olfactory, gustatory and tactile signals as well as variations in temperature or humidity. The sensilla often appear as cuticular expansions and are found all over the body, either separated or grouped together in specialized organs. The chemoreceptor sensilla (olfactory and gustatory) transfer stimuli to the sensory neurons: the olfactory receptor neurons (ORNs) and the gustatory receptor neurons (GRNs). The olfactory sensilla are mainly located on the antennae, as well as in smaller numbers on the maxillary palps of dipteran species (60 sensilla on the palps, compared to more than 400 on the antennae in fruit flies) and on the labial palps and the ovipositor of various species. Although antennae primarily function as an insect's "nose", they also carry structures for detecting taste, vibration, humidity, temperature, and sometimes even sounds, as in mosquitoes. Antennae come in a wide variety of shapes and sizes according to insect orders and life styles (Steinbrecht 1999), and even according to sex. Sexual dimorphism in the antennae is typically observed in moths: males often have larger antennae, sometimes with lateral branches ("pectinate" antennae), and they carry more olfactory sensilla, most of which are sensitive to the sex pheromone emitted by females. This gives males exceptional pheromone response thresholds.

The gustatory sensilla are found in many places on an insect's body: the mouthparts, legs, antennae, wings and genitalia (Vosshall and Stocker 2007), which means that, strictly speaking, insects do not have a single "taste organ". The distribution of gustatory structures reflects the many roles that taste plays in insects. Taste is crucial in feeding, assessing the chemical composition of food and distinguishing food sources from toxic substances, as well as for reproduction, when recognizing a sexual partner or choosing an oviposition site, and for social interactions, such as when social insects recognize a fellow colony member.

Sensilla are classified into different types based on their external morphology and function (Altner and Prillinger 1980; Zacharuk 1985). They range from 5 to 600 μm long and 1 to 5 μm in diameter, which is one-tenth the diameter of a human hair. The

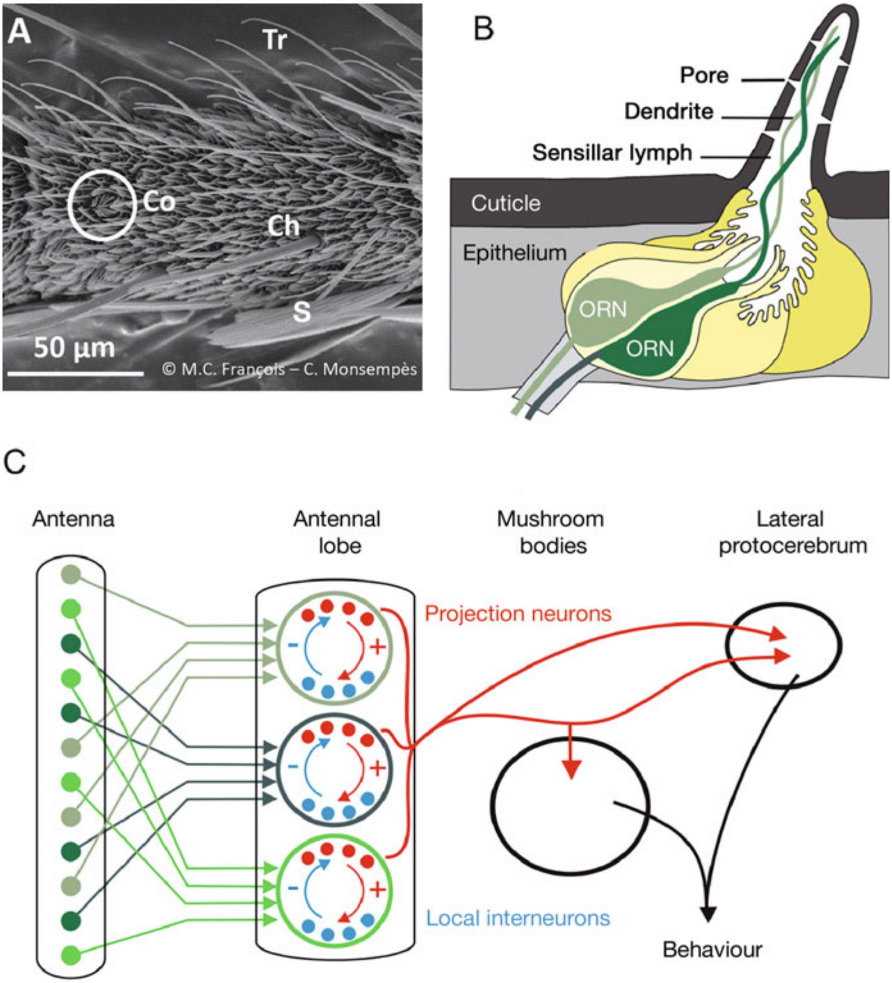

Fig. 16.1 Olfaction: peripheral odour detection and central signal integration. (**a**) Detail of an antenna segment from the Egyptian cotton leafworm (*Spodoptera littoralis*) under a scanning electron microscope. (**b**) Diagram of an olfactory sensillum. (**c**) Diagram of the insect olfactory system

(**a**) The antenna's ventral surface carries a large number of olfactory sensilla: *Tr*: Trichoid sensilla, with olfactory receptor neurons (ORNs) expressing odorant receptors; *Co*: coeloconic sensilla, with ORNs expressing ionotropic receptors. Large so-called chaetic sensilla (*Ch*) are mechanosensory. The dorsal surface is covered with scales (*S*) and has few sensilla. (**b**) The olfactory receptor neurons (*ORN*) are surrounded by three sheath cells (*in yellow*) that surround the sensillar compartment. Odorants enter the sensilla through cuticular pores and interact with odorant receptors located in the dendritic membrane of the ORNs. (**c**) All ORNs (*in green*) of the same type (expressing the same odorant receptor) project towards the same glomerulus in the antennal lobe. At this level, the signal delivered by the ORNs is integrated by a network of local inhibitory interneurons (*in blue*) and excitatory projection neurons (*in red*). The projection neurons project into one or more nerve tracts to the mushroom bodies and to the lateral horns (lateral protocerebrum) olfactory sensilla are characterized by the presence of a very large number of pores on their surface, with a diameter of 10–100 nm, which allow odorant molecules to enter. There are four main types of olfactory sensilla: trichoid (hair-like), basiconic (shorter and larger), coeloconic (small sensilla in the centre of a cuticular invagination) and, in coleopterans and hymenopterans, placoid (plate-like). In moths, the sex pheromone is detected by the ORNs in the long trichoid sensilla, present in very large numbers on the antennae (more than 60,000 in the tobacco hornworm *Manduca sexta*), while non-pheromonal odours, such as volatile plant compounds, are detected by the short trichoid, basiconic and coeloconic sensilla.

The gustatory sensilla have a single pore with a diameter of 0.01–0.2 μm located at their tip. These sensilla often have a basal articulated socket, which reflects their dual taste and mechanosensory functions. They are not as numerous as the olfactory sensilla. For example, there are a total of about 260 in the fruit fly, with 62 on the labium (analogous to our tongue). Males' forelegs have about 50 gustatory sensilla, whereas females have 37. This sexual dimorphism is due to the presence of specialized sensilla in males to ensure the detection of pheromones emitted by females.

At the larval stage, insects also have chemosensory detection capabilities but with limited receptor neurons. For example, an adult fruit fly will have about 1300 ORNs and 300 GRNs on its head, while a larva will have only 21 ORNs and 80 GRNs. The inversion of the number ratio of ORNs to GRNs between these two developmental stages corresponds to the ability of the larvae to move short distances while adults travel long distances.

16.2.2 Chemoreceptor Neurons

Although chemoreceptor sensilla come in many different shapes, their cellular organization remains the same (Fig. 16.1b) (Keil 1999). ORNs and GRNs are bipolar neurons: their dendrite extends into the sensillum and carries the chemoreceptor proteins; their cell body is located at the base of the sensillum; and their axon connects directly to the brain. The cell bodies of chemoreceptor neurons are surrounded by three sheath cells that separate the intra-sensillar compartment and control the ionic and protein composition of the sensillar lymph in which the dendrites of the sensory neurons are bathed.

The olfactory sensilla generally have one to five ORNs, but there can be as many as 50 in migratory locusts and over 100 in social insects. The gustatory sensilla usually have four GRNs and one mechanoreceptor neuron, but fruit flies have sensilla with two GRNs and one mechanoreceptor neuron.

16.2.3 Brain Centres of Chemosensation

The axons of the antennal ORNs form the antennal nerve that projects into the antennal lobe, the primary olfactory centre in insects (Fig. 16.1c). This structure handles the initial processing of information collected by the ORNs. The antennal lobe is a network of neurons composed of the axonal endings of the ORNs, local interneurons and projection neurons. The antennal lobe is organized into glomeruli, spherical zones in which the numerous synaptic contacts between these different neuron populations are concentrated (Su et al. 2009).

All ORNs that have the same odorant receptor protein (see Sect. 16.3.1 "Chemosensory receptors"), and therefore those that are sensitive to the same odorant molecules, project into the same glomerulus: this is called odotopic organization. Thus, in insects there are as many glomeruli as there are types of odorant receptors. Sexual dimorphism is observed in the glomeruli involved in the sex pheromone perception. In moths, the macroglomerular complex, which is comprised of macroglomeruli that are sensitive to different pheromone molecules, is observed in the antennal lobe of males but not females (Hansson et al. 1991).

The ORNs are connected (via synapses) within each glomerulus to projection neurons that integrate the information delivered by the ORNs. The convergence of many ORNs towards few projection neurons (100–1000 ORNs for one projection neuron) means they are highly sensitive with a high signal processing speed. This anatomical organization is the reason why the insect olfactory system is so quick to detect changes in odour concentrations. Projection neurons project from the glomeruli into the mushroom bodies, which are the centre of multi-sensory integration and learning, and to the lateral horns, which are responsible for innate behavioural responses to particular stimuli. Centrifugal neurons, present in small numbers and with only the axon in the antennal lobe, modulate this structure's activity.

The local interneurons are confined to the antennal lobe where they each innervate a large number of glomeruli. They are generally inhibitory and participate in signal integration by reducing the activity of ORNs, thus ensuring gain control of the information that is transmitted to the projection neurons (Olsen and Wilson 2008). However, there are also local excitatory neurons.

The central nervous structures responsible for the integration of taste information are not as well described as those involved in olfactory integration. In keeping with the dispersal of the gustatory sensilla over nearly the entire body of the insect, the GRNs have projections in several areas: the suboesophageal ganglion, the thoracic ganglia and the terminal abdominal ganglion (Stocker 1994). The suboesophageal ganglion nevertheless plays a central role in integrating gustatory information. In fruit flies, it receives afferents from the GRNs of the proboscis, mouthparts and legs via the thoracic ganglia. Despite the lack of glomerular organization of the suboesophageal ganglion, the projections of these GRNs are segregated according to the organ they come from (proboscis, mouthparts, legs), even for GRNs with the same receptor protein (Wang et al. 2004). The projection logic of GRNs (organotopic organization) therefore differs from that of ORNs (odotopic organization). This organization explains how behavioural responses to the same taste stimulus can vary according to the organ stimulated. For example, activation of the GRNs in the legs can induce extension or retraction of the proboscis, and activation of the GRNs in the proboscis leads to ingestion or rejection of food. Additionally, GRNs from the same organ, but which are sensitive to different taste modalities (sweet or bitter), project into different areas of the suboesophageal ganglion (Thorne et al. 2004; Wang et al. 2004). The spatial pattern of activation of this ganglion thus seems to provide the brain with information on the type of taste stimulus detected and where its detection occurs on the insect's body.

16.3 Molecular Mechanisms of Chemosensory Detection

16.3.1 Chemosensory Receptors

The first step in the perception of a chemical signal by an insect is its conversion into an electrical message at the level of the receptor neurons: this phenomenon is known as signal transduction. It is made possible by receptor proteins, located in the membrane of these neurons, that are capable of binding to one or more semiochemicals (Fig. 16.2). The bond between a semiochemical (whether an odorant or a tastant) and its receptor causes channels to open in the membrane; ions then pass through, modifying the potential of the neuron, and the electrical message is created. If this electrical phenomenon, known as the receptor potential, exceeds a certain activation threshold, the receptor neuron's axon then emits signals called action potentials, which are transmitted to the brain. While the olfactory and gustatory receptor neurons of all animals work in this way, the type of membrane receptors involved varies. In insects, they belong to three families of proteins called odorant, gustatory and ionotropic receptors (Fig. 16.2).

16.3.2 Odorant Receptors (ORs)

These are the main receptors for volatile organic compounds in insects (Hallem et al. 2006; Leal 2013). Each ORN generally expresses only one type of odorant receptor, which determines its response spectrum. Insect ORs are proteins with seven transmembrane domains expressed in olfactory receptor neurons. The ORs function through an association with a conserved co-receptor, which is specific to insects. This OR/co-receptor complex forms an ion channel that is activated when the odorant binds to the receptor, which causes cations (positively charged ions) to enter the neuron, allowing signal transduction.

16.3.3 Gustatory Receptors (GRs)

These receptors are expressed in the GRNs and also have seven transmembrane domains. They are involved in detecting sugars and bitter substances, as well as certain non-volatile pheromones detected by contact (Hallem et al. 2006). One

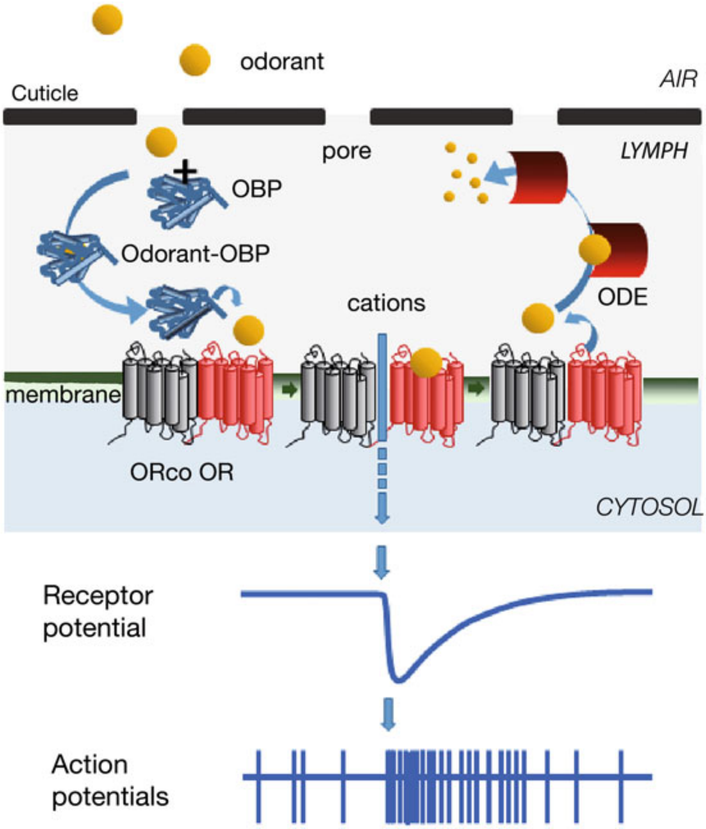

Fig. 16.2 Molecular mechanisms of olfactory detection in insects. The odorant molecules penetrate the olfactory sensillum through the cuticular pores and are taken up by the odorant-binding proteins (*OBP*) to cross the sensillar lymph and reach the membrane of the olfactory receptor neurons. They are recognized in the membrane by the protein complex formed by an odorant receptor (*OR*) and its corresponding co-receptor (odorant receptor co-receptor, *ORco*). This complex forms an ion channel that opens as a result of the interaction with the odorant molecule, allowing cations to enter the neuron, thus modifying the neuron's potential. The chemical message is converted into an electrical message. The response is first amplitude coded (receptor potential) and then frequency coded (action potentials). If the receptor potential exceeds a certain activation threshold, the receptor neuron axon emits action potentials, which are transmitted to the brain. Finally, the odorant molecules are broken down by odorant-degrading enzymes (*ODE*) to complete the response

fundamental difference with ORs is that several GRs are located within the same neuron. For example, in fruit flies, bitter-sensitive GRNs can express up to ten different receptors to detect a wide range of molecules.

16.3.4 Ionotropic Receptors (IRs)

Ionotropic receptors are expressed in both ORNs and GRNs (Rimal and Lee 2018). Like odorant receptors, they function as complexes, with one or more co-receptors. Although their function requires further study, the ionotropic receptors expressed in the ORNs seem to be involved in the detection of volatile organic compounds with an acid or amine functional group, and those expressed in the GRNs in the detection of amino acids, salt and calcium.

16.3.5 Soluble Proteins

Proteins other than receptor proteins also play an important role in chemical signal detection in insects (Leal 2013) (Fig. 16.2). This is especially true with odorant-binding proteins (OBPs), which are secreted in very large quantities in the olfactory sensillar lymph. OBPs solubilize odorants, most of which are hydrophobic and therefore insoluble in an aqueous medium such as the sensillar lymph. They thus facilitate odorant detection by the ORNs and enhance olfactory system sensitivity. The gustatory sensilla also have soluble proteins that belong to another family, the CheB proteins, which are involved in gustatory detection of sex pheromones in fruit flies (Starostina et al. 2009).

In addition to the OBPs, the olfactory sensillar lymph also contains many odorant-degrading enzymes (ODEs) belonging to different enzyme families (Leal 2013). ODEs allow rapid signal inactivation once the odorant is linked to its receiver, which is essential in the olfactory system's response dynamics. Additionally, these enzymes allow the sensillar lymph to be "cleaned" by eliminating foreign molecules that could enter the sensillum and accumulate, thus disrupting the detection of the odorant signal.

16.3.6 Identifying Chemosensory Receptors in Insects

The chemosensory receptors in insects differ from those of mammals: although their function is the same, their evolutionary origin is different. It was only after the first sequencing of an insect genome – that of the fruit fly in the early 2000s – that the first receptors for semiochemicals were described in insects. Since then, advances in sequencing technologies have greatly accelerated the discovery of receptors in many insect species. The sequences of genes that code for chemosensory receptors can now be identified by analysing the genome of a single insect, or more simply by analysing the transcriptome, i.e. the repertoire of genes expressed within an organ, in this case olfactory or gustatory (Montagné et al. 2015). For example, by 2019, the odorant receptors of more than 70 species of butterflies, 50 species of flies and 20 species of ants had been identified.

Regardless of the receptor family considered, their numbers vary considerably between insects (Robertson 2019). Thus, the number of odorant receptors ranges from 10 in lice to more than 350 in some ants. The number of gustatory receptors also varies widely, with up to several hundred in cockroaches or caterpillar crop pests. Finally, the number of ionotropic receptors is often lower, but the recent discovery of nearly 900 IRs in a cockroach shows that their role in chemical communication could be predominant in certain insects. Beyond their number, the structure (and therefore the function) of receptors also differs substantially from one species to another. This is the result of very rapid diversification along insects' evolutionary path and their adaptation to different ecological niches. For example, the chemosensory receptors of bees, mosquitoes and butterflies are very different, and the knowledge gleaned about one insect's receptors is not easily transposable to another species.

16.3.7 Functional Studies of Odorant Receptors

Insects use their many chemosensory receptors to communicate with each other and interpret their chemical environment. Understanding the functions of these receptors is therefore essential to gain insight into their ecology and, in the case of pests, what determines their food choices. However, functional studies to ascertain which semiochemicals are detected by these receptors are still restricted to very few species, and mainly focused on odorant receptors (Montagné et al. 2015). This is due to the difficulty of carrying out functional analyses and to the great diversity of odorants and tastants likely to be detected by insects. Various methods are used to determine the response spectrum of an odorant receptor, the most common being to functionally express the OR in a heterologous system. In this case, the receptor is expressed in vitro in cultured cells or *Xenopus* eggs, or in vivo in fruit fly ORNs. The cells expressing the OR to be tested are stimulated by an odorant and their electrical response is measured using different electrophysiology techniques to determine whether the OR is able to bind to this odorant. These techniques have been applied for example to ORs that detect sex pheromones in moths, cuticular pheromones in an ant, and allelochemicals in the fruit fly, a mosquito and the crop moth pest *Spodoptera littoralis* (de Fouchier et al. 2017).

16.4 Neural Coding of Chemical Signals

Like all sensory neurons, chemoreceptor neurons translate the detection of stimuli into electrical signals. Variations in the frequency of action potentials emitted by these neurons encode the qualitative, intensive and temporal properties of the stimuli they detect (Wilson 2013). This is called peripheral olfactory coding.

Each species has as many functional types of ORNs as there are receptors capable of binding to odorant molecules. An insect with 50 receptors (ORs and IRs combined) therefore theoretically has 50 functional types of ORNs, but can detect and distinguish many more than 50 different odorants. This is because most ORNs detect a variable number of odorants (with the exception of ORNs that are almost exclusively pheromone sensitive), and most odorants are detected by several types of ORNs that have partially overlapping sensitivity profiles. The identity of an odorant molecule is therefore coded not by the activation of a single population of ORNs but by the activation of a specific combination of them (Fig. 16.1c). This combined activation of a few dozen types of ORNs allows an insect to identify hundreds of odorants. The response dynamics depend on the type of ORN and the nature and concentration of the odorant detected. The range of concentrations that can be detected by an ORN – from the sensory threshold to response saturation – covers two to three orders of magnitude, and up to six orders of magnitude for ORNs responsible for sex pheromone detection in male moths. These pheromonal ORNs are the most sensitive ORNs in the animal kingdom. In the silkworm moth *Bombyx mori*, it was estimated that a single pheromone molecule was sufficient to trigger the electrical response of an ORN sensitive to this compound. The ionotropic function (without activation of a second messenger cascade) of insect odorant and ionotropic receptors makes odorant detection faster. For example, the ORN response time observed in fruit flies is 3 ms, which allows a behavioural response within 70 ms of odorant stimulation (Gaudry et al. 2013). This response dynamic allows flying insects to efficiently orient themselves within the intermittent structure of a turbulent odour plume (Vickers et al. 2001).

The classical method of recording the electrical activity of GRNs is to place a glass capillary over the sensillum to serve as both a recording electrode and a stimulation pipette (see Chap. 15). As a result, very little data exists on the activity of GRNs at rest, their response dynamics or possible responses by inhibition of action potential discharge. Overall, GRNs are less sensitive or discriminatory than ORNs. Sensory thresholds are generally lowest for antifeedant molecules – substances that are potentially toxic. Peripheral and central taste coding seems to pass through dedicated neuronal pathways, known as labelled lines, which is consistent with the presence of neurons specialized in the detection of different flavours (e.g. sugars, salts) and with their segregated projections in the suboesophageal ganglion. This has been well documented in fruit flies for the coding of sweet and bitter stimuli (Marella et al. 2006). Whereas the olfactory combinatorial coding allows insects to distinguish among hundreds of different odours, the responses of its gustatory system most often lead it to a binary choice – to feed or not. This does not require an extremely fine level of taste discrimination. However, while GRNs specialize in detecting specific compounds, responses to mixtures of compounds are often lower than the sum of responses to individual compounds (this is known as mixture suppression). Thus, bitter compounds can reduce, or even totally inhibit, a GRN's response to sugars, even if the exact mechanisms are not fully understood (French et al. 2015). The coding of bitter compounds is therefore the result of the combination of activation of GRNs to bitter compounds and inhibition of GRNs to sugars.

16.5 Chemosensory Plasticity

16.5.1 Olfactory Plasticity

As in mammals, the olfactory response in insects can be modulated by their physiological state (age, satiety level, circadian rhythm and mating), their olfactory experience or other stimuli (Gadenne et al. 2016). Age-related olfactory plasticity concerns the detection of fellow species members, hosts, food and oviposition site odours. It can lead to either enhanced detection, synchronous with maturation of the sexual organs (e.g. of sex pheromones by certain species of male moths, of kairomones by female mosquitoes), or the opposite, with a reduced response (e.g. to the aggregation pheromone in desert locusts or to the queen mandibular pheromone by worker bees). Although age-related olfactory plasticity is sometimes observed at the peripheral level, it generally originates in the antennal lobe and is thought to be controlled by hormones (juvenile hormone and ecdysteroids). Nutritional status also modulates olfaction, particularly in haematophagous insects (mosquitoes, kissing bugs) but also in fruit flies and herbivorous caterpillars, and depends on the activation of abdominal mechanoreceptors and factors circulating in the haemolymph that modulate the functioning of the peripheral olfactory system. The circadian rhythm can also modify olfactory responses in insects. The expression of clock genes in the antennae, variations in ORN responses and in the expression of olfactory genes (OBPs, ORs) indicate that this plasticity takes place as early as at the peripheral level. Finally, mating can generate a significant change in olfaction, which is generally transitory. In the black cutworm *Agrotis ipsilon*, this change affects the antennal lobe neurons, but not the ORNs, and is characterized by reduced pheromonal perception associated with an increase in responses to host plant odours (Barrozo et al. 2011).

Modulation of sensitivity to sensory signals through experience is essential for animals to adapt to a changing environment, and helps insects exploit resources more efficiently. Many examples indicate experience-related olfactory plasticity related to olfactory signals from food, oviposition sites or sexual partners. Brief exposure of male *Spodoptera littoralis* moths to sex pheromones results in sensitization after 24 h of the macroglomerular complex neurons and induces better orientation to the pheromone (Anderson et al. 2007). Exposure to non-olfactory signals that have a strong biological significance can have the same effect (insectivorous bat sounds, sweet stimuli). Plasticity, which is linked to olfactory experience or the type of tasks performed by social insects, is generally accompanied by an increase (although sometimes a decrease) in the size of the glomeruli involved in odour processing. This is due to increased synaptic density rather than to adult neurogenesis. This experience-related plasticity has either a peripheral origin, with deregulation of the OR expression at times, or more generally at the antennal lobe level, and could depend on various neuromodulators (biogenic amines, neuropeptides).

16.5.2 Gustatory Plasticity

Like olfaction, taste shows considerable plasticity in insects and is linked to physiological state and experience (Glendinning 2015). In orthopterans, the opening of the sensilla terminal pores is regulated by the secretion of a plug, which limits the entry of tastants and the stimulation of GRNs by palatable compounds, and leads the insect to stop feeding. In fruit flies, dopamine modulates the proboscis extension reflex in response to sugar, but not to bitter compounds or water. This modulation seems to depend on the level of satiety via the activity of a dopaminergic neuron that innervates the suboesophageal ganglion. Variations in the concentration of specific nutrients, such as amino acids and sugars, specifically modulate the response of the GRNs that detect them. The development of caterpillars on a particular plant can induce a food preference. Various mechanisms likely contribute to this preference, such as habituation to repellent compounds, activation of detoxification enzymes, or changes in GRN sensitivity (Bernays and Weiss 1996). For example, tobacco hornworm caterpillars (*Manduca sexta)* are specialists in plants from the Solanaceae family. When raised on nightshades, they develop a very strong preference for nightshade foliage and have GRNs that are much more sensitive to a specific compound of these plants than caterpillars fed on other plants (del Campo and Miles 2003).

16.6 Conclusion

Although insects smell and taste like mammals and the main combinatorial coding principles are the same, the structures involved and the molecular mechanisms at work are different. In contrast to our invaginated olfactory epithelium in the nasal cavity (which means that we can only smell if we breathe in) or the taste buds on our tongue (we can only taste what we ingest), insects' olfactory and taste structures are outwardly directed. These systems are much faster, allowing insects to monitor their olfactory environment in real time. Insects have thousands of olfactory sensilla that act as miniature noses with different detection properties, and their neurons are organized according to a very precise morphological and physiological pattern for each species. Another surprising difference compared to mammals is that insects can taste their food or recognize their fellow species members with their mouthparts, legs, abdomen and antennae. Finally, the molecular components involved are also different (receptors, binding proteins) and their evolutionary origin is completely distinct in insects and mammals.

The insect chemosensory system has inspired neurobiology and the design of odour-tracking robots, and as we will see in the next chapter, its functioning can be manipulated to develop biocontrol solutions.

Chapter 17
Semiochemicals and Insect Control

Sylvia Anton and Emmanuelle Jacquin-Joly

17.1 Introduction

The importance of semiochemicals in inducing insect behaviour has driven the study of these substances for more than 50 years with the aim of controlling harmful insects (disease vectors, pests of agricultural and horticultural crops, forests, and stored products). Pheromones are currently the most widely used semiochemicals due to their effectiveness (active at very low doses), specificity (these signals are species specific, which helps protect beneficial insects) and lack of toxicity (Witzgall et al. 2010). As a result, several hundred pheromones have now been identified and are used for monitoring or controlling pest populations (see El-Sayed 2011b for a large inventory). They can sometimes be combined with other semiochemicals such as kairomones to reinforce expected behaviours. Different combinations of semiochemicals are possible, always based on the aim of disrupting natural behaviour.

Research into semiochemicals for biological pest control is being spurred on today by many phenomena, such as global change, which has led to many invasive or newly adapted species; the rise in international trade, a source of introduced species; and the development of resistance to chemical insecticides and the general desire to reduce their use in farming to protect biodiversity and limit environmental pollution. This chapter first describes the different ways semiochemicals are currently being used and then explores new avenues of development driven by research.

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17.2 Different Uses of Semiochemicals for Insect Control

17.2.1 Insect Pest Population Monitoring

This method is used in many countries as a decision support tool, and is proving to be very effective in monitoring the arrival and subsequent population density of lepidopterans, coleopterans and dipterans. This approach mainly uses sex and aggregation pheromones. It consists in artificially reproducing the natural attractant mixture, which is prepared with synthetic molecules and diffused from a trap (Fig. 17.1a). Individuals of the target species (only one of the sexes in the case of a sex pheromone, both in the case of an aggregation pheromone) will be attracted by this lure and trapped; the trapped individuals are then counted to assess the infestation rate. If this rate remains below an economically acceptable threshold, there is no need to apply insecticides, and preventive or unnecessary treatments can be avoided.

The overall amount of insecticides can thus be drastically reduced since they are only needed to be applied when and where the pest appears, or when the population

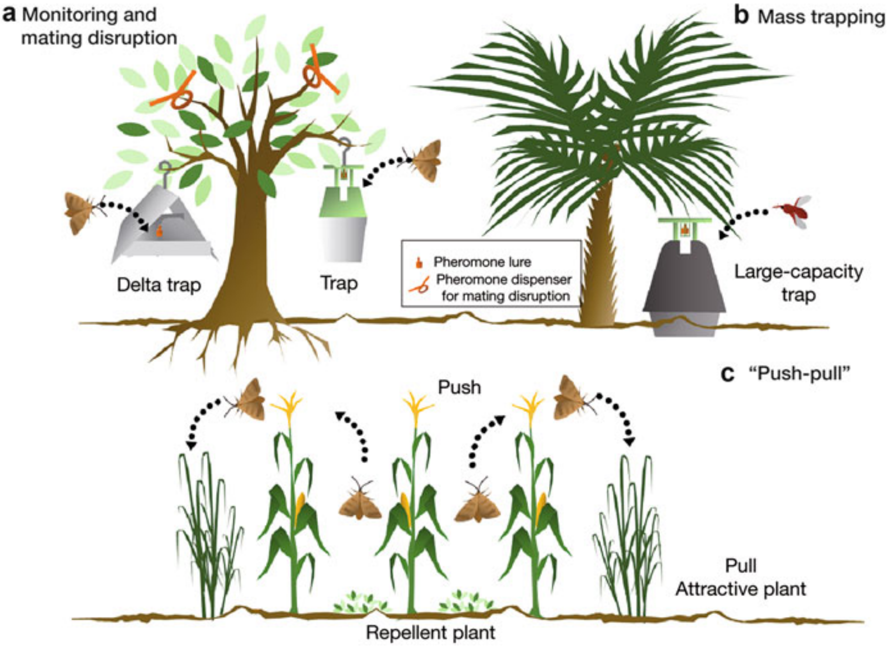

Fig. 17.1 Illustration of the main methods of using semiochemicals for plant protection. (**a**) Monitoring. Different types of traps can be used to attract insects (e.g. using synthetic sex pheromones) and estimate infestation rates. For mating disruption, dispensers are placed throughout the orchard or vineyard to saturate the air with sex pheromone: the natural signal is scrambled. (**b**) Mass trapping. The attraction signal is combined with a large-capacity trap. (**c**) Push-pull. For this strategy, companion plants that repel insects are grown between crop rows (here, maize) to push them away and attractive plants are grown outside the plot to pull insects outside the crop

rises significantly (McNeil 1991; Witzgall et al. 2010). This approach can be combined with biological control methods, such as the use of natural enemies and pathogenic microorganisms: monitoring populations with pheromones helps to ensure the effective use of different control methods with the aim of only treating them when necessary (Witzgall et al. 2010).

Monitoring populations with pheromones is particularly effective for species that are difficult to monitor using other methods because they are very small or lead a hidden life. Known examples include the monitoring of populations of saddle gall midge *Haplodiplosis marginata* (Rowley et al. 2017), or the tomato leafminer *Tuta absoluta* (whose caterpillar attacks the leaves and fruits of nightshades) for which pheromones are used both for population monitoring and mass trapping.

17.2.2 Mass Trapping and the Attract-and-Kill Approach

Based on the same principle, a large proportion of a pest population can be trapped to reduce their numbers. Lures are combined with large-capacity traps (Fig. 17.1b). These traps may contain insecticide products or natural entomopathogenic agents (fungi, viruses, etc.): this is the attract-and-kill strategy. Insects can be attracted either by a synthetic pheromone (sex or aggregation pheromone), by kairomones (e.g. food odours), or by a combination of the two that can act synergistically. In addition to the odours used, the trap shape and colour are optimized for maximum attractiveness and trapping.

The attract-and-kill strategy was successfully implemented against the Mediterranean fruit fly *Ceratitis capitata* in citrus orchards (Alonso-Muñoz and Garcia-Marí 2013). Mass trapping is used to control many beetles (such as the coffee berry borer beetle and various bark beetles in North America and Asia). In China, the bark beetle *Ips duplicatus* has been effectively controlled through pheromone mass trapping, with a substantial reduction in their populations in a forest monitored over a 20-year period. The number of dead trees dropped from an average of about 550 per year to around 100 (Schlyter et al. 2001). Mass trapping has also been used to combat the red palm weevil (*Rhynchophorus ferrugineus*), which has devastated palm trees in Asia, the Middle East, Africa and the whole Mediterranean area, including southern France. Using an aggregation pheromone combined with kairomones from the host plants (Al-Dosary et al. 2016), nearly 20,000 palm weevils were trapped between April and October 2018 around the Antibes/Sophia-Antipolis metropolitan area. These methods are especially useful when insecticides are ineffective (most of the beetles mentioned above live sheltered in trunks or fruit), but unfortunately, they do not always have the expected impact. For example, although used in combination with fungi and entomopathogenic nematodes, trapping was unable to save all the palm trees in the Côte d’Azur.

17.2.3 Mating Disruption

This is the most widely used method using semiochemicals. Large quantities of sex pheromone are released into the environment, disorienting the receiving individuals, which can no longer track the individuals emitting the pheromone (Fig. 17.1a). Because sexual communication is severely disrupted, reproduction becomes more difficult and rare.

Mating disruption has been used for several decades to protect vineyards and orchards against lepidopteran pests such as grapevine moths (*Lobesia botrana*, *Eupoecilia ambiguella*) or the codling moth *Cydia pomonella*. It is of interest for crops with high added value because of the cost and the time needed to set up the dispensers (about 500 dispensers per hectare of vineyard). This method is especially effective if it is implemented collectively: it requires consultation and agreement from all farmers in the area to be protected, as well as the collaboration of various stakeholders (researchers, associations, farmers, etc.). Other factors must also be taken into consideration: the terrain must be suitable, the regional climate and geography must be favourable, the costs of synthesizing pheromones must not be too high and the dispensers must be effective. In all of France, only 30,000 ha of vineyards (4% of the total vineyard area) use mating disruption for pest control, but the Trentino region in northern Italy, where 22,100 ha of orchards and 10,450 ha of vineyards are effectively treated with mating disruption to replace insecticides, is a good example of success (Ioratti and Lucchi 2016). Another large-scale example is the Slow the Spread (STS) programme in the United States against the invasive gypsy moth *Lymantria dispar*, which causes defoliation in oak. Mating disruption has been used to treat more than three million ha of forests through this programme (Lance et al. 2016).

In the long term, this approach has been proven to reduce pest populations, both directly and indirectly; as an environmentally friendly technique, it also protects pests' natural enemies.

17.2.4 The Push-Pull Strategy with Companion Plants

The push-pull technique combines repellent substances or companion plants that push pests away from crops and pull them towards attractive substances or companion plants, possibly combined with traps placed at field borders to eliminate pests (Fig. 17.1c). A companion plant is a plant that is not grown to be harvested but rather to provide a particular service (here, attracting or repelling an insect). An important factor in the choice of companion plants is the selection of attractive varieties for the pull function. For example, a variety of maize has been identified as specifically attracting aphid vectors of potato virus Y, and is recommended as a field margin plant (Schröder et al. 2015). These methods are very effective in small cropping systems. They are widely used in market gardening in sub-Saharan Africa; for instance, stemborers are controlled by intercropping maize with repellent legumes of the genus *Desmodium* and planting elephant grass *Pennisetum purpureum* at the field margin. Elephant grass attracts and traps insects with a sticky substance (Pickett et al. 2014). Considerable efforts are being made to use this technique more extensively, and in combination with other biological control methods (Gebreziher 2020). On a larger scale, a push-pull technique using verbenone (a compound produced by non-host trees) as a repellent and aggregation pheromone of the bark beetle as an attractant is being used to protect Canadian pine forests (Lindgren and Borden 1993). In Europe, the push-pull system has been developed to control pests such as the cabbage root fly *Delia radicum*, whose larvae damage the roots and are therefore difficult to control with conventional insecticide treatments. In this particular case, individual compounds are used: dimethyl disulphide as a repellent and *cis*-3-hexenyl acetate, alone or combined with Chinese cabbage, as an attractant associated with a trap. To date, this method has not yet been used commercially to control this fly (Lamy et al. 2018).

17.2.5 Olfactory "Resistant" Cultivars

Different varieties of the same crop species produce bouquets of different volatile compounds and therefore vary in their attractiveness to insect pests. One way to reduce damage is to use less attractive varieties: these can be found among existing varieties or can be obtained by selection or even genetic transformation of the volatile emission biosynthesis pathways. Different varieties of rapeseed, for example, are more or less attractive to the cabbage root fly *Delia radicum* depending on their emission rates of β-caryophyllene and humulene (Kergunteuil et al. 2015). A variety of wheat that was genetically modified to emit the aphid alarm pheromone β-farnesene has been shown to repel several aphid species and attract the aphid parasitoid *Aphidius ervi* (Bruce et al. 2015).

17.3 Future Prospects

17.3.1 Better Exploiting Plant-Insect and Plant-Plant Communication

Kairomones, which were previously mentioned with regard to their ability to attract natural enemies, are also involved in attracting insects to plants. This offers as yet unexploited prospects for trapping insects. Although research is active in this field, it is complicated by the chemical diversity and the abundance of potentially effective signals. Since kairomones are often complex mixtures, artificially reproducing them can be challenging. In the case of the specialist broad bean weevil *Bruchus* *rufimanus*, certain compounds emitted by the host plant have been identified as attractive to adults. Following these observations, a highly effective odor mixture was developed and patented to trap the weevils after a two-year field experiment (patent from INRAE, Terres Inovia and Arvalis-Institut du vegetal). Attractants for mated European corn borers (*Ostrinia nubilalis*) are being studied based on the same principle. The findings have yet to be applied to tangible crop protection solutions.

When a pest attacks a plant, the plant's defence system is activated and generates or increases the emission of specific compounds. These substances are perceived by nearby plants as well as insect pests and their natural enemies, and can help control the infestation. Plants that perceive signals from another plant under attack can activate their defence mechanisms before being attacked themselves (Baldwin 2010). The emitted substances tell herbivorous females that a plant is already "occupied" and to go somewhere else to lay their eggs, while also informing the pest's natural enemies where they might find their host/prey. For example, parasitoids and predators of the cabbage root fly *Delia radicum* are attracted by compounds that are produced when the larvae attack the plant's roots (Goubert et al. 2013). The signals emitted by the plants depend on the type of attack and give precise information to the insects concerned. Thus, when a pest lays its eggs on a plant's leaves, this will stimulate the production of substances that attract egg parasitoids, while an attack by herbivorous larvae will activate the production of other substances that are attractive to larval parasitoids (Hilker and Fatouros 2015).

New pest control methods are being studied based on this knowledge. For example, the use of defence stimulators such as jasmonic or salicylic acid is being considered to artificially increase plant production of volatile compounds that signal an attack by herbivores and repel them. Apple trees treated with a homologue of salicylic acid emit α-farnesene, which repels the rosy apple aphid *Dysaphis plantaginea* (Warneys et al. 2018). Effective solutions can be variety-based: different rice varieties treated with jasmonic acid produce compounds that are more or less attractive to parasitoids of the brown planthopper *Nilaparvata lugens* (Lou et al. 2006).

Finally, recent discoveries on plant sensitivity to olfactory cues open up an unexplored field of research. Although the mechanisms are still poorly understood, it is clear that plants perceive and respond to olfactory stimuli. Thus, goldenrod *Solidago altissima* produces a defensive response in the presence of *E,S*-conophthorin, an odour emitted by the goldenrod gall fly *Eurosta solidaginis* (Helms et al. 2017).

17.3.2 Microorganisms and Olfaction

Herbivorous insects are interested in more than just plant odours. Studying the relationships between insects and microorganisms creates new prospects for biological control. While it has long been known that fruit flies feed on yeasts found on fermenting fruit, it has recently been discovered that such yeasts are an important diet component of other herbivorous species, such as codling moth caterpillars (*Cydia pomonella*): the odours emitted by these yeasts attract females and stimulate egg-laying (Witzgall et al. 2012). The tritrophic relationships among insects, plants and microorganisms (yeasts, fungi and bacteria) are much more widespread than initially thought. By delving deeper into these relationships, researchers will be able to better define the chemical signatures used by pests and to develop new attractants and even repellents.

17.3.3 Reverse Chemical Ecology: Understanding the Molecular Mechanisms of Olfactory Detection

Today, understanding the molecular mechanisms of insect olfaction opens up promising avenues to optimize and develop new semiochemical-based biological control methods. Chapter 16 describes these mechanisms, which involve different types of proteins, such as odorant-binding proteins (OBPs) and odorant receptors (ORs). OBPs solubilize and transport molecular signals before they are recognized by the ORs.

The development of high-throughput RNA/DNA sequencing methods has produced a flood of transcriptomic and genomic data on insect pests, for which an inventory of OBPs and ORs has been established (Montagné et al. 2015). Once expressed in a host organism (e.g. cultured cells), researchers can study the function of these proteins – i.e. identify the odorants that activate them – using in vitro screening (de Fouchier et al. 2017).

Such screening is much more efficient than behavioural screening, which requires live insects in good physiological condition. However, behavioural studies still serve an important purpose – if an odorant activates an OR, its behavioural effect (attractive? repellent? neutral?) must be identified – but they are limited to odorants that are known to be actually perceived by the insect. Thus, the discovery of the odorants recognized by the ORs of the Egyptian cotton leafworm *Spodoptera littoralis* caterpillar guided the study of larval behaviour, and researchers identified several new attractive compounds (de Fouchier et al. 2018). This chemical ecology is said to be “reversed”, since the proteins themselves are the starting point. Researchers can study all the ORs of a species (about 70 ORs are expressed in noctuid antennae, for example) or focus on specific ORs involved in key insect behaviours, such as sex pheromone receptors (Bastin-Héline et al. 2019).

Knowledge of these proteins can be used in modelling approaches to predict new receptor-ligand interactions and speed up the discovery of new ligands, which may be more active than the natural ligand or block the receptor response. When tested experimentally, modelling sometimes proves to be remarkably effective (30–90% of the predicted substances were found to be active on fruit fly ORs; see Boyles et al. 2013). Research on the CO_2 receptor in *Aedes aegypti* mosquitoes – a signal that determines host attraction – has led to identification of new attractants and repellents for this mosquito (Tauxe et al. 2013).

Such compounds are promising because they may be easier to synthesize and are therefore cheaper, more stable over time, more effective than traditional semiochemicals, and of course are selected from a range of natural, non-toxic substances. They can also be insect specific, since insect ORs have no homologues among other living organisms (see Chap. 16). Moreover, each insect order has a repertoire of ORs specific to its ecological niche: insect ORs have an average protein sequence identity of only 20–30%. For example, bees do not share any ORs with noctuids or mosquitoes. It is thus possible to target the ORs of one species while protecting beneficial insects as well as overall biodiversity. While the practical application of ORs still requires research and field tests, the coming years should bring about innovations in this area.

Finally, these olfactory proteins could be used to develop a new generation of artificial noses: they are grafted onto an electronic interface and emit a signal when they come into contact with their ligands. Such biosensors may prove to be extremely sensitive and thus allow early detection of pests, even when hidden. Early detection of a species is crucial, because it is the first step towards effective control, as indicated above in the Sect. 17.2.1 “Insect pest population monitoring”.

17.4 Conclusion

Some 20 million semiochemical dispensers are used worldwide every year. To make these approaches more effective, we must have a good understanding of the biology of the target species (identification of its pheromone(s), kairomone(s), life cycle, circadian rhythm, sexual maturation, function of its odorant receptors, etc.), as well as the local climate conditions that can influence compound diffusion (wind, humidity, temperature). Similarly, research in chemistry, fluid mechanics and materials physics can help reduce the costs of synthesizing useful compounds and improve dispenser characteristics by making them more durable or stabilizing their emissions. Another important area of research is the monitoring of the development of resistance to semiochemical-based control methods. Monitoring of moth populations exposed to high doses of sex pheromone has shown that rare phenotypes of females producing unusual pheromone compound ratios may increase, or that males may respond to blends of unusual compounds, indicating adaptation to treatment (Evenden and Haynes 2001; Tabata et al. 2007). Phenomena such as the selection of such “resistant” individuals, the appearance of mutations that modify communication channels, and the rapid evolution of odorant receptors must not be neglected if semiochemicals are to be used sustainably.

Part VI
Conditions for Successful Biocontrol and Its Large-Scale Deployment

Thibaut Malausa

Chapters 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, and 17 provide an overview of research related to extended biocontrol, from current and promising techniques to their application possibilities. However, while some appealing methods seem to meet the needs of agriculture, they may not always be used in the field. Chapters 18, 19, 20, and 21 delve into the challenges associated with deploying biocontrol methods, and more specifically, their successful use by many stakeholders over large areas. In this part of the book, we will explore several aspects of deployment: the barriers to moving from the lab to the field, factors and technologies that can facilitate implementation at different scales, diffusion of innovations in the value chain, etc. We will also attempt to tackle the challenge of deployment for extended biocontrol, including all types of biocontrol products, methods and strategies (hereafter called “methods” for simplicity’s sake) that rely on the beneficial action of microorganisms, macroorganisms, semiochemicals and animal-, plant- and mineral-based substances. Some of these methods use organisms and substances as inputs (sometimes sold as commercial products) or revolve around practices that support the positive impacts of organisms that live on crops (typically known as conservation biological control).

Chapter 18 outlines the deployment challenges and positions the different categories of extended biocontrol described in the previous chapters in relation to this challenge. Chapters 19, 20, and 21 then focus on three main sets of factors influencing biocontrol deployment we consider especially important, but which receive less attention than the biological and ecological aspects seen in the previous chapters. Chapter 19 focuses on socio-economic factors, while Chap. 20 looks at the importance of designing cropping systems suited to different biocontrol methods. Chapter 21 examines three key types of technological challenges: the use of

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biocontrol on seeds and seeds as a biocontrol vector; formulation technologies used to develop biocontrol organisms and substances; and the expected contribution of robotics (and agricultural equipment in general) and digital technologies in biocontrol deployment.

The aim is to detail the difficulties involved in biocontrol deployment and explain why the pluri- and interdisciplinary research efforts behind it are as challenging – if not more so – as developing the methods themselves.

Chapter 18
The Challenge of Biocontrol Deployment

Cédric Bertrand, Thibaut Malausa, and Philippe C. Nicot

18.1 Introduction

The level of operational deployment of biocontrol methods has increased in recent years, but remains low overall. Biocontrol products still account for a very modest share of the plant protection market – below 5% globally – with substantial variations between countries and continents (Cock et al. 2009; van Lenteren 2012). The ecosystem services provided by beneficial organisms (conservation biological control, see Chaps. 6, 7, and 8) display a considerably positive impact on agriculture, but this impact is rarely quantified and the practices that support it are seldom identified and replicated at large scale. However, there are major differences between cropping systems. For example, protected cropping systems (greenhouses) make much greater use of biocontrol. Biocontrol has become standard practice in these systems, along with preventive methods (e.g. climate control to limit the risk of epidemics, resistant varieties and pollination by installing bumblebee hives, etc.). Peppers grown in greenhouses in Spain are an emblematic example of this generalized deployment. After some synthetic chemical insecticides were banned, agricultural stakeholders successfully implemented integrated pest management programmes using mites and predatory insects in just 2–3 years (Calvo et al. 2012). In unprotected (open field) fruit and vegetable cropping systems, a significant number of biocontrol methods have also become more widespread, such as baculoviruses against Lepidoptera (Beas-Catena et al. 2014; Haase et al. 2015). Biocontrol deployment is least

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advanced in field crops, although there are rare exceptions, such as the use in France of oophagous parasitoid wasps of the genus *Trichogramma* against European corn borer (*Ostrinia nubilalis*), ferric phosphate against slugs and sulphur against aerial diseases in many plant species. While these last two examples fall within the French definition of biocontrol, they fall outside our view of extended biocontrol and do not fit the International Organisation for Biological and Integrated Control (IOBC) definition commonly used internationally. In South America, other examples of success in field crops include the parasitoid micro-wasps *Cotesia* sp. and *Trichogramma* sp. against stem-boring Lepidoptera (especially in sugar cane), and the beneficial fungi of the genus *Trichoderma* against root diseases in various open field crops (van Lenteren et al. 2018).

Deployment is a complex challenge because it covers several scales (field, landscape, geographical regions), mobilises various levers (new technologies, cropping system redesign, public policies, etc.) and involves many stakeholders (from farmers to consumers), and because each type of biocontrol (organisms, substances) comes with its own set of challenges. To describe this challenge, we will break down the complexity and discuss the factors of success or failure in using biocontrol at each possible scale and for each type of biocontrol.

18.2 Field-Scale Biocontrol Deployment: Success Factors

The challenge of deploying biocontrol can first be seen at field level. Successful deployment at this scale requires mastery of biocontrol, from precisely identifying organisms and substances and their modes of action to understanding field application techniques.

Although there are commonalities between the different biocontrol methods, we will consider three categories of extended biocontrol here:

- organisms (macro- and microorganisms) used as inputs;
- management of pest control services provided by native and introduced organisms;
- natural substances (including semiochemicals, although they may have a distinct regulatory status in certain countries).

18.2.1 Introduced Macroorganisms and Microorganisms

With introduced organisms, the main modes of action are predation (including parasitism) and, to a lesser extent, competition. A special case concerns autocidal control (sterile insect technique, see Chap. 5). We will not go back into detail on the modes of action and the main related research questions, since they are covered in Chaps. 2, 3, 4, 5 (macroorganisms) and 12 (microorganisms). The factors of success and failure in the deployment of organisms have been relatively well identified. Current research and innovation activities are focused on the relative importance of these factors and how to develop practical applications from their identification.

A first key deployment objective for biocontrol organisms is to maintain them in the field for a long enough time to ensure the target pests and diseases are adequately managed. One group of the success factors are those that influence an introduced population's ability to persist in the agricultural system. These are closely intertwined with a second group of factors, the agricultural system's characteristics that influence the population's persistence. These two groups of factors cover two broad areas of research and innovation. The first includes the characteristics of biocontrol agents likely to impact population dynamics once introduced (phenotypic traits that increase the selective value depending on climate conditions, the presence of other organisms, and the target pest's characteristics) and population mechanisms (governing population dynamics and genetics). For macroorganisms, these characteristics are discussed in detail in Fauvergue et al. (2012) and in Chap. 2. The second group of factors refers to the design of sustainable systems that help natural enemy populations establish – i.e. providing them with resources and habitats that maximize their selective value or at least their temporary survival. For macroorganisms, certain aspects of this are discussed in Chap. 6. For microorganisms, the main determining elements (described in Chap. 11) include abiotic environmental conditions (microclimate at the level of plant tissue or soil, concentrations of nutrients or compounds, volatile or not, with varying degrees of antagonistic or stimulating effects), and very likely an essential role of the native microbiota into which the biocontrol agent must be established, all under the influence of the crop genotype.

The second key objective is to ensure the economic feasibility of the introduction. Many biocontrol agents have been identified and the pest control service they provide has been demonstrated at least on a small scale. However, this does not mean these organisms are available or affordable for most farmers. Cost effectiveness is an especially important deployment factor when it comes to biocontrol agents. The crop protection budget for a crop and the level of biocontrol use are directly related. In greenhouse systems, budgets often exceed €5000/ha. In these systems, growers combine numerous macroorganisms to control most pests. Meanwhile, in field crops, where plant protection budgets are about 90% lower (although there are wide disparities among sectors), biological control agents are rarely used. In France for example, the only macroorganism used on more than 100,000 hectares is *Trichogramma brassicae* wasps on maize, most likely because the per-hectare product cost (between €30 and €50/ha) is compatible with the economic constraints of certain production systems.

The final cost to farmers is influenced by many factors, each relating to one step in the biocontrol agent supply chain. Producing biocontrol agents is often expensive. Biocontrol macroorganisms are most often predators or parasitoids that require the production of three trophic levels: a plant resource or nutrient medium for a phytophagous insect, the phytophagous insect itself, and the biocontrol agent feeding on the phytophagous insect. Since the biocontrol markets and companies (or subsidiaries) are small, the innovation sector of macroorganism biocontrol has so far failed to invest heavily in making mass production processes efficient. The pathways (rearing automation, design of nutrient media and feeding systems that do not require one or both of the lowest trophic levels) are generally known (Morales-Ramos et al. 2013) and have been for several decades, but breakthrough innovations are scarce, with most research and development projects leading to modest improvements compared to the issues at stake. However, the complexity of the problems to be solved should not be underestimated. Rearing live macroorganisms remains a challenge because there are many population mechanisms to be managed, organisms are susceptible to pathogens, and rearing methods do not necessarily favour the characteristics of organisms that are important for effective biocontrol in the field (see also Chap. 5). The characteristics that are both desirable for field effectiveness and measurable in the laboratory are not well understood. The links between genotype, phenotype in the laboratory, phenotype in the field and effectiveness in the field are a vast and complex field of research that is still emerging. Technical improvements in insect rearing are therefore sometimes based on vague or irrelevant objectives, and are particularly difficult to develop. It should also be noted that some biocontrol agents have never been successfully produced in controlled environments. For example, the micro-wasp *Torymus sinensis*, used to manage oriental chestnut gall wasp populations in western Europe (Borowiec et al. 2018; Ferracini et al. 2019), has yet to be produced in the laboratory. Individuals wintering in areas with high species density had to be collected and then released in target areas. With microorganisms, the situation is qualitatively similar (production cost remains a key criteria for commercial success), but mass production seems to be better controlled, and enthusiasm for these products, which can be used in the field in a more similar way to chemical substances than macroorganisms, has generated greater investment, infrastructure development and advances in production technologies.

Once the biocontrol agents are produced, the other challenge is to get them to the field. Several factors come into play:

- the ability to store many organisms following production or near the field before they are used by farmers;
- the ability to transport them without negatively affecting the characteristics that are key to their success in the field;
- the ability to introduce biocontrol agents when their mode of action will give optimal control of the target pests;
- the availability of suitable agricultural means for efficacious and cost-effective field application (labour, equipment costs and use).

Macroorganism and microorganism applications differ considerably in these respects. Most macroorganisms cannot be stored for long periods of time, withstand transport or the related temperature fluctuations, or be applied in the field with equipment similar to that currently used for conventional pesticides. However, decision support tools are now starting to be used for macroorganisms to better position releases according to the target phenology (see the example of

Trichogramma wasps below). With microorganisms, the situation can be the opposite. Although keeping them alive and fit is also essential, their long-term storage and transport are easier, with formulations (e.g. wettable powders, liquids; see Chap. 21) that are often similar to those of chemical pesticides. However, they are generally invisible to the naked eye, which makes it very difficult to monitor their establishment and performance in a crop, and therefore to define the population thresholds for effective protection in the field. At best, only the dose (the number of living cells per square metre) applied to the crop is known. This also makes it hard to assess in the field the impact of crop management practices or fluctuations in environmental conditions on these microbial populations, and the consequences on their protective effectiveness. These questions generate important, specific research needs. For example, tools must be developed that are quick and easy to use in the field to quantify the microorganisms of interest on the plant or in the soil, as well as models to predict their fluctuations according to the main factors. A key issue here is the creation of decision support tools to enable farmers to optimize the use of microbial biocontrol agents. The need to design agricultural equipment specifically adapted to applying microbial agents may not seem as pressing as macroorganisms, because microorganism formulations are sprayed in the field with methods used for conventional chemicals. However, certain specificities must be considered with regard to microorganisms' biological properties (e.g. the impact that sprayers may have on their survival or fitness) and their formulations (see also Chap. 21).

The case of *Trichogramma* wasps, one of the few macroorganisms widely used in field crops, offers a good illustration of the factors outlined above. *Trichogramma* can be produced on an alternative host (which can also be used to produce other biological control agents to achieve economies of scale): the Mediterranean flour moth *Ephestia kuhniella* or the grain moth *Sitotroga cerealella*. These species can be produced on a nutrient medium, without using living plant matter. The commercial prospects for *Trichogramma* have led R&D stakeholders to invest in relatively sophisticated production facilities compared to other macroorganisms. In addition, diapause can be induced in *Trichogramma* at low temperatures, so the wasps can be stored for several months before being used in spring and summer. They are relatively easy to transport as they are shipped as eggs from parasitized alternative hosts that are relatively resistant to stress. The phenology of the target pest – the European corn borer *Ostrinia nubilalis* – is relatively well known, and expertise provided to assist the temporal positioning of releases has reached a very satisfactory level, although it could still be improved. *Trichogramma* therefore boast several success factors, which is likely why they are widely used. The only drawback at present is the very limited availability of field application equipment: most applications are carried out manually, making use on large farms unrealistic. Technological developments (drones and other aircraft, quads, dispensers positioned on conventional sprayers) are under way, but their routine use still poses challenges for the emerging biocontrol industry: Who should finance this equipment and its upkeep? Who uses it? What training do users need? Is it necessary to develop specialized equipment fleets for biocontrol?

18.2.2 Management of Pest Regulation Services Provided by Native and Introduced Macro- and Microorganisms

As we saw in Chaps. 4 and 7, there are a growing number of examples of cropping techniques or resources being added (flower mixes, sugars, etc.) to encourage the action of native or introduced biocontrol agents in a field. As with biocontrol inputs, there are considerable disparities in practices, techniques and products aimed at improving this action. In protected systems, banker plants or substrates for temporary or seasonal establishment of biological control agent populations (native or introduced) are increasingly common (Messelink et al. 2014). In other systems, the scientific literature provides quite a few examples of the beneficial effects of techniques such as hedges or companion plants, particularly for macroorganisms (Gillespie et al. 2011; Horgan et al. 2017). Aside from their demonstration in the scientific literature, it is difficult to estimate the frequency of use of such local ecological infrastructure, and we are unaware of any mechanisms or tools that would facilitate their widespread or systematic use. There is also an obvious gap in terms of an equivalent of conservation biological control in microbial communities. Understanding the factors that influence the dynamics of the crop microbiota and its effects on the soil and crops is, however, a challenge that is well identified by the scientific community and is the subject of growing research efforts (see Chaps. 9 and 10).

18.2.3 Natural Substances (of Animal, Plant or Mineral Origin) and Semiochemicals (Pheromones, Kairomones)

Substances of plant (including algae) or microbial origin can be divided into two subgroups: purified compounds such as pelargonic acid and acetic acid, which are used as natural herbicides, and spinosyns A and D (microbial metabolites) registered as insecticides. These substances are treated in registration dossiers as conventional active ingredients. The second group consists of more complex extracts and includes preparations that are well characterized with proven effectiveness, particularly as insecticides, such as pyrethrum extract. Substances of animal origin are rarer; for example, sheep fat is used as a roe deer repellent. Finally, semiochemicals (pheromones, kairomones), which are most often mixtures of compounds secreted by macroorganisms (insects), then identified and chemically synthesized, are in a category all their own.

Several natural substances and semiochemicals are already at an intermediate level of deployment. Entomotoxic Cry proteins produced by *Bacillus thuringiensis* are also widely used around the world and are generally sold in mixtures with bacterial spores that may or may not be inactivated. Using pheromones for mating disruption in orchards and vineyards is also common practice. The factors explaining why natural substances are not used on a broader scale are not as obvious as for organisms. All the categories of factors seen above apply, but *a priori* pose fewer constraints: it is much simpler to produce many substances than organisms, and their storage and transport are easier to manage. Their application could also make use of existing infrastructure. We will not go into detail on semiochemicals here, because they require dispensers to be installed in the field and therefore deployment depends highly on an area-wide management strategy.

Besides the need to create sustainable channels guaranteeing access to plant biomass for the production of plant extracts, there is another key factor here: the very limited knowledge of most substances' modes of action, which hampers the development of application methods and positioning support. COS-OGA, an oligosaccharide complex that induces plant defences, is one example that merits attention. According to studies (van Aubel et al. 2016), this complex appears to penetrate the plant via the stomata and so must be applied in a specific way to optimize penetration and activity. Unfortunately, today the modes of action of substances used against plant pathogens are limited to induction of plant defences, fungicidal or bactericidal activities, and very few mechanistic studies are available. New "omics" approaches should enable researchers to identify the metabolic pathways affected by these substances in the plant or phytopathogen and ultimately characterize the cellular targets. Among natural extracts, the mode of action of certain insecticidal essential oils is now being addressed to characterize the synergies observed in these complex natural mixtures (Tak and Isman 2017).

18.3 Area-Wide Deployment of Biocontrol

18.3.1 The Deployment of Certain Methods Must Be Considered at an Area-Wide Level

Often, the effectiveness of a biocontrol method at field scale is highly dependent on its level of deployment on an area-wide scale. A method applied to a small proportion of a mobile pest population may be ineffective, with the rest of the population becoming a source of re-infestation. An area-wide pest management strategy to reach a single population or several connected populations is particularly effective with methods that do not have a direct biocidal effect and work by disrupting reproduction. This is the case for mating disruption using semiochemicals (pheromones) and autocidal control (release of sterile insects). These methods can be very successful against highly mobile insects (Lepidoptera, Diptera) if the entire area affected by a population is targeted; otherwise, they may not be successful. Since the two methods above have no direct effect on females, any absence of sterile males or pheromones disrupting reproduction in a neighbouring field will cause an inflow of fertilized females that will contribute to the establishment of a pest population despite the local use of biocontrol.

Area-wide introduction strategies are more generally relevant for biocontrol agents that can move between multiple fields (macroorganisms). However, these strategies are rarely implemented and are not based on knowledge of population dynamics.

Although microorganisms rarely have an intrinsic capacity for motility, managing their use on an area-wide scale remains a topic of interest. They can be disseminated effectively over various distances by different vectors (e.g. wind, rain, animals, soil particles and crop residues, agricultural equipment, seeds and plants). These phenomena have been well studied for plant pathogenic microorganisms, but there is little documentation when it comes to microbial biocontrol agents. The possibility of beneficial microbial populations spreading from treated fields, or those naturally present on crops or weeds, is therefore possible, but still largely unexplored.

18.3.2 Managing Ecosystem Services at the Landscape Scale

Since Chaps. 6 and 7 deal broadly with this aspect of biocontrol, we will simply state here that conservation biological control is above all a landscape-level challenge. Any land-use planning to promote a landscape organization that supports biological pest and disease management will affect an entire region not just a small local area. In terms of deployment, two particularly complicated issues arise. How can inter-field and landscape organization structures be developed to effectively promote biological control? Once proposals grounded in science are put forward, how can they be implemented? Regarding the first question, the scientific literature shows that it is extremely difficult to generalize proposals, because the links between landscape, habitats, biocontrol and crop damage hinge on the biological situations in each region and the ecosystems considered (Karp et al. 2018). As regards the second question, to our knowledge no mechanism or tools yet exist to coordinate territorial stakeholders to organize agricultural areas and landscapes supporting biocontrol in line with recommendations based on scientific data. An active research community does tackle issues related to the links between landscape and biocontrol (see Chaps. 6 and 7). However, the paths towards operational variations seem particularly complex because they entail a collective approach involving many stakeholders. These two observations raise questions for research stakeholders: What should be expected, at the operational level, from landscape ecology research on general issues concerning the links between landscape, biodiversity and biocontrol? To go as far as operational variations in the field, is it necessary to carry out large-scale studies on each target territory, without looking for generalities, but by designing ecological infrastructure adapted to the local situation? What social science research could facilitate the design and implementation of territorial public policies or collective initiatives of local actors, considering the interests and reasoning of all stakeholders in the territory?

18.3.3 Biocontrol Depends on Area-Wide Pest Management Strategies to Prevent Epidemics

Although the term “extended biocontrol” used in this book covers a broad range of methods, which makes it difficult to generalize, most biocontrol methods aim to manage pest densities rather than eradicate the pest. Biocontrol methods are therefore not usually suitable in conditions where pest densities are high. Hence, biocontrol development is closely linked to a more general use of preventive measures, in order to reduce epidemic frequency. However, although preventive measures can be used at field level (e.g. resistant varieties), the stakes seem to be the highest mainly at the territorial level, such as the need for stakeholders to work together to organize crop rotations or alternate varietal choices geographically and timewise. Once again, these approaches, which could be a factor in successful biocontrol deployment, are not put into practice (with rare exceptions) and are still limited to research.

18.3.4 How Sustainable Would Extended Biocontrol Deployment Be?

Adverse effects related to the widespread deployment of a biocontrol agent or substance can be expected, such as the development of resistance in the target pest due to increased and more spatially homogeneous selection pressure. This phenomenon, well understood for chemical control, requires further study for biocontrol agents (see Chap. 23). This knowledge could be useful in implementing specific resistance management strategies, as in the case of synthetic chemical pesticides (e.g. those recommended by the Fungicide Resistance Action Committee – FRAC 2021).

18.4 Diffusing Innovation Across the Value Chain

Until now, we have mainly dealt with the challenge of deploying biocontrol based on technical considerations (organism biology, field delivery techniques, etc.). However, it is undoubtedly the economic and social dimensions of this challenge that have the greatest impact on biocontrol development. These aspects will be presented in detail in Chap. 19, but we will mention a few elements here in connection with the technical elements previously outlined.

All the technical factors described so far and the barriers to deployment they may pose do not seem insurmountable. Usually the technologies needed to address them exist, and the pathways for improvement seem to be well defined, and have been for several decades – see for example Elad and Chet (1995) on the challenges of deploying biocontrol against plant pathogens. It therefore would appear that the challenge is above all to allocate sufficient research and development efforts to produce more knowledge about organisms, substances and modes of action, and to develop tools to effectively integrate this knowledge in the field.

Why is the biocontrol innovation sector unable to allocate the necessary resources? To answer this question, it is important to begin by not underestimating the effort required. Biocontrol deployment requires the creation of new infrastructure, including industrial production, storage and distribution capacities; adapted regulations; stakeholder expertise; etc. To date, such infrastructure has been built around the conventional pesticide model developed after the Second World War. Although the required investment to build this model has not been rigorously assessed, it seems colossal: for example, it is likely in the hundreds of billions of euros in France, where it has been strongly subsidized by the French government and has led to a profound shift in agriculture (see Chap. 19). By comparison, the current level of risk-taking by value chain stakeholders (agriculture, agri-food, distribution, etc.) to build new infrastructure suited to biocontrol is rather low. This leaves the burden of investment to the biocontrol innovation sector and public investment. However, the biocontrol innovation sector is small and has only modest R&D resources. For instance, the biocontrol industry's annual turnover is around €100 to €150 million, which translates to an R&D capacity of around €10 to €20 million a year, assuming R&D investment of 10–15% of turnover as generally observed in the sector, according to the International Biocontrol Manufacturers Association France (IBMA France 2017). Public funding for research and innovation is mostly indexed to private investment, which strongly limits its impact on biocontrol deployment given the low level of private investment in the sector. For example, in France, a research tax credit is calculated according to the percentage of R&D investment declared by companies. Support for industrial R&D is provided through co-financing or advances, which are also a percentage of private investment, while many collaborative funding projects between the academic sector and industry are built around private investment. Given the situation, the main challenge lies in creating public policies to enable increased risk-taking across the entire value chain (from farmers to consumers) and to support the public investments needed to create an agroecological infrastructure on which private investment can then develop. It is important for this type of public policy to be enacted at the international level; if not, large economic players may not change their level of risk-taking because of the political positioning of one or several countries. Stakeholder training, an essential component of new infrastructure that is more supportive of biocontrol in agroecological systems, also plays a key role and is the subject of initiatives by most stakeholders, both public and private.

The success of broad deployment will also require breaking away from classic R&D schemes linked to conventional pesticides, i.e. searching for an innovative active substance formulated for use with standard agricultural equipment and tested under conditions developed for conventional products in line with conventional marketing requirements. This restrictive scheme is unsuitable for certain biocontrol solutions, and it excludes the development of ranges of solutions such as those based on microbial consortia or fragile microorganisms (such as spores that cannot be conserved, etc.), or successive and synergistic applications of biocontrol solutions. This "conventional" R&D scheme also tends to confine research efforts to innovation on commercial plant protection products (even when considered biocontrol). It also limits research that seeks to develop service offers involving the management of endemic species that are beneficial to plants, which would require a holistic approach and territorialized diagnosis and recommendation.

Regulation is also a key factor for deployment, and the positive and negative impacts it has are regularly highlighted by biocontrol stakeholders (Barratt et al. 2018). The negative impact most often cited by biocontrol companies is a slowdown in R&D activities, linked to the obligation to submit product marketing authorization dossiers (in application of European Commission Directive 2001/36/EC). Country-specific regulations can also be prohibitive: for instance, in France companies must submit introduction authorization dossiers for macroorganisms (in application of French decree no. 2012-140 of 30 January 2012 relating to the conditions of authorization to introduce exotic macroorganisms useful to plants into the country and into the environment, particularly in the context of biological control). Substantial costs are involved in compiling these dossiers, especially for microorganisms and substances, and obtaining a marketing authorization is a somewhat lengthy process. Returns on investment are already relatively distant due to the low market penetration rates of developed products (notably due to the absence of infrastructure facilitating their deployment), and regulations can further discourage investors. Regulations are also penalizing for biocontrol solutions based on blends or mixtures of organisms or substances, each of which must be thoroughly characterized, aside from a few semiochemicals. Furthermore, most criteria and procedures in force have not been designed for biocontrol products. National and international working groups are currently drawing up proposals for testing and evaluation procedures that are better adapted to the specific needs of biocontrol. In addition, the Convention on Biological Diversity, and especially access and benefit sharing (covered by the Nagoya Protocol), is also cited as an additional factor of complexity to be considered when developing products based on organisms identified in the environment.

The above-mentioned elements suggest a mainly negative view of regulation. However, regulations on environmental protection or the use of plant protection products are also a powerful lever for promoting biocontrol. Applying strict criteria for the definition of biocontrol products protects their image and their integration into a framework for sustainable agriculture. Like any other method, the impacts of biocontrol methods are investigated by the scientific community (Thomas and Willis 1998) and rigorous regulatory procedures are an initial step in ensuring they are safe to use. Moreover, the widespread deployment of biocontrol in greenhouses is concomitant with decisions to ban the use of certain chemical substances. For instance, when France enacted a law (known as the Labbé law) that banned the use of chemical pesticides in garden and public green spaces, the biocontrol market surged in these areas (+25% according to IBMA 2017). France has also established new regulations to create positive discrimination for biocontrol, since products on the national biocontrol list (updated monthly by the French Ministry of Agriculture) are subject to exemptions from restrictions that apply to other plant protection products (such as a ban on commercial advertising). They are also not subject to the obligation to reduce use in the context of the introduction of “white certificates” for low chemical-input plant protection methods (known under the acronym CEPP in France). Finally, in many more established industries, marketing application procedures are seen not only as constraints, but also as a way to coordinate R&D activities and players.

Chapter 19
Biocontrol in France: Prospects for Structuring a Developing Sector

Manuel Boutet and Aura Parmentier-Cajaiba

19.1 Introduction

In this chapter, we will look closely at the managerial, institutional and political choices and alternatives facing extended biocontrol today. The term "biocontrol" has become more settled recently (Eilenberg et al. 2001), even if it is poorly understood by the public, and has been extended to include substances that are not necessarily on the French government's official list of allowed "biocontrol products". However, there is a way for us to better characterize biocontrol based on paradoxical needs:

- to replace synthetic plant protection products with biology-based techniques;
- to drive change in agriculture to create a more agroecological approach.

The paradox is that biocontrol techniques must fit into the agricultural and even agri-food systems as they exist, while biocontrol is also expected to establish a new paradigm.

To better understand the situation, we felt it was important to examine the current institutional organization of the agricultural sector, the yield and predictability conventions that underpin the sector, and the more recent trends towards the financialization of agriculture. From this viewpoint, we can see that there is a specific social and managerial logic to implementing techniques – a technique cannot be generalized without first being integrated into the organizations and markets that regulate the production and allocation of natural and social resources. It is not

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enough for chemicals to be identified as dangerous and for alternatives to exist for all of agriculture to change. Our goal is to identify the main points of resistance. We chose not to address the well-known exogenous factor of lobbying by the chemical industry (the Monsanto Papers affair is one recent illustration) and instead will focus on systemic points of resistance that are more often overlooked or underestimated. More specifically, we will examine the conventions of the sector, the role of regulation, the limits of the product-based format, the many possible development models, and finally several important factors in disseminating biocontrol at the farm level. To illustrate this more clearly, we will use France as a case study, starting with structuring of the agricultural sector from the late 1950s. This offers a broad overview of the integration of successive innovations in the field of biocontrol.

19.2 Talking About Biocontrol: An Analysis Based on Scientific, Media and Institutional Discourse

Following the controversy over the effects of synthetic chemical products used in agriculture (Chateauraynaud and Debaz 2017), the idea that we must reduce use of these products, or even find alternative solutions, is now firmly rooted in society. Extended biocontrol is considered here as a plausible alternative because it is based on plant protection products being perceived as "natural in origin". This is a narrative that makes biocontrol development more accessible to regulators, politicians and the public. When we talk about "societal demand for biocontrol", this is the narrative we mean. However, this approach is also at the heart of a series of problems for biocontrol development.

On the one hand, biocontrol is analysed through the framework created for chemicals. For example, Hélène Ledouble's research on the terminology used by the press to talk about biocontrol shows that it draws from the vocabulary of eradication and war (Ledouble 2019). Although she concludes that there is a lack of rigour among journalists, we might very well wonder whether alternative narratives exist in the scientific literature or even in popular science magazines. Journalists have more facts than they do stories; they have the scientific data, but scientists have not done the work of building stories from this data.

On the other hand, the press is not alone in taking liberties with scientific definitions. For example, consider the scientific definition of biological control proposed by Eilenberg et al. (2001: 390): "The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be". The central tenet of this definition is the idea of *reducing* the pressure of the pest population – not *eradicating* it. This idea rarely is found in the press, but it is not any clearer in legal texts either. For instance:

> "The [Ecophyto] plan sets out measures to develop biocontrol products, which are agents and products that use natural mechanisms within the framework of integrated crop pest management." (French Rural and Maritime Fisheries Code, Article L. 253–6).

The definitional fluidity is not confined to France. Consistent with our definition of extended biocontrol in this book, and unlike Eilenberg et al. (2001), the

International Organization for Biological Control defines biological control as using living organisms *and* their products.

In practice, biological control definitions vary according to the stakeholders. For instance, since microorganisms have been included in pesticide regulations, some institutional stakeholders exclude them from their definitions of biological control, a term they apply strictly to macroorganisms, hence this somewhat absurd discussion:

> [Investigator] "Since macroorganisms can affect pest populations, aren't you afraid they will end up being regulated like microorganisms?"
>
> [Institutional stakeholder] "No, you are confusing pesticides with biological control."

Biological control, which is a method, is not antithetical to "pesticide", which refers to a product whose purpose is to kill a crop pest. There are, of course, biopesticides.

Viewing biocontrol through the lens of chemical categories has consequences not only on its image, but also on the possibility of highlighting its many specific properties. For example, biocontrol products are more sensitive to temperature and need to be packaged differently, which means rethinking how they are stored and transported. Their properties are more systemic and biocontrol is linked to the climate and the soil. The overall approaches are therefore more targeted and less universal. Certain properties are perceived negatively within the chemical paradigm (storage, formulation, sensitivity to temperature), whereas they make it possible to envisage a more systemic approach to agriculture (adaptable to particular climates, involving different modes of action and cultivation, less toxic for biodiversity and the environment).

If bringing the products to market and their subsequent promotion have proven difficult in the current context, we must once again question the role scientific discourse has played. One very simple example – the environmental benefits of biocontrol products and their effects on biodiversity – shows that it is clear that no single measure has achieved consensus.

On the industry side, environmental gains remain implicit. Industry players promote the idea of "natural" or "natural origin", which suggests that these products are better than products produced with synthetic chemistry. This is reinforced by French and European regulations, which prohibit advertising on environmental benefits. But, when we raise this with industry players, they say they are uninterested, noting the additional expense of an assessment of environmental benefits – the existing legal requirements are already very costly – or the lack of an effective method. This lack of method seems likely to last, given that institutional stakeholders are not tackling this issue. It also brings up the question of the few resources available to integrate and guide research, regulation and public incentive activities in order to anticipate and measure the environmental benefits and risks specific to biocontrol.

19.3 The Role of Biocontrol in Agricultural Processing

In this context, biocontrol is an innovation. Advances in biological knowledge explain this in part, but the impression of novelty that biocontrol conveys today goes beyond that. Biological control methods for crops have been around since the advent of agriculture: hedges to support beneficial organisms, fish in rice paddies, agroforestry practices, etc. To understand the apparent "novelty" linked to biocontrol, and the public's difficulty in understanding it, we must look back to a very particular time in the history of agriculture. Between the 1950s and 1980s, with the proactive and radical attempts to modernize what is now known as "conventional agriculture", it would seem that the agricultural sector – and society as a whole – had developed amnesia, only able to draw from the past with considerably difficulty (Mendras 1992 [1967]).

In France, productivist agriculture was introduced with the "Green Revolution" starting with the reconstruction of the 1950s, under the direction of the *Commissariat général du Plan*, tasked with setting out the country's economic planning through five-year plans. The scale of the economic and social transformation undertaken at the time was staggering. To put it into perspective, we must first consider the change in world view that had occurred in agriculture. Prior to this period, uncertainty mainly stemmed from the vagaries of weather. However, it had shifted to market variations: for example, today farmers choose the crops they grow based on price forecasts. Such an approach is only possible once the internal uncertainties of production methods have been reduced – requiring innovation, investment and debt – which leads to financialization and increased dependence on markets.

That this new agriculture was called "conventional agriculture" also marks an oversight: that of the small-scale farming that had developed in France until the 1940s. There were no economic or social laws at play here, and the timing was different compared to other developed countries. For example, in England rural flight began in the nineteenth century, and around the 1929 stock market crash in the United States. With this type of transformation, new knowledge eclipses old knowledge; systemic knowledge and know-how is lost. Large operators replace small farmers and have less need of detailed agronomic knowledge, which is replaced by the planning systems that are put into place. More specifically, the use of biological control declines – both because such methods are considered "less effective" (not *in*effective) in terms of performance, and because biological and conventional control methods are somewhat incompatible.

19.3.1 The Green Revolution

To truly understand the situation today, we must look to the past. In France, from the 1950s to the 1980s, the share of the French population directly employed by the agricultural sector fell from one-third to a few percent. The French situation is quite specific; in England, for example, the first rural exodus emptied the countryside as early as the nineteenth century during the first Industrial Revolution. The French rural flight, largely deliberate and planned, came with new compromises – the disappearance of small farmers, the merging of farms, and a new model of the average farm, which was still largely family-based. These compromises balanced economic objectives (importance of the agri-food industry's exports) and social objectives (combating rural desertification). For public authorities, the goal was to achieve two aims: modernization and food autonomy, under the constraints of regional planning (Hervieu 1995). These aims are supported when necessary by national and European public policies.

The chemical pest control used in conventional agriculture enabled agricultural work to be systematized. The agronomic, biological and social sciences were largely involved in this modernization movement, and work on farmers' resistance to change regarding new seeds was groundbreaking in the sociology of innovation (Flichy 2003; Griliches 1957; Joly and Lemarié 2000). This systematization developed gradually and was well described by Henri Mendras in his book *La fin des paysans* (*The vanishing peasant: innovation and change in French agriculture*) published in 1967 (1992 [1967]), where he analyses this transformation of agricultural systems. The introduction of hybrid seeds was the first step. This initial association of seeds and chemical inputs (synthetic fertilizers and soil amendments) to maximize their potential resulted in a subsequent shift in logic of merging fields to make input purchases profitable. These successive aggregations, combined with higher per-plant yields, ultimately encouraged farmers to buy agricultural implements. Farmers took on debt, which was a new possibility thanks to the predictability offered by this planned system. Until that point, farm-level indebtedness was an unusual practice in the agricultural world.

Today, farmers seem to have no other choice. A central argument in this debate, in particular against organic farming, is the universal value of "feeding the world" (which appears to be a recent metamorphosis of the strategic and national objective of "food autonomy"). However, this "feeding the world" narrative is fragile, since there is neither "one" type of agriculture at the global level, nor even a universal model of agriculture. In developed countries, the circular economy is growing and, in developing countries, agriculture organized around yield and export for international markets is not the agriculture that feeds people (Jarosz 2000). The fact remains that, in an agricultural system organized around a central conventional measurement – yield – real or supposed drops in yield, associated with changes in practices, are thrown up like red flags when deviations from the predominant system are noted.

19.3.2 Externalities

This brief overview of French agricultural history highlights two aspects we feel are central to understanding where biocontrol now stands. First, the agricultural sector as we know it today is not only the product of economic or social logics; it is also the result of national political drive and several decades of proactive institutional development. Second, this sector is organized around a central conventional measurement: yield. For example, crop planning methods include forecasts of both the climate and anticipated market prices of agricultural commodities when selecting the varieties to be grown. To protect farmers, European regulations include yield in their considerations, unlike regulations in other countries (e.g. the United States, Australia, New Zealand), which are limited to public health aspects. For instance, in Europe plant protection products can only obtain marketing authorization if they are "sufficiently" effective, effectiveness being defined by studies designed to measure agricultural production performance when the plant protection product in question is used. This creates a barrier for approaches aiming for systemic efficiency, which would take into account, for example, the particularities of an area, the various associated cropping techniques and their synergies.

This applies to the product and to the cropping system, which we can illustrate with two examples. First, the logic of purification of chemical substances is enshrined in the regulatory framework – each substance must be approved separately and must therefore prove its effectiveness independently. This makes it difficult, if not impossible, to approve and therefore market products based on bacterial communities with synergistic actions. Second, products must be effective on their own at very high rates in order to be registered, a requirement designed to protect farmers. However, today this rule excludes products that could be useful if integrated into a particular cropping system as one contribution among others to reduce pest pressure on crops. As these examples show, some of the current debates around agriculture combine economics, regulation and epistemology, and the opposition between reductionist and systemic approaches in particular – which concerns legitimate ways of establishing the validity of knowledge – has far-reaching industrial consequences.

As we have pointed out, yield is a core element of the trade-offs around which the agricultural sector is organized today, namely due to the need to balance economic efficiency while supporting family-based farms (Comby et al. 2016).

When evaluated based on other measurements, the rationality of this organization is questionable. Thus, synthetic products perfectly fulfilled the role for which they had been designed – to increase agricultural performance in terms of quantity and predictability – but, from the 2000s onwards, other measurements not taken into account in their design entered the public debate and revealed externalities of agricultural activity: occupational diseases among farmers; environmental effects, particularly ecosystem effects; and food residues. As is often the case in metrology, improvements in evaluation techniques that make more precise measurements possible provide critics with ammunition – the effects criticized today as "residues" likely could not have been identified in the 1950s.

19.3.3 Markets and Intermediaries

Once the peasantry is replaced by an agri-food production sector – rebuilt around varietal selection, mechanization and chemical inputs – the sector will become progressively more vertically integrated,[1] with the same player acquiring related

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[1] "An economic activity is said to be integrated when all the operations relating to it are carried out under the authority of a single decision-making centre" (Le Bihan 1960).

production and distribution activities to control the value chain. The sector is first organized into circuits of commodity flows, and these into a system, alongside the existing regional agricultural systems (Jarosz 2000). In the 1990s, the value chain was coordinated all the way to the household level. For example, the demand for flower products dramatically changed the structure of horticultural farms in Africa – forming a global commodity chain from supermarkets to producers of various sizes in Kenya (Gibbon 2001). But this industrial production logic was gradually supplanted by one of corporate performance – a "global value chain". This phenomenon is furthered by the organizational concentration of economic players through mergers and acquisitions conducting similar activities in one or more industries (also known as horizontal integration), at all levels of the logistics chain, leading to a natural selection of the most profitable players (Temple et al. 2011). Corporate performance – which has now become inseparable from agricultural production – means that financial profitability indicators take precedence over all other considerations. The paradox is that the integration (both vertical and horizontal) and the financialization of the sector have made it increasingly difficult for national and European policies to guarantee previous social advantages, particularly decent wages for farmers (Villain 1984). Considerable efforts have been made to find new, economized forms of these social advantages, as shown by the work on the "commons" (Thomas et al. 2018) and on "ecosystem services" (Therond et al. 2017), which support the establishment of new institutional systems.

Downstream, food prices are traded on world markets where the whims of free competition play a role. But for this to happen, the entire chain of cooperation for each sector must be integrated, including the upstream inputs and machinery and the downstream processing and distribution operations. All this requires the development of standards, norms and regulations. More specifically, this integration is synonymous with rationalization at the farm level, which means that all the organizational needs of the value chain are constraints on productive decisions. This logic is embodied by the well-known figure of the agricultural advisor, who often works with farmers to select crop varieties, based in particular on economic expectations of market prices for agricultural commodities at the end of the season.

Considering the development of agriculture in France and beyond, the current situation cannot be viewed as set in stone; the resulting shifts are not only technical. They also happen through the transformation of the systems themselves because they are ultimately institutional.

When biocontrol products appear, they do not simply arrive on a market. They must fit into a fairly complex system of collaboration at the level of the sectors. For example, farmers may have to carry out certain treatments on their fields, sometimes for insurance reasons, or because a cooperative has requested them, or because they are linked to investment loans, or for regulatory reasons (for example, compulsory control of the grapevine flavescence dorée vector, using several chemical insecticide treatments). Conversely, certain demands from supermarkets or agri-food industry players have forced farmers to rethink their farming methods.

19.4 Can Biocontrol Be Reduced to a Product Format?

This complexity of the agri-food sector has prompted biocontrol industry players to bend to fit existing formats. The International Biocontrol Manufacturers Association (IBMA) organizes a yearly conference that typifies this situation, where a range of "products" are presented and the most "universal" among them are highlighted. In other words, the aim is to develop products that can be applied on field crops in all countries. In this business-as-usual world, where market size is crucial, it soon becomes difficult to understand why biocontrol would ever be preferred to synthetic chemistry. Even promoters of biocontrol products acknowledge the limitations: they are not as effective and are less predictable, transport is challenging, storage is not always possible, application requires labour, products depend on weather and soil, and so on. This depiction of biocontrol products as incomplete or imperfect is not surprising, because they are held up to chemical standards – durable, stable conservation, high short-term biocidal effects, etc.

One underlying question is the relevance of developing biocontrol as "products". The difference between experimentation and a product is that a product must undergo passivation so customers can be sure the product's properties will be stable during use (Callon 2013). Considerable efforts have been made over the last 15 years to design such products. Nevertheless, biocontrol is still often associated with an ancillary "service" that companies provide but do not promote to customers. When performance depends not only on the product itself but also the advice provided on using it, this implies a different use that changes farmers' practices. For example, farmers may need to shift from a curative approach to a preventive approach to maintain a balance of pest and biocontrol agent densities. In practice, this shift in logic may cause application delays due to introductions of biocontrol agents throughout the year. These introductions must be made dynamically, based on knowledge of the ecology of pests and the diagnosis of the agricultural system, rather than in reaction to observed pest outbreaks or according to pre-established treatment schedules.

To overcome these difficulties, two aspects should be considered: the scale of intervention and the value of biocontrol. First, how should products and their associated services be designed? How can changes in practices at the farming-system level be suggested?

Second, biocontrol must be valued for what it is. If it continues to be judged based only on existing standards and remains dependent on the global supply chain that structures agriculture today – where a farm's predicted yield is the first link guaranteeing the sector's financial performance – then it would seem difficult to see the value in using these products and techniques more widely. Measurements could be developed to objectively assess the hypothetical gains from substituting biocontrol products for chemical inputs.

19.5 Regulation: An Unsuitable Obstacle for Biocontrol Players, Uncertainty for Civil Society

In Europe, regulation is a major obstacle to widespread biocontrol use. Besides financial performance indicators, regulation of agricultural inputs has grown since 1943 (Prieur 1987) to ensure product quality for consumers. Agricultural inputs are historically associated with the chemical industry. Directive 2001/36/EC groups some biological control products with pesticides (specifically microorganisms) and allows their regulation. Macroorganisms are still regulated by the individual Member States in Europe. When no national regulations exist, companies must follow supranational standards when placing these products on the market (e.g. those of the International Organization for Biological Control (IOBC) or the European and Mediterranean Plant Protection Organization (EPPO)). This makes it difficult for small companies that do not always have the legal expertise to penetrate markets with different legal frameworks and which depend on different institutional levels.

Today, biopesticides, including microorganism- and pheromone-based products, are regulated by Europe in the same way as chemically synthesized products. The regulatory system uses a set of indicators to assess the environmental effect of an input: treatment frequency index (TFI), maximum residue limit (MRL), median lethal dose (LD_{50}), etc. These indicators are developed based on studies and observed outcomes. Products are characterized according to their possible use, which has a significant impact on the dissemination of biocontrol products.

Regulation is hotly debated by biocontrol industry players because the studies required by regulations entail financial costs and delays in placing products on the market. Once again, this penalizes small and medium-sized companies without sufficient funds to overcome immutable regulatory timetables.

These managerial concerns are compounded by the fact that input markets at the European level are defined, in part, through criteria specific to chemical plant protection products. For example, it is impossible to obtain approval for a mixture of microorganisms. It is questionable whether the toxicity measurement alone is adequate for measuring the long-term effects of releases of living organisms. Such regulations are certainly not an incentive for market players to think systemically.

Beyond the cost for companies and biocontrol players to get their products approved – a genuine barrier to entry – the regulatory system does not take into account the specific characteristics of biocontrol products. As such, it does not allow consideration of the potential problems that could arise from these innovations, which creates uncertainty about the large-scale dissemination of these techniques.

19.6 Biocontrol and Placement on the Market: Moving Towards Pluralist Business Models

One problem raised in the previous sections is the consideration of the "specific characteristics of biocontrol". What we mean by this is that there is a link between the technique and the managerial and socio-technical agencement[2] (Callon 2016; Muniesa et al. 2007) it fits into. The most obvious example today is the interdependence among mechanization, chemical inputs and monocropping; the current system is partly based on this interdependence. Implementing each biocontrol technique has prerequisites and application conditions that make it easier to integrate in certain systems (see Table 19.1). We are not suggesting a deterministic outcome, but rather that there are reasons to consider the coexistence of several business models according to the technical systems of different types of biological control.

The marketing of chemical inputs is relatively unambiguous. Generally, the product formulation will be sold and associated with a single or repeated application dose that the user undertakes to respect. There is a catalogue of preparations that varies according to the relevant sectors, crops and varieties.

Eilenberg et al. (2001) describe four types of biological control. We will show that deploying these methods requires a socio-technical network with specific characteristics, which implies variable business models that differ to those for chemical inputs.

19.6.1 Classical Biological Control and Inoculation Biological Control: Towards an Alternative Business Model

Classical biological control is "the intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control" (Eilenberg et al. 2001: 391). This method of biological control requires specialized scientific knowledge to identify the organism that will regulate the new

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[2] Sociologist Michel Callon uses the term "agencement", noting that "the term *agencement* is a French word that has no exact English counterpart. In French its meaning is very close to 'arrangement' (or 'assemblage'). It conveys the idea of a combination of heterogeneous elements that have been carefully adjusted one another. But arrangements (as well as assemblages) could imply a sort of divide between human agents (those who arrange or assemble) and things that have been arranged. This is why Deleuze and Guattari (1998) proposed the notion of agencement. Agencement has the same root as agency: agencements are arrangements endowed with the capacity of acting in different ways depending on their configuration" (Callon 2006). Here, by socio-technical agencement we mean a set of heterogeneous elements that form a system because they have been arranged together: laws, institutions, conventions, groups of actors, technologies, physical artefacts, etc.

Table 19.1 Possible agencement between technical systems and business model elements by type of biological control

Type of biocontrol	Conditions of implementation	Business model elements
Classical biological control	Find a new long-term balance. One-time intervention. Need for applied science.	Service: identification of the balance (scientific expertise). Reproduction service (production expertise). Single distribution and release service. Area-wide application. Public and/or private stakeholders. **Logic far removed from conventional pest management.**
Augmentative – inoculation	Find a medium-term balance. Occasional, spaced-out interventions. Need for applied science.	Service: identification of the balance (scientific expertise). Reproduction service (production expertise). Single release service. Area-wide application. Public and/or private stakeholders. **Logic far removed from conventional pest management.**
Augmentative – inundation (and use of natural substances)	Recurrent intervention. Frequent intervention. Passivation of products.	Revenue source: commercial product. Revenue source: associated service. Private stakeholders. **Logic similar to conventional pest management.**
Conservation	Detailed knowledge of the local area. In-depth knowledge of alternative practices and products. Logic of continuous improvement.	Service: consulting on regional planning. Service: consulting on current and innovative practices. Service: supporting change. **Logic removed from conventional pest management.**

pest population, followed by the introduction of this organism, which will then become established in the ecosystem. Such a considerable investment on an occasional basis would seem to rule out a classic business model designed around a product that can be sold on a recurring basis. It is thus clear why the existence of a public agricultural research institute may be one condition for implementing this method. The easiest way to develop classical biological control would be to promote it as a public service or to reflect on the conditions of a commercial service that would be carried out by multiple stakeholders and could, perhaps, be applied on a regional scale. This different conception of pest management calls for identifying potential stakeholders and understanding their value systems in order to propose ad hoc business models.

Inoculation biological control is similar to classical biological control, but differs in two respects: pest control does not involve an exotic agent, and control is not meant to be indefinite, as the definition proposed by Eilenberg et al. (2001: 393) makes clear: “the intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently.” The socio-technical agencement could therefore be similar to that envisaged for classical biological control. Since inoculation implies few repeated treatments, it is difficult to consider exploiting it in the same way as conventional agricultural inputs.

19.6.2 Conservation Biological Control: A Focus on Services

For conservation biological control, which consists of a “modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests” (Eilenberg et al. 2001: 396), the associated business models could be developed based on combining products and services. Such products and services could be implemented by experts in conservation biological control and specialists who support changes in agricultural practices (product use and new habits). Options could include variable configurations ranging from providing a public service to supplying various services or even products. The issue of coordination among stakeholders and their relevance would arise, more than for other types of biological control. Accordingly, it might be possible to consider the development of a marketable product or service, but this would require considerable “investment in forms” (Thévenot 1986): this approach assumes the joint development of structures and standards to support the development of these products and services.

19.6.3 Inundation Biological Control: Translating the Conventional Model

Inundation control, like the use of natural substances, can be easily developed through products, based on the conventional input model. It is now the most represented type of biological control in the growing biocontrol market. In essence, it takes the form of a solution with stable properties that can be applied according to a precise quantity determined by agricultural advisors. As Eilenberg et al. (2001: 395) define it, inundation biological control is “the use of living organisms to control pests when control is achieved exclusively by the released organisms themselves.” This means that like with chemical products, it requires repeated applications to prevent or control a pest outbreak, since the specific nature of this control method depends on the services provided by the released agents. It can therefore be included in a catalogue and ordered every year or even several times a year. The need for regular applications makes it an interesting asset for a profit-based company, but biocontrol companies show little interest in promoting and billing the related services required for this type of solution. This could be an important point to explore in business models for inundation biological control. For example, proposing a range of services could showcase a company's expertise and provide a revenue stream that could be spread over the year. Services can also create engagement with customers, but they are relatively expensive to offer. Digital activities could be a middle ground to promote the development of these services and minimize costs. Although inundation biological control seems like it would fit more naturally into the current agricultural system, it is essential to not underestimate the importance of developing business models that meet the specific needs of biocontrol products, and especially offer them as realistic and valuable solutions for customers. The high production costs, use conditions and immediate efficacy rates of some extended biocontrol methods, which differ significantly from standard biological control, should also be considered when tackling the necessary changes to cropping systems to address current ecological problems and for which biological control seems well suited (see Chap. 20).

Table 19.1 links the conditions of implementation (technical systems) with several plausible elements to aid the development of business models for biological control.

Unlike synthetic chemical plant protection products, biological control products involve rethinking the cropping system. They necessitate a different kind of activity: biological control requires attention to biological processes. The focus is on reintegrating the observation of fields and animals into practices, adopting preventive rather than curative logics (or curative logics to be applied at the right time), and possibly replacing the strict rigour of planning with careful and patient professional support.

19.7 Conclusion

Because of the importance of the institutional context, markets and socio-technical networks in which agricultural activities are embedded, research that attempts to identify the pathways to transform modern agriculture focuses on the integrated nature of the "regional agri-food system". Claire Lamine thus identifies three elements that facilitate transitions: the possibility of niche experimentation, the hybridization of dominant and alternative players, and the combination of top-down and bottom-up organizational processes (Lamine 2017). In other words, user-centred and democratized innovation (Von Hippel 2006) is important in agriculture, within a context of open innovation, or at least where dissent and controversies contribute to increasing the collective intelligence (Chateauraynaud and Debaz 2017). It is possible that the doubts about modern agriculture – to which science has largely contributed through innovations that increasingly favour performance – may lead to new expert roles to enhance the most scientifically grounded solutions with farmers' knowledge of their local area and practices.

Research that explores the diffusion of innovation emphasizes the importance of communication. For Everett Rogers (2003), the diffusion of innovation is a communication act. More than a technique's effectiveness, the diffusion of innovations will depend on users having access to reliable information. In other words, innovation introduces uncertainty, and anything that reduces that uncertainty supports decision-making. This is doubly important in an agricultural context where, as we saw earlier, predictability is expected of farmers.

At the farm level, there are two main sources of uncertainty regarding biocontrol. First is the interaction between techniques. A new technique does not exist in a bubble – it must be integrated into a technical environment. Not only will it replace certain existing techniques, it must also be compatible with all techniques used to address other issues. Second, a new technique, however effective, changes the way things are done. What may seem positive at the farm level may not be for farm workers, whose routines and work organization are disrupted and who may not have the necessary skills.

Farmers also face additional constraints, such as having to apply techniques and products according to specifications set out by a customer, or constraints imposed by global agricultural markets, which may even dictate the type of seed for each situation. Introducing biocontrol approaches implies a more systematic and systemic way of thinking, and may even require thinking on a smaller scale. Organic farming shows the importance of agricultural knowledge to establish more balanced cropping systems than monocropping systems.

Chapter 20
Integrating Biocontrol into Cropping System Design

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20.1 Introduction

While biocontrol has been very successful on high added-value crops (those grown under cover, viticulture, arboriculture, horticulture or floriculture crops), its applications in field crops remain very limited: *Trichogramma* wasps against the European corn borer *Ostrinia nubilalis*, Contans® WG against the fungus *Sclerotinia* in rapeseed, and biofumigation against the fungus *Rhizoctonia* in sugar beet or take-all caused by *Gaeumannomyces tritici* in wheat.

But regardless of the type of biocontrol, any solution based on interactions between organisms and the crop will only provide partial protection, unlike the instantaneous pest control achieved with chemical control. For this reason, and to ensure that biocontrol solutions are more effective, we believe it is necessary to develop biocontrol strategies that consider how farming practices, cover crops, pests and biological control agents all interact within the agroecosystem. This systemic approach to biocontrol requires managing the interactions between the biocontrol means used (substance, organism) and the properties of the entire agroecosystem:

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microclimate that promotes the action of a biocontrol agent, survival of the organism used, risk of proliferation and invasion of an introduced organism, combination of solutions with partial effects to fight the pest. Many farming practices (tillage, organic and mineral amendments, crop sequences, intercropping cash crops with companion plants, species mixtures) already play a role in the climatic, soil and biological characteristics of crop fields. These farming practices must be considered and modernized if we want to optimize the use of a biocontrol agent.

Choosing a biocontrol method therefore raises questions about the redesign and evaluation of cropping systems, and so we will begin this chapter with an overview of methods for redesigning low-pesticide cropping systems. The second part of this chapter will illustrate, through various examples, the interactions within the agricultural system and the need for a systemic approach to best leverage the chosen solutions. We will wrap up with the challenges of integrating biocontrol, both with regard to cropping system assessment and in terms of regulations and public policy.

20.2 Systemic Agriculture Concepts and Methods

20.2.1 Cropping Means Implementing Practices in a Systemic Way

The concept of the cropping system refers to the set of agricultural techniques implemented on a field (or on several fields treated in a consistent way) during several successive years (Sebillotte 1990). By using this expression, agronomists mean that all the techniques together “create a system”, i.e. that they are closely linked, to the point that often one cannot be modified without also having to modify several others. This coherent cropping system is developed by farmers, and can be linked to their production objectives, available resources, knowledge and know-how, the information they collect, and their interactions with many other players, who often strongly influence their technical choices (Meynard et al. 2001).

The first reason for coherence among cultivation techniques is that farmers implement them by considering them as part of their overall framework of objectives and available resources. Thus, barley for malting and barley for animal feed are not grown the same way: varieties, sowing dates and nitrogen fertilization methods differ between the two, depending on the specific quality requirements of the malting market. Similarly, a livestock farmer and a cereal farmer who both grow wheat will not do so in the same way. The first has livestock manure for fertilizer, while the second generally only uses chemical fertilizers; their workloads are not balanced in the same way according to the periods of the year, so one will defer certain activities (e.g. for the livestock farmer, spring plant protection treatments may compete with fodder harvests) that the other can carry out at optimal times.

A second reason for coherence among the techniques applied successively on the same field lies in how the agroecosystem itself functions. A direct line cannot always be drawn between techniques and the agroecosystem characteristics (of the environment, organism populations) that are affected; one technique can affect several characteristics. Tillage, for example, can alter water levels and aeration in the soil; modify the location of crop residues, which affects their decomposition as well as their infectivity; and strongly affect the soil's biological activity – destruction of macrofauna, stimulation of aerobic microbial populations, etc. – which is a major factor in soil development. A single agroecosystem characteristic can be influenced by several techniques. For example, the weed population of a cereal field depends on the crop sequences, tillage, sowing dates, weed control and competitiveness of the cereal, which itself is conditioned on its variety, density, fertilization, etc. This results in strong interactions among techniques, which must be considered in multifunctional agroecosystem management (Clermont-Dauphin et al. 2014).

Farmers have developed know-how in managing these interactions. The complex associations in "Creole garden" systems, which are common in places such as the Antilles and French Guiana, where the various species play complementary roles (attraction of beneficial organisms, nitrogen fixation, shading, etc.), are an extraordinary example of this. The knowledge on which this systemic management is based has diverse origins: agricultural knowledge disseminated by development organizations, producers' own personal experiences, traditional knowledge, etc. In general, the portrayal of the agroecosystem associated with industrial agriculture is extremely simplistic: for each "limiting factor" there is a corresponding input to manage it. Thus, mineral fertilization prevents nutrient deficiencies, pesticides control pests, and water deficits can be overcome with irrigation. Meanwhile, other means that could be used to manage water and mineral supply to crops or control pests fall by the wayside. The technical packages of the Green Revolution, as well as those related to intensive monocultures (maize, bananas, etc.), were built on these principles.

Farmers who do not understand a process or are unaware of these interactions within the system will not consider the full system in their technical reasoning. Biocontrol methods can easily fall into this trap. Pesticides cannot simply be swapped out for biocontrol products. Several techniques must be changed when implementing a biocontrol solution, not only to offset the less complete protection offered by biocontrol (less persistent than chemical pesticides and not as immediately curative), but also to optimize its effectiveness. For example, to reduce the use of fungicides and insecticides on wheat, simply adopting disease-resistant varieties will not suffice; farmers must also promote ground beetle populations through grass strips or reduced tillage. Sowing should be delayed to stagger the crop cycle in relation to that of pests. Early nitrogen fertilization, which promotes powdery mildew and septoria leaf blotch, should be reduced. Usually each technique used in an integrated pest management strategy is only partially effective, and the result is not nearly as dramatic as with pesticides. It is by combining several of these partial techniques and capitalizing on their interactions that it becomes possible to manage pests. How can cropping systems be designed to integrate biocontrol techniques as one means among many others?

20.2.2 Growing Crops with Biocontrol Means Redesigning the Agricultural System

The design of innovative cropping systems cannot be confined to a normative approach, which would have to be followed as a matter of course. Undertaking design work implies adapting the approach to the specific situation and the local context, borrowing from previous work with pragmatism and rigour. Meynard et al. (2012) note that the design of innovative cropping systems is categorized into two main families of approaches: "de novo" or "step-by-step".

De novo design seeks to invent high-performance systems without worrying, at least initially, about the transition involved in moving from the current system to the innovative one. The aim is to disrupt the system. Design workshops (Reau et al. 2012; Berthet 2013) offer very favourable conditions for de novo design, and make it possible to apply the range of knowledge associated with various trades or disciplines and their complementarity. Agronomic models also offer interesting options to support de novo design. Designers can use them to explore a huge array of technique combinations, sometimes far beyond what the top experts know, and gain insight into the long-term behaviour of the systems being created. These models can help pinpoint the combinations of technical choices, among a multitude of possibilities, that meet a given specification in terms of production, income, labour or environmental impact. Current research focuses on how to apply non-scientific knowledge to de novo design. For example, Martin et al. (2012) created a serious game called Rami fourrager® (meaning "forage rummy" in French) to aid farmers and agricultural consultants in designing forage systems.

In step-by-step design, the focus is on organizing change. The existing system is a starting point; it is gradually modified, based on learning loops (Meynard et al. 2012), into an innovative system that was not known in advance. The design work begins with a diagnosis to identify the key points of the cropping system to be improved (Which indicators are not satisfactory? Which processes are at play?), and to imagine the changes in practices or organization likely to improve the situation. Once these changes have been implemented, a new diagnosis is run to check whether the situation has improved and to determine new priorities, thus sparking a genuine loop of continuous improvement. Compared to de novo design, exploration is more cautious (sometimes too cautious), but has the advantage of adapting to the specific constraints of each farm. Step-by-step design lends itself well to gradually getting producers on board through a development approach. Producers, often supported by a technician or group of peers, develops their new system year after year while learning how to operate it at the same time. They gain an understanding of the system's benefits and can gradually reorganize their work and means of production (Mischler et al. 2009).

Whatever the chosen approach to designing low- or no-pesticide systems that incorporate biocontrol solutions, there will inevitably be some back and forth between identifying actionable knowledge and generating solutions that must be evaluated in silico (model-assisted design) and/or in the field. When biocontrol solutions replace conventional options, the link with the rest of the cropping system is fairly easy to organize. But when they must be seamlessly integrated with other farming practices – which is often the case – these design approaches are indispensable, and need to be based on knowledge of the interactions within the agricultural system. With this in mind, we outline below several innovations: those still being studied but not widely used, although we do already know how they fit in with other practices, or those that are routinely used but which require major system adaptations.

20.3 The Importance of the Systemic Nature of Biocontrol Solutions: Some Examples

20.3.1 In Horticulture, Releases of Organisms That Must Be Supported

There are many biocontrol solutions available in horticulture. A wide range of macroorganisms are sold commercially for different uses. The predatory mirid *Macrolophus pygmaeus* Rambur, for example, has been used for about 30 years to control pests in tomatoes grown in greenhouses. This polyphagous mirid prefers to feed on whiteflies (Lykouressis et al. 2009), but it can also attack mites (Enkegaard et al. 2001), aphids (Perdikis and Lykouressis 2002) and moths such as *Tuta absoluta* (De Backer et al. 2014).

However, the effectiveness of inundative releases of *M. pygmaeus* remains partial. To make protection strategies more robust, this approach often needs to be combined with other techniques such as mass trapping, parasitoid releases, mating disruption, microbial control, and the use of natural substances or semiochemicals (Trottin-Caudal et al. 2011; Pérez-Hedo et al. 2017).

For this natural enemy to establish quickly, it requires sufficiently high temperatures (Perdikis and Lykouressis 2000, 2002), food (prey, host plant or alternative resources) and compatible farming practices. However, certain interventions, such as deleafing (Bonato and Ridray 2007), the application of plant protection products (Martinou et al. 2014), sprinkler irrigation or the use of sticky traps, can disrupt the mirids' establishment. Conversely, other practices, such as heating greenhouses, late planting or providing supplementary food sources (*Ephestia kuehniella* moth eggs or *Artemia* sp. brine shrimp cysts), encourage the establishment of *M. pygameus*. The protection strategy must therefore consider these positive and negative interactions between practices to be effective.

Finally, conservation biological control is also possible used in combination with inundation biological control. *M. pygmaeus* is naturally present in the Mediterranean region and several studies have shown that its abundance in the environment can be promoted by planting flower strips, semi-natural areas and reservoir plants (Perdikis et al. 2011; Aviron et al. 2013; Alomar et al. 2002; Bresch et al. 2014; Messelink et al. 2014; Balzan 2017).

20.3.2 In Field Crops, Developing Biocontrol Means Rethinking the Whole System

Biocontrol is rarely used to protect field crops from pests; with regard to diseases in these crops, it is still mostly at the research stage and there are few commercial products on the market. For insect pests, botanical compounds and semiochemicals have been put forward as promising solutions for integrated pest management programmes (Vet and Dicke 1992; Pappas et al. 2017). For example, DL-β-Aminobutyric acid (BABA) has been shown to significantly reduce aphid populations (green peach aphid *Myzus persicae* and cabbage aphid *Brevicoryne brassicae*) and the larvae of two Lepidoptera species (cabbage looper *Trichoplusia ni* and diamondback moth *Plutella xylostella*) on Brassicaceae (Hodge et al. 2006), but once again, researchers are still studying these solutions to determine how they work. Finally, although natural enemies such as predators and parasitoids have been shown to reduce pest dynamics in arable crops (Bianchi et al. 2006), many of these solutions are still at the research stage, particularly with regard to increasing knowledge of interactions (see Chaps. 6 and 7).

20.3.3 Inundative Releases of Trichogramma *and Supporting Their Populations*

There is, however, one well-known example for maize growers: the egg parasitoids of the genus *Trichogramma*. These micro-wasps, which are discussed in detail in Chaps. 4 and 18, can successfully manage the European corn borer, *Ostrinia nubilalis*, when applied at the right time (Razinger et al. 2016). Inundative releases of *Trichogramma* are effective at controlling *O. nubilalis* populations, but can reach their limits in situations where other pests must be treated with plant protection products. Thus, chemical control must be well organized (by choosing the least toxic plant protection products and applying a minimum number of treatments) to affect these parasitoids as little as possible and not jeopardize their establishment in fields. Similarly, preventive treatment and regular monitoring are essential for the successful implementation of this biocontrol method. Other factors that can reduce the effectiveness of inundative releases of *Trichogramma* – unfavourable environmental conditions, poor dispersal of the micro-wasps and poor timing of releases with moth development (Collier and Van Steenwyk 2004) – can be circumvented by combining releases and habitat management (conservation biological control). Similarly, beneficial plants, whether used in intercropping or in a mixture, can also be a way to make the environment more suitable to the survival and dispersal of *Trichogramma*.

20.3.4 Rethinking the Field and the Surrounding Area for Aphid Control Means a Comprehensive Redesign

20.3.4.1 In the Field Itself

France banned the insecticide imidachloprid (a neonicotinoid) on 1 September 2018, resulting in an urgent need to address compatibility between different alternative methods of controlling cereal aphids.

Thus, using aphid-resistant varieties combined with aphid control by natural enemies deserves further examination: is synergy consistently observed? Biswas and Singh (1998) demonstrated a very interesting synergistic effect between a maize variety resistant to the sugarcane aphid (*Melanaphis sacchari*) and a parasitoid hymenopteran of the Braconidae family. The parasitoid had a higher reproductive rate on the resistant variety compared to the susceptible control variety. However, a recent study by Leybourne et al. (2019) showed instead a negative effect of partial plant resistance in a barley species on the oviposition rate of the generalist parasitic wasp *Aphidius colemani* (Viereck), which instead favoured the bird cherry-oat aphid *Rhopalosiphum padi*. The effect was not so much related to the expected decrease in the number of nymphs of the parasitoid due to the plant's resistance to the aphid, but rather to an indirect effect of the host plant in relation to its plant architecture.

One known strategy for managing cereal aphids is to diversify the crop cover by planting several plant species in the same field, specifically legumes alongside cereals. Farmers must follow timing and spacing patterns that can vary considerably, such as sowing different crops simultaneously or at different periods, using permanent cover or companion plants, etc. (see for example Iverson et al. 2014). However, if plant diversity within the field is increased, particularly by including legumes, the nitrogen input strategy will need to be completely revised, with a possible sharp overall decrease. Similarly, in terms of tillage, plant cover complexity limits the possibilities of mechanical equipment passing through during the crop season, particularly for weeding, in a context where it is assumed that no herbicides are used. This is why plant cover diversification often goes hand in hand with the implementation of conservation agriculture, characterized by longer rotations, permanent soil cover and no tillage. Thus, Chabert and Sarthou (2017) demonstrated in a conservation agriculture context that only the combination of these three pillars of conservation agriculture resulted in genuinely effective biological control of cereal aphids by natural enemies.

20.3.4.2 Around the Field

Implementing conservation biological control by taking the habitats around cultivated fields into account (see Chap. 7) is even less suited to simply substituting one practice for another and requires considering the full complexity of interactions within trophic chains (see Chap. 6). The result is that we must rethink the cropping system as a whole, and integrate it into nearby landscape components as well as at the territorial scale. A growing body of literature underscores that controlling key pests needs to happen by combining conservation biological control and other strategies, such as using parasitoids and trap plants (e.g. *Brassica rapa*; see Cook et al. 2013), which are planted to attract, divert or retain target insects or pathogens to reduce damage to the main crop. Additionally, flower-rich field margins have been proposed to improve natural pest control, as they can provide food sources, shelter and overwintering habitats (see Chap. 7). However, these approaches remain complex: the success of natural pest control of cereal aphids by natural enemies within the cultivated field has been observed to vary substantially depending on the species growing in the field margins, and not all natural enemies are equally supported.

It is clear from these examples that biocontrol solutions cannot be implemented as a single, one-off approach, precisely because, as explained in the “Systemic agriculture concepts and methods” section of this chapter, practices have concurrent effects on systems. And yet, to date, there are no studies that have explored all these intra-field practices together; studies investigating them at the intra- and supra-field levels are still in the nascent stages (see Chaps. 7 and 8). Biocontrol alternatives to pesticides can easily be adjusted incrementally, just as solutions in a conventional system. De novo innovative design will be essential for introducing more systemic solutions that impact both outputs (yields), objectives (pesticide-free) and evaluation criteria (acceptable losses, associated costs, satisfactory levels of effectiveness, etc.).

20.4 New Issues, New Methods and New Regulations

Biocontrol is one lever among many in an integrated crop protection strategy. As such, it cannot escape thorough consideration on how it is assessed and adopted. Research and development now faces new challenges given the various situations and possible strategies along with the likely diversification of future cropping systems (see examples above). The aim is not so much to develop “turnkey” innovations or ready-to-use biocontrol solutions but rather to create design methods, “integrated biocontrol solutions” and analysis tools that economic operators can use in innovation processes based on the characteristics of the systems in which they operate and their specific strategies. One of the first tools is assessment.

20.4.1 Biocontrol Use Calls for Revising How Cropping Systems and the Solutions Themselves Are Evaluated

How can we design "innovative systems" that are truly ground-breaking, but with the most solid and broadest possible multi-criteria assessment of their potential performance? How can assessment tools support innovative design processes and transitions to new solutions by serving as a mediation tool between designers, but also with users? Agricultural scientists have been exploring these issues for a long time now as farming systems become more complex and their sustainability is questioned. Over the last 15 years, there has been a real need to innovate and explore, as widely as possible, protection systems that break with current practices to reduce pesticide dependence. To support designers creating these more sustainable systems, several tools have been developed for ex-ante assessment, such as the DEXiPM model, which was designed to assess field crop systems according to the three pillars of sustainability: environmental, social and economic (Lichtfouse et al. 2009). Since experimentation is cumbersome and costly, designers can select the most promising candidate systems by drawing from information available upstream of system experiments from experimental stations or agricultural plots.

However, when introducing biological control solutions – the simplest of which may bring a more coherent perspective to the systems, or even be based on ecological processes at landscape level – merely assessing the basic pest mortality or yield improvement will not suffice. Measuring the "effectiveness" of biocontrol is more complex. First, this is because several pests are often targeted; because the survival of the introduced natural enemy and the survival of a community of "dependable" organisms in a landscape must be considered; and because these effects can be detected over the long term. Second, some solutions to be explored must be based on techniques that have not yet been finalized. Finally, biocontrol solutions may not be, in the short term, economically viable, ecologically efficient or socially acceptable, but could become so under certain conditions, with appropriate public policies or properly adapted supply chain strategies. How can the services rendered by these biological pest control mechanisms be assessed? How can we evaluate the performance of these systems, which are unthinkable today but will be indispensable tomorrow? Which indicators will be useful and usable? And for which stakeholders? In this context, the issues around assessing new technologies and biocontrol solutions – which cannot simply replace other options – in terms of their economic, social and environmental effects come into sharp focus. The aim is to shift from an incremental approach to assessment, based on the effectiveness/safety ratio of an individual technique, to a systemic and dynamic approach that takes into account a combination of techniques, a broader set of criteria and several environments. This is a real challenge for research and regulatory authorities in terms of:

- methods: new scientific approaches, methods and tools are needed for deeper understanding as well as a capacity for ex-ante and post-ante assessment of the systemic effects of new technologies and practices, including (and especially) when they break away from current practices;

- knowledge and its source: as cultural systems become increasingly complex, knowledge will most often be fragmented and sometimes limited to expert opinions; as such, there is a strategic component to combining qualitative and quantitative data in the same assessment process;
- how assessment tools are designed: stakeholders are closely and directly involved in the innovation process and have their own inclinations and assessments of innovative practices; given the range of strategies and preferences, this affects assessment methods and questions the normative nature of current regulatory procedures relating to marketing new technologies and innovation dissemination.

20.4.2 Reconsider How to Design and Include Farmers' Innovations

Given the major challenges of agriculture, and especially the need to reduce pesticide use, open innovation – defined by Chesbrough and Bogers (2014) as "a distributed innovation process based on purposively managed knowledge flows across the boundaries of functional structures" – has become increasingly important. The many stakeholders involved (farmers, research institutes, R&D and consulting firms, agricultural supply and processing companies, local authorities, environmentalists, consumers) are organized in networks where several objects (e.g. cropping and production systems, decision-making tools, agricultural equipment) are designed in a coordinated manner, through exchanges of knowledge and inventions. We are thus witnessing a real change in the innovation process, illustrated by the sharp rise in forums and interactive online tools (GECO-Écophyto, Agricool), thanks to the development of innovation networks that put farmers front and centre (economic and environmental interest groups, the DEPHY farm network in France, Base, etc.); R&D efforts that are focused on farmers' innovations to encourage others to adopt new methods (innovation tracking, workshops with farmers, etc.); the emergence of territorial "living labs"; and consumers working hand in hand with farmers and industrial players to develop innovative sectors. The creation of open innovation networks in particular explores systems agronomists' contributions to developing agricultural system design approaches suited to a range of situations, in order to support the agroecological transition (Salembier et al. 2018).

Recognition of producers' innovation capacities is an essential component of this framework (Prost et al. 2017). Farmers' resourcefulness will enable us to explore original biocontrol methods, resulting from their observations and hands-on learning, as well as to enrich our collective vision of the synergies among pest and disease management techniques. For example, Salembier et al. (2015a, b) highlighted the ingenuity of market gardeners in short supply chains who, having to produce many vegetable species to satisfy their customers, plant them together in complex arrangements to support natural enemy populations and limit the transfer of pests from one species to another. This goes back to our ability to identify, analyse and decontextualize innovative farming practices, taking into account not only the climatic, edaphic and landscape elements that condition their success, but also the logic of producers' actions, i.e. the indicators and decision-making rules they rely on and their reasoning. Methodological progress on these different aspects is essential (Salembier et al. 2015a, b).

20.4.3 Revise Economic Tools to Promote Biocontrol in Cropping Systems

Promoting biocontrol is not easy because, as this book shows, the problems associated with crop protection and the biological control solutions themselves depend on ecological mechanisms operating at scales involving complex field management as well as landscape composition and structure. Farmers must therefore act at both the field and landscape levels. At these scales, farmers' activities have both positive and negative externalities on neighbouring farmers. For example, practices employed by some that may be unfavourable to biodiversity but which support their chosen biocontrol techniques may limit the effectiveness of others' beneficial practices (Pagiola et al. 2004). The ecological results must be assessed over the medium term, with an increase in the variability of yields and profits. With a lack of certainty regarding yield outcomes, since biocontrol effectiveness is being reconsidered, farmers will be reluctant to adopt related practices. A regulator (public or private) wishing to promote biocontrol must consider these elements when choosing the economic instruments to implement.

Supporting biocontrol requires reducing practices with a negative impact on beneficial biodiversity (using plant protection products, destroying favourable habitats) and encouraging practices that improve biological control (redesigning cropping systems, using biocontrol products, developing agroecological infrastructure, etc.). Biocontrol products can be promoted through a more attractive tax system than that for plant protection products. Given the sector's negative view of binding regulations and taxation of plant protection products (Lockie 2013), subsidizing biocontrol products could lift certain barriers to their inclusion in cropping systems. However, tax policy alone cannot ensure that farmers will use these products, even if they are economically advantageous, because they may not be considered as simple substitutes for plant protection products, due to a lack of information or training on their use, or because their effect is more uncertain. Authorities will also need to provide information, advice and training. Using insurance mechanisms can also help limit the risks associated with the transition to biological control.

For conservation biological control, geographically clustered changes in practices (Donald and Evans 2006; Hartig and Drechsler 2009) can be promoted through payment schemes (subsidies), agro-environmental measures or payments for environmental services (Engel 2016), on the condition that practices that support biocontrol are implemented (e.g. agroecological infrastructure), and including an agglomeration bonus design (Parkhurst et al. 2002) or a multi-round auction (Reeson et al. 2011). Combining such measures also helps support the sustainability of natural enemy species by ensuring that farms maintain or add semi-natural elements. One interesting mechanism is to offer either cooperative contracts in which payment is granted if the targeted environmental level is achieved overall (Dupraz et al. 2009), or schemes that combine a voluntary commitment to a common environmental objective with the threat of a tax or subsidy reduction if the objective is not achieved (Segerson 2013). Encouraging collective commitment through an economic and environmental interest group (MAAF 2017) reduces the problems of externalities and provides a forum for learning and support to encourage farmers to adopt exploratory approaches and identify viable solutions (Stallman 2011).

Since farmers can generally expect an economic gain from biocontrol, permanent monetary aid is unnecessary, whereas advice and information are indispensable. Payments may eventually be phased out due to the positive impact of biocontrol on farms' future finances. Support through public payments compensates changes in the productive system rather than simply covering production losses due to lower yields.

20.5 Conclusion

This chapter demonstrates that biocontrol can only be integrated into current systems if done in a systemic way. Within agricultural systems, practices must be considered with respect to each other in order to ensure introduced organisms survive, to maintain those already present, and to stimulate plant defences. Designers must work with and support farmers to come up with effective combinations of solutions to limit chemical pesticides. Economic and policy-based support are also necessary.

Chapter 21
New Technologies for the Deployment of Extended Biocontrol

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21.1 Introduction

Among the challenges related to biocontrol deployment outlined in Chap. 18, several point to a need for technological development on at least three key and interdependent fronts:

- technologies and know-how combining biocontrol and seeds, with seeds as both an object of protection and a possible application vector;
- formulation technologies applied to biocontrol substances and organisms;
- developments in agricultural equipment, robotics and digital technology to optimize and control biocontrol application on a local and regional scale.

These three fronts converge towards the same need: to use biocontrol in the right place and at the right time. In this chapter, we attempt to lay out the conceptual and technical principles for each of these fronts to show why they are important for extended biocontrol, to identify the most promising prospects, and finally to underscore their interdependence.

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21.2 Seeds: A Target of Treatment and a Biocontrol Application Vector

21.2.1 The Strategic Importance of Seeds

Uniting the biocontrol innovation and seed sectors is key to biocontrol deployment for several reasons. To begin with, the scientific and industrial communities dealing with seeds are working to develop new strategies to maintain seed and seedling quality without using substances that are harmful to human health or the environment. This should turn the seed market into a powerful vector for innovation in biocontrol. Additionally, using seeds for biocontrol entails technologies (including formulation) that will facilitate the general development of biocontrol methods. Finally, seeds themselves could also be a vector for transferring organisms or biocontrol substances to the field, or a means of modifying local microbiota to favour certain communities that are beneficial to crops.

In this chapter, we will discuss several levers that are relevant to developing biocontrol solutions, with a focus on the influence of the mother plant on seed quality and the use of seeds as a carrier for biocontrol solutions. All these aspects contribute to protecting seedlings and the future plants during crop establishment.

21.2.2 Activating Immunity in Seed Plants and Seeds: A Lever for Extended Biocontrol

A first lever to counter contamination is the immunity of the different tissues of the seed itself. The activation of immune defences in the early stages of embryogenesis was demonstrated in the interaction between the bacterial species *Xanthomonas campestris* pv. *campestris* and barrel medic (*Medicago truncatula*) seeds (Terrasson et al. 2015). In seeds, basal defences are activated from embryogenesis onwards and constitutively expressed during imbibition, particularly with the production of secondary metabolites (e.g. phytoalexins) or cell wall reinforcement (Ahuja et al. 2010; Bolingue et al. 2010). Varietal selection is one possible way to increase the effectiveness of these defensive responses.

A second solution to reduce contamination and the spread of diseases can be achieved by stimulating plant defence mechanisms during vegetative development (also known as priming). Priming the defence mechanisms is a low metabolic cost measure, because defence responses are not (or only slightly and transiently) activated by the priming stimulus (Martinez-Medina et al. 2016). However, after stress is perceived (trigger stimulus), plants deploy their defence responses faster, stronger and/or more durably (potentiating effect). Recent studies have shown that defence priming can be transmitted over several generations, indicating an epigenetic component to the phenomenon. This priming improves the defensive response in seeds produced by the stimulated plants, and is likely to decrease contamination at the seed level.

However, these solutions cannot prevent external contamination of seeds, either in the field or post-harvest. To reduce such risks, crop management sequences are developed to decontaminate or reduce the bacterial and fungal load on seeds.

21.2.3 Seeds as a Target and Vector for Biocontrol Solutions

The first seed treatment techniques date back to antiquity, and since then these techniques have evolved and diversified (for a review, see Sharma et al. 2015). They aim to reduce the load of naturally occurring pathogenic microorganisms as well as protect and physiologically prepare seeds for better germination and seedling establishment. There are different treatments: heat-based (steam or immersion in hot water baths), radiation-based (such as gamma radiation, UV light, laser and more recently cold plasma) (Siddique et al. 2018), and pelleting and film coating (Sharma et al. 2015).

Pelleting and film coating differ in the thickness of the matrix applied. Pelleting is characterized by a larger matrix that makes handling easier (standardized size and weight) (Pedrini et al. 2017). Both pelleting and film coating aim to deliver active substances. Various active ingredients have been described in the scientific literature (Pedrini et al. 2017; Scott 1989). They can be classified according to their effects on plants:

- protective substances: conventional plant protection and biocontrol products (Bamisile et al. 2018; Sharma et al. 2015);
- nutrients: macro- and micronutrients (Scott 1998);
- plant stimulant microorganisms: plant growth promoting rhizobacteria (PGPR) or plant growth promoting fungi (PGPF); for a review, see Le Mire et al. (2016);
- adjuvants, which locally modify the physicochemical conditions of the soil (Mangold and Sheley 2007); and
- dyes, which are used to differentiate between treated seed batches and deter certain pests (Pedrini et al. 2017).

The scientific literature points to numerous avenues for plant protection against pathogens by microorganisms or botanical substances applied to seeds (Sharma et al. 2015). However, few studies include more than one model plant in their trials (Mao et al. 1998; Tinivella et al. 2009) or the genetic diversity of the plant species considered (Dubey et al. 2007). Similarly, little attention is paid to pathogen diversity within a species or the synergy of different biotic and abiotic stresses (Bardin et al. 2015; Lamichhane 2015). However, these aspects are important for generalizing biocontrol solutions and assessing the sustainability of protection mechanisms (Bardin et al. 2015). When developing seed treatments using plant defence stimulators, applying elicitors such as jasmonic acid can generate increased resistance to various types of pests, including aphids, caterpillars, mites and fungal diseases (Worrall et al. 2012).

Seeds represent a particularly interesting stage of cultivation to enhance the value of the microbiota, which have demonstrated beneficial effects on plant nutrition and health (see Chaps. 9 and 10). The use of endophytic microorganisms for their potential as biocontrol agents is promising (Card et al. 2015; Orozco-Mosqueda et al. 2018). These microorganisms, which can colonize the tissues inside the plant, evade the strong competition within the rhizosphere (Sessitsch et al. 2019). Their proximity to the plant tissues would give them a greater influence than rhizosphere microorganisms on plant physiology (Orozco-Mosqueda et al. 2018). Plant protection microorganisms could be incorporated into the seed microbiota when the plant is in the floral stage, by sprinkling (Mitter et al. 2017) or via infected pollen (Hodgson et al. 2014) or pollinating insects (Prado et al. 2019; Shafir et al. 2006). However, these modes of application do not guarantee the same level of colonization for each seed. Alternative processes can be considered, such as injecting microorganisms into the plant tissue (e.g. see the patent WO2017162758A1 for “seed injection”; Orozco-Mosqueda et al. 2018).

In the future, a combination of different biocontrol solutions will guarantee seed quality. Actions can be carried out via the plant (defence gene activation, secondary metabolite production, microbiota optimization, etc.) as well as via the seeds, by developing treatments that combine several mechanisms of action: for example, optimizing the physicochemical conditions of the soil near the seed to promote germination or directly supplying beneficial microorganisms and nutrients likely to guide the rhizosphere microbiota (Ab Rahman et al. 2018).

21.3 The Challenge of Formulating Biocontrol Substances and Organisms

21.3.1 Formulation: An Overview

Formulation is the art of turning an active ingredient into a stable, effective and easy-to-use product. The process includes all operations involved in mixing or shaping ingredients (Table 21.1). Formulation can provide:

- stability: the active ingredient can be stored longer by farmers and remains active longer when used. As a result, manufacturers can better manage production while farmers can use the same product from one year to the next with better results;
- performance: the active principle is more effective. Typically, the aim is to better target the plant or pest, avoid leaching and promote contact between the active principle and the target pest, etc.;
- ease of use: the active ingredient is simple to use. Conventional synthetic chemicals are often very user friendly: simply pour a container of product into

Table 21.1 A few examples of formulations used in agriculture

Name	Description
Wettable powder (WP)	A powder formulation that forms a suspension when mixed with water prior to spraying.
Emulsifiable concentrate (EC)	An active ingredient dissolved in a solvent with emulsifiers.
Suspension concentrate (SC)	A solid active ingredient dispersed in water.
Soluble liquid (SL)	A homogeneous liquid formulation intended to be applied after being diluted in water as a true active ingredient solution.
Water dispersible granulate (WDG)	A solid, non-dusty granular formulation which disperses or dissolves quickly when added to water in the spray tank to give a fine particle suspension.
Oil dispersion (OD)	A solid active ingredient dispersed in oil.
Microemulsion (ME)	A stable oil-in-water or water-in-oil emulsion with a droplet size between 0.01 and 0.05 μm.
Capsule suspension (CS)	An insoluble active ingredient dispersed in water.

Source: Definitions adapted from Croda Crop Care (2021)

a sprayer. The objective is the same for biological control: to make product use as easy as possible;

- controlled release: the active ingredient must be released at the right time (or gradually) and in the right place. Farmers would much prefer products that only have to be applied once for a long-lasting effect to cut down on labour and fuel costs;
- protection: with regard to the compound against the environment (some active ingredients are sensitive to oxidation or photo-oxidation) or the environment against the compound (one typical example is protecting users from dust);
- combined options with other products.

21.3.2 Formulation for Extended Biocontrol: A Challenge for Industry

The active substances used in conventional agriculture are most often small organic or inorganic compounds. Agricultural chemistry has developed a wide range of formulations. The equipment used to apply treatments (which are mainly liquid based) is designed with a system for incorporating, mixing and spraying these active ingredients on crops. However, there are two major drawbacks to these sprayer systems, which condition product applicability. First, sprayers have an extremely high level of filtration to limit clogging in the nozzles. Second, the pressurized system has valves (pump, regulator, etc.) that can generate heat, which can be harmful to certain encapsulated products or living organisms (Beck et al. 2013).

The active substances used in extended biocontrol – biomolecules, macroorganisms (insects or nematodes) and microorganisms – are very different to conventional compounds. Biomolecules include natural extracts (see Chaps. 12, 13, and 14) and semiochemicals such as pheromones (see Chaps. 15 and 17). While some active ingredients work in a similar way as conventional pesticides (instantly effective), others have a unique mode of operation. Pheromones are a good example: they must be diffused throughout the entire flight of the adult pests (e.g. pine processionary, *Thaumetopoea pityocampa*) or cover several flight periods to effectively protect a crop, sometimes over periods of several months. Such constraints differ considerably from a treatment that works immediately upon application, and agrochemical formulation technologies are not suitable because they have been developed to protect compounds from oxidation rather than to diffuse them over several months. For pheromones, manufacturers have developed formulations for use with dispensers with a wax or polymer medium (Pherobank, BASF, M2I). However, these dispensers often must be set up by hand at the right time of the season. This is rather labour intensive and has resulted in calls for technological developments that we will cover in last part of this chapter: automated field deployment; the design of specific equipment for users, which must take into account the specificities of product formulation; diagnostic and decision support tools to facilitate use at times and places that optimize target pest management.

Macroorganisms, such as natural enemies or nematodes, are emblematic of the differences between biocontrol products and conventional products. Macroorganisms can be stored from a few days to a maximum of 3 months at temperatures not exceeding 10 °C. Typically, nematodes must be kept in moist, aerobic conditions while preventing the growth of bacterial contaminants; they do not survive freezing and rarely survive drying (Peters 2016). A few innovative formulation possibilities have been investigated, notably using alginate beads (Patel and Vorlop 1994), but without success. Immobilization in a gel that a farmer would destabilize before spreading by adding a compound has been put forward, before being abandoned because it is too complex (Peters 2016).

For microorganisms used in biocontrol, formulation plays a particularly important role in compound stability. The challenge here is to immobilize living organisms over long periods (ideally several years) without loss of viability, so that they become active once applied in the field and are effective against their target despite climate condition variability. Some of the first biocontrol microorganism products sold commercially were wettable powder formulations. Farmers have been slow to adopt them because they are difficult to use (the finely-ground powders are dusty and hard to mix in the tank) and produce inconsistent results. Manufacturers then developed dispersible granule formulations, which are simpler to use. However, such progress has often come at the expense of reduced microorganism concentrations, with a corresponding drop in performance. This is explained on the one hand by the need to add material to form the matrix, and on the other by the granulation process (which involves a rise in temperature, which affects microorganism viability). However, only a small fraction of microorganisms are applied in this way. The encapsulation techniques developed by the agrochemical industry, i.e. trapping within a polymer matrix, are not suited to microorganisms. Most of these capsules are actually smaller than the microorganisms themselves (by a few microns). These processes also often involve mechanisms that are not compatible with living organisms. Microorganisms impose certain constraints in terms of stability, both during storage and in the field. The right formulation and process must be identified to be able to keep microorganisms viable for several years, and possibly protect them from UV light in the field.

Sustainable agriculture emphasizes the use of biobased and biodegradable active ingredients and materials that minimize environmental and health impacts. This affects the active ingredients and rules out nearly all conventional agrochemical formulations. As a result, manufacturers are increasingly turning to compounds such as oils, waxes and biobased polymers to formulate active ingredients. This also implies managing natural resources. In France for example, brown algae, from which alginate is extracted, is monitored by the French National Institute for Ocean Science (IFREMER), which grants harvest quotas to seaweed farmers. Achieving sustainable formulations is a major challenge that is often given little attention in the biocontrol debate. In some countries, kerosene is still used to formulate spores of beneficial fungi of the genus *Beauveria* (Biaggioni Lopes and Faria 2019).

21.3.3 The Future of Formulation in Extended Biocontrol

Three trends are emerging in the biocontrol innovation sector and are likely to address the challenges related to formulation.

First, the development of robotics, which is described at the end of this chapter, is an interesting avenue that could lift various formulation constraints. For example, a drone can deposit pheromone dispensers, thus freeing farmers from this laborious process.

As is often the case in this type of context, some players are able to think totally outside the box and offer different approaches that generate new needs. Companies such as BVT (Bee Vectoring Technology 2021) and Lallemand Plant Care (Lahdenperä 2020), in partnership with the company Biobest, have developed products that are distributed by pollinators (known as entomovectoring). Other options of formulating substances or microorganisms that must pass through and possibly persist in macroorganisms are currently being investigated.

Finally, we would like to underscore that research and innovation on formulation raise specific issues. Research on formulation technologies is quickly becoming competitive for industry and has mainly been kept in-house by manufacturers (especially in agrochemical industry). As things now stand – likely because of this situation – there are relatively few academic research teams working on formulation technologies for sustainable agriculture. Those that are tend to revisit known methods, including immobilization in hydrogels (Szczech and Maciorowski 2016). This method has been known for more than 15 years (Fravel et al. 1998), but industry players remain unconvinced. Nor do manufacturers appear interested in any of the other methods listed in a recent review (Vemmer and Patel 2013), including thermal and ionic gelation, spray drying, complex coacervation, silicon-based sol-gel chemistry, thermal gelation and emulsion polymerization, and layer-by-layer coating. The review's authors point out that one prospect would be to develop new copolymer gels for the controlled release of active ingredients. It is of interest to note that this option is being successfully explored in the pharmaceutical industry by MedinCell, with its BEPO® technology. Meanwhile, there have been several recent advances in France resulting from skill transfers between the public and private sectors and public-private R&D collaborations. For example, a start-up called Kapsera, created in 2018 in partnership with ESPCI Paris (Paris school of industrial physics and chemistry), is working on an encapsulation technology designed to be universal (the capsules can contain an aqueous or oily phase) and specifically adapted to each microorganism. Another example is an emulsion method produced by the company M2i that stabilizes pheromones during storage and allows them to be released over several months (Locatelli-Champagne et al. 2017).[1] M2i is very active in creating and manufacturing innovative pheromone-based formulations. It has developed a product that remains active for 4 months to control pine processionary (*Thaumetopoea pityocampa*),[2] and is currently testing a product to control vine moth (*Eupoecilia ambiguella*).

21.4 Agricultural Equipment, Robotics and Digital Technology to Optimize Biocontrol

When biocontrol agents are formulated to be compatible with the applicability specifications for conventional plant protection products, they can be applied with an acceptable level of efficiency using standard crop treatment equipment. "Applicability" refers to all the properties of solubilization, foaming power, clogging limitation and ease of cleaning. Certain substances used to stimulate natural plant defences or small microorganisms (e.g. bacteria such as *Bacillus thuringiensis*) are generally compatible with these characteristics. Nevertheless, they do bring up original research questions depending on their mode of action. Issues related to distributing deposits within the plant canopy generally appear to be more complex in terms of ensuring optimal effectiveness. Products with a contact mode of action have a shorter protection period and are more sensitive to weather conditions (risk of rain leaching) than products with a more systemic mode of action. Finally, for larger organisms such as nematodes, the spraying process leads to questions about the

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[1] https://agri-startup-summit.com/wp-content/uploads/2017/06/general-presentation-of-M2i-1.pdf (accessed 15 June 2021).

[2] https://ec.europa.eu/environment/awards/downloads/EUBusinessAwards_M2i-LD.pdf (accessed 15 June 2021).

possible loss of viability due to passage through various sections of the hydraulic system, and in particular the risk of shearing at the nozzles (Beck et al. 2013).

Specific problems, such as clogged filters and nozzles, may also occur. With larger organisms, there may be issues regarding optimal drop size for transport to their target and the behaviour of drops loaded with particulate organisms on impact. Air-injector nozzles traditionally used to produce large drops at low flow rates also come with their own set of challenges. These considerations are stimulating research on alternatives, such as nozzles equipped with pulse width modulation valves ensuring discontinuous spraying at frequencies of several dozen hertz. The prohibitive workload – a barrier when it comes to biocontrol products with a short duration of action – could benefit from research on solid set canopy delivery systems placed over or in the foliage of perennial crops (Sinha et al. 2019) and help to further develop this technology. However, what is certain is that spraying, a major vector of conventional crop protection, can only cover a small range of expressed needs to ensure the full potential of biocontrol deployment.

Digital and robotic tools offer many opportunities to develop technologies that could be used with extended biocontrol. The main possibilities are absolute positioning, digital sensors and smart devices.

The widespread use of absolute positioning accuracy gives global navigation satellite systems an important role in automating farming tasks. The ability to deploy biocontrol agents on a large scale at the right time is one guarantee of their performance. Automating the trajectory and required action opens the door to overcoming the inherent workload constraints and implementation costs. Applying *Trichogramma* wasps to manage the European corn borer (*Ostrinia nubilalis*) using drones is emblematic of these new possibilities. The swift development of a range of services to apply capsules containing parasitoid eggs is a more targeted alternative to helicopter releases and less tedious than manual releases. It could soon become competitive in this market and should, as the technology matures and components are standardized, facilitate access to this type of biocontrol.

Rapid developments in optics and digital imaging, as well as signal processing techniques and algorithms for extracting the relevant information, create additional prospects for extended biocontrol. In the field of disease surveillance, spectral information is used to monitor plant health by exploiting reflectance variations induced by biological changes in plant organs, while spatial information makes it possible to quantify the severity of attacks and refine pathogen identification. Regarding crop pests, stakeholders can rely on imaging to identify and count attacks, mainly by arthropods, whether at trap level or in the crop itself. Some new technologies aim to use automated identification to manage differential physical destruction of pests, such as by using a laser transmitter (Mullen et al. 2016). However, detection principles and methods can be extended to the various pest phyla currently used for counts (see for example the Limacapt connected sensor for monitoring slugs sold by De Sangosse).

Connectivity is an important paradigm shift in farming, where information can be exchanged automatically and facilitated by cloud storage. As a result, there has been an explosion in the supply of commercial solutions for connected sensors for agrometeorological observations that centralize data at the local farm level via an online platform. Among the usual measurements, rainfall, soil water content and dew condensation are data for which farmers need to have the most relevant local information possible to feed into decision support and warning tools. Continuous measurement of parasite pressure is a priority objective to complement the range of local information collected automatically by these devices. Pest population dynamics can be measured via automated counting of insects in connected traps or slugs in the field. Similarly, for microorganisms, various projects aim to detect pathogen spores in the air to measure parasite pressure and thus optimize intervention timing. This information is recognized as necessary for optimal risk management using biocontrol agents, which offer smaller windows of opportunity for treatment than the preventive protection from conventional solutions. They also allow for shared risk management on a regional scale that would tolerate crop losses in unusual climatic or parasitic situations.

Connected devices also create new opportunities for applying biocontrol agents. The case of gaseous agents is emblematic of the potential contribution of these technologies. Semiochemicals (see Chaps. 15 and 17) provide many pest control options, from scent disruption to attracting or repelling insects. Conventional approaches to diffusing semiochemicals from porous matrices are easy to implement, but come with a lack of emission controllability. As a result, these compounds, which can be expensive, are not necessarily emitted at the desired rate at the right time based on needs. Furthermore, because semiochemicals are often composed of mixtures of various substances with different vapour pressures, the emitted composition is likely to vary over time, thereby altering effectiveness. Mega-dispensers, in which the mixture is stored in a pressurized container, partly address these challenges by determining the emission at the desired times. Since the costs associated with purchasing and implementing dispensers limit the number of economically viable emission points, optimizing dispenser position, emission rate and timing could be useful. The development of diffusers connected to low-speed communication networks offers the possibility of mobilizing chemical ecology knowledge to adapt semiochemical diffusion to protection objectives. In atmospheric dispersion models using data from meteorological stations, air concentrations can be controlled by taking meteorological conditions into account. Moreover, the possibility of adjusting semiochemical emission makes it possible to deploy strategies on a wider scale by coordinating several local networks over the Internet, such as based on warnings on epidemiological thresholds.

21.5 Conclusion

The three areas of technological research presented above are only a small part of the vast field of research that must be cleared to ensure that all extended biocontrol methods used in sustainable production systems can flourish. However, we have highlighted them here for two specific reasons. First, these areas harness skills that are still under-represented in the biocontrol research-innovation community, which means that specific actions from both industrial and public players are required: training, recruitment, new collaborations, acquisition of subsidiaries, etc. Second, these areas break down the silos between research and innovation on biocontrol with regard to the other main agroecological levers: plant genetics (seeds and biocontrol), robotics, digital technology and agronomy, with the design of cropping systems (for the other two areas covered in this chapter).

Part VII
Is Biological Control a Sustainable Crop Protection Method?

Christian Lannou and Thibaut Malausa

At a time when society, backed by public authorities, is calling on agricultural stakeholders to reduce their use of synthetic chemical pesticides, biological control methods are most often perceived as more environmentally friendly plant protection alternatives. The argument is that biocontrol uses organisms or substances that are naturally present in the environment. Compared to synthetic pesticides, which are formulated with active ingredients that are chemically modified to be more biocidal, persistent and resistant to climate conditions, one might expect that natural, unmodified substances would be less harmful for many reasons: perhaps they would be more rapidly degradable and more easily metabolized by exposed communities, with fewer unpredictable effects because they are composed of molecules that many living organisms are already exposed to, etc. Such assumptions, although intuitive, are not always supported by scientific data, due to the lack of research on these topics. There are also natural substances that are known to be highly hazardous to health (e.g. arsenic) and some biocontrol organisms have become invasive species in certain cases (e.g. the Asian lady beetle *Harmonia axyridis*).

In addition to their effects on non-target organisms, pesticides have another drawback that the public is less aware of: their frequent use leads to the emergence of resistance in pest populations. A parallel can be drawn with the emergence of antibiotic resistance in human pathogens. This is not a problem for practitioners alone – a drop in effectiveness can result in a need for higher doses or more frequent applications, or even a return to products that are more harmful to the environment.

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Can biocontrol methods enable agriculture to maintain the long-term technical effectiveness of the solutions used while simultaneously protecting the environment?

This is a critical issue that can both support the credibility of the approaches that are or will be put forward and produce sound standards regarding their sustainability. The previous chapters showed the potential of biocontrol to make agriculture more environmentally friendly. However, we must be able to anticipate possible risks or deal with lower product effectiveness. Chapter 22 focuses on the health and biodiversity risks associated with the use of the bioinsecticide *Bacillus thuringiensis*, one of the most widely used bioinsecticides in the world, as well as its derivatives. Chapter 23 then examines whether biocontrol solutions are sustainable, particularly with respect to evolving pest populations.

Chapter 22
Health and Biodiversity Risks Linked to a Major Bioinsecticide: *Bacillus thuringiensis*

Mathilde Bonis and Armel Gallet

22.1 Introduction

Agricultural practices are shifting towards reduced chemical pesticide use in favour of biological control alternatives, which are considered more environmentally friendly. Biopesticides are already increasingly being used to fight pests and weeds. Although biopesticides currently account for only 5–7% of all plant protection products used worldwide, they are projected to reach a 50% share of the global pesticide market by the early 2050s (Damalas and Koutroubas 2018; Willer and Lernoud 2018). Various government incentives seek to support this shift. For example, France's Ecophyto 2 action plan aims to halve the use of chemical pesticides by 2025, which would result in a significant increase in the use of biopesticides and therefore their release into the environment.

22.2 *Bt* Biopesticides

Bacteria of the genus *Bacillus* are a promising source of effective biocontrol agents. Indeed, many of them have properties that are useful in agriculture, such as stimulating plant growth, activating plants' natural defences, or protecting them against various pests by producing antibiotics, antifungals and entomopathogenic substances (Shafi et al. 2017). In addition, *Bacillus* species are generally used in the form of spores, which are easy and inexpensive to produce and store. One of them,

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Bacillus thuringiensis (*Bt*), tops the world's biopesticide market, with more than 50% of the total market share (Lacey et al. 2015). It is used on 69% of certified-organic cropland (Le Douarin 2017). The uses of *Bt* extend beyond simply controlling various insect pests (mostly Lepidoptera and Coleoptera) in conventional or organic agriculture. It also has important biocidal applications in vector control against mosquito larvae (genera *Aedes* and *Culex*), as well as in forestry against processionary moths (*Thaumetopoea processionea* and *pityocampa*).

Bt was first isolated in 1901 in Japan in silkworms (*Bombyx mori*), and then re-isolated about 10 years later near Thuringia, Germany, from a population of Mediterranean flour moths (*Ephestia kuhniella*). The bacterium was quickly characterized for its entomopathogenic properties due to the presence of crystalline toxins produced during sporulation, thus demonstrating its potential usability. The specificity of action of certain *Bt* strains against insect pests then led to the selection of these strains and their commercial use by as early as the 1950s in the United States (Milner 1994). *Bt* products consist of a mixture of *Bt* spores and crystalline toxins (50–80% of the formula), along with adjuvants (such as naphthalene-1-sulfonic acid) to enhance product wettability and dispersion during application. *Bt* bioinsecticides target the digestive tract of the target insect larvae. After larvae ingest the product, the crystalline toxins are dissolved at basic pH and the toxins are activated by proteases in the gut. Once they bind to specific epithelial receptors, they alter the epithelium via cell lysis, allowing the bacteria to cross the gut barrier and penetrate the internal environment. The insect dies within a few days from septicaemia.

22.3 Possible Links to Health Risks Associated with *Bt* Products

Bt products are applied by terrestrial or aerial spray application techniques. The more they are used, the higher their presence in the air, water and food – three potential sources of contamination for humans and animals. Furthermore, because the crystals are unstable, additional applications are often required, resulting in the accumulation of spores and a higher bacterial load. The real effects, both short and long term, of exposure to *Bt* products, even at low doses, with regard to the health of non-target organisms and the environment therefore seem to be a legitimate concern. As early as July 2013, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) highlighted the dearth of studies on the long-term effects of *Bt* bioinsecticides and the probable underestimation of the involvement of *Bt* in foodborne outbreaks (ANSES 2013), an opinion shared by the European Food Safety Authority (EFSA) in 2016 (EFSA Biohaz 2016). This hypothesis is supported by the fact that *Bt* belongs to the ubiquitous *Bacillus cereus* (*B. cereus*) group of bacteria, considered to be the second leading cause of foodborne outbreaks in France (Santé publique France 2017) and the third in Europe after *Salmonella* spp. and *Staphyloccus aureus* (EFSA and ECDC 2018). In 2017, EU Member States reported

298 cases of foodborne outbreaks due to *B. cereus*, linked to 3184 human cases, 189 hospitalizations and one death. *B. cereus* can also cause non-intestinal diseases such as eye infections (Callegan et al. 2017) or septicaemia associated with nosocomial infections in older or immunocompromised people (Bottone 2010; Glasset et al. 2018; Lotte et al. 2017).

Foodborne outbreaks due to *B. cereus* are generally associated with two main types of clinical manifestations: the first, emetic syndrome (due to the ingestion of cereulide, an emetic toxin produced by *B. cereus*) and the second, diarrhoeal disease (linked to the synergistic action of the enterotoxins Nhe, Hbl, Hly and CytK secreted by *B. cereus* in the host intestine) (Berthold-Pluta et al. 2015). Diarrhoeal symptoms are prompted by the ingestion of *B. cereus* spores. The acid pH of the stomach and the local immune defences of the upper part of the small intestine eliminate the vast majority of vegetative bacteria. But *B. cereus* spores can survive these hostile conditions and eventually reach the lower part of the small intestine, where the conditions are suitable to their germination and even proliferation. However, the secretion of enterotoxins by the vegetative cells of *B. cereus* in the intestine does not seem to be systematic. Several factors can modulate the diversity and quantity of enterotoxins produced: the number of vegetative (i.e. non-spore-forming) cells, the physicochemical environment, and the commensal flora (Ibid.).

To date, several studies have shown that *Bt* is capable of producing major enterotoxins associated with diarrhoeal-type foodborne outbreaks (Cytk2, Nhe and Hbl), suggesting that it may have the same virulence potential for humans as other *B. cereus* species (Damgaard et al. 1996; Johler et al. 2018). Although strongly suspected, *Bt*'s involvement in the occurrence of foodborne outbreaks has not yet been specifically proven. Routine laboratory tests carried out during foodborne outbreak investigations cannot distinguish *Bt* from other *B. cereus* species, so its potential implication is likely underestimated. However, several studies have produced strong evidence. For example, a Canadian study re-analysed 39 cases of foodborne illness attributed to *B. cereus*. The researchers identified *Bt* in four cases (10%) and mixed contamination (*Bt* and *B. cereus*) in 11 cases (McIntyre et al. 2008). One highly publicized case was a 2012 episode of a foodborne outbreak in a family in Germany, where a commercially available *Bt* strain (in this case, SA-11), isolated at a level of 3×10^4 CFU/g[1] of lettuce, was strongly suspected to be the cause of the associated symptoms (EFSA Biohaz 2016). However, this conclusion is still under debate, particularly in light of the probable concomitant ingestion of another *B. cereus* species by other family members (Raymond and Federici 2017). Additionally, a study in New Zealand of nearly 300 people living in an aerial *Bt* treatment area found that 61% of people complained of diarrhoea and stomach upset (Petrie et al. 2003).

Bacteria from the *B. cereus* group are also considered to be opportunistic pathogens in mammals. Thus, like *B. cereus*, *Bt* has been identified in pulmonary infections or superinfections in mice and humans (Barfod et al. 2010; Ghelardi

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[1] CFU: colony-forming unit.

et al. 2007), as well as in cases of nosocomial infections with respiratory complications or diarrhoea reported in humans (Bottone 2010; Celandroni et al. 2014). Furthermore, *Bt*, like *B. cereus*, is naturally resistant to a number of antibiotics, including those from the penicillin family, making it difficult to treat patients (Luna et al. 2007). Finally, it is important to point out that numerous studies have shown the harmlessness of crystalline toxins to mammals, including humans, over a short period of time (i.e. absence of acute toxicity) (Rubio-Infante and Moreno-Fierros 2016). The symptoms described would therefore be attributable more to the bacteria itself than to the presence of its crystalline toxins.

22.4 Possible Sources of *Bt* Contamination

Because *Bt* is naturally present in soils (soilborne species), the hypothesis of an agricultural origin of *Bt* strains isolated from food is sometimes also questioned. But several studies do support this hypothesis. For example, Johler et al. analysed *Bt* isolates from seven foodborne outbreak episodes in 2018 and showed that five of the *Bt* isolates were indistinguishable from certain commercial strains (based on toxin and genetic characterization profiles), as were eight of the 24 *Bt* isolates collected from processed foods and vegetables, irrespective of the context of the outbreaks (Johler et al. 2018). Among the five cases of food poisoning, one involved the same strain as the reported case of the German family, for which the traceability of the lettuce batch allowed the producer to confirm the application of the suspected *Bt* product (EFSA Biohaz 2016). Danish scientists also observed that a large proportion of "*B. cereus*-like" species (39%), isolated from local fresh fruit and vegetables, belonged to the *Bt* species and that almost half of these *Bt* bacteria were similar to two commercial strains: ABTS-351 and GC-91 (Frederiksen et al. 2006). Another study investigated the presence of commercial strains of *Bt* in 54 pasteurized whole milk samples, 40 ice cream samples and two green tea drinks randomly purchased in supermarkets and grocery shops in China. Of the 146 *B. cereus* isolates identified, 19 were *Bt* strains, 18 of which were indistinguishable from *Bt* products marketed in the country. It should be noted that the amount of *B. cereus* found in food and beverages is often well below alert thresholds (Zhou et al. 2008). At present, *Bt* is not regulated according to any specific food safety criteria in Europe, except for dried infant formulae (EU Commission Regulation (EC) 2073/2005). However, this is not necessarily the case in all Member States. For example, in France, authorities set an alert threshold of 1×10^5 CFU/g of food (Directorate General for Food, DGAL/MUS/N2009-8188, 2009), based on the fact that diarrhoeal syndromes linked to *B. cereus* were generally associated with a contamination of 10^5 to 10^8 cells/g of food (EFSA Biohaz 2005). Reported cases of *B. cereus* food poisoning have occasionally been caused at lower levels (EFSA Biohaz 2005), and *Bt* levels identified in food are often below the alert threshold (Rosenquist et al. 2005). This suggests that this alert threshold and even the regulation could be subject to re-evaluation in the light of new data on *Bt* toxicity.

22.5 Persistence of *Bt* in the Environment and the Digestive Tract

In many studies, the absence of mortality due to acute toxicity obscures the lack of data related to chronic low-dose exposure to a given substance. In fact, there is little information on the potential adverse effects of long-term exposure to small amounts of *Bt* or its toxins. This is all the more troubling since spore-forming bacteria can withstand extreme environmental conditions over a long period of time (Setlow 2014).

Bt spores are no exception. Studies have shown that 72 h after spraying Chinese cabbage with a *Bt* bioinsecticide, nearly all spores colonized the leaf surface (Bizzarri and Bishop 2008). In addition, *Bt* takes 28 days to disappear from the surface of Chinese cabbage after treatment (Raymond et al. 2010) and can persist in the soil for more than a year (Vettori et al. 2003). After mosquito control with *Bt* bioinsecticides, *Bt* spores were found in near treatment-dose quantities for at least 10 weeks, and in some places several years after spraying (Tilquin et al. 2008).

These data were produced from analyses performed after a single treatment. However, the European Union authorizes between three and eight applications spaced 1 week apart (SANCO/1541/08 – rev. 4 and SANCO/1543/08 – rev. 4) and up to 12 applications have been reported for a single crop (ANSES 2013). The actual amounts of *Bt* in the environment may therefore be higher than assumed. *Bt* spores have also been shown to persist in the digestive tract of many organisms: at least 18 days in the intestines of human-biota-associated rats (Wilcks et al. 2008) and up to 30 days in the intestines of mice (Oliveira-Filho et al. 2009). *Bt* was also detected in faecal samples from farmers 5 days after cessation of use of any *Bt* product (Jensen et al. 2002).

The ability of *Bt* spores to persist could affect the development and health of many non-target organisms, especially since exposure and intake of these products is expected to increase. One of *Bt*'s possible underestimated effects on animal and human health is the development of chronic intestinal pathophysiologies beyond food poisoning, which is an immediate consequence of *Bt* ingestion. The presence of non-commensal bacteria in the gut is now recognized in both mammals and invertebrates as an underlying cause of a pro-inflammatory state, which can lead to the development of chronic inflammatory bowel diseases such as Crohn's disease or ulcerative colitis, and in some cases even develop into cancer (Gersemann et al. 2011; Rizzo et al. 2011). As an opportunistic non-commensal bacterium, *Bt* is capable of disrupting gut homeostasis and inducing inflammation (Loudhaief et al. 2017), which means that chronic ingestion of even a small dose could have an impact on the development of digestive tract diseases.

22.6 The Environmental Impacts of *Bt*

What are the potential impacts of *Bt* bioinsecticides on the environment? Although the effects of *Bt* products on wild mammals (in natura) are likely similar to those demonstrated above in mice, rats and humans, they have yet to be described. Nevertheless, a few scarce investigations have been carried out on the effects of *Bt* on non-target arthropods, and it would appear that entomopathogenic toxins are more likely the cause of adverse effects than the bacteria themselves. When toxins are found in run-off, they are thought to induce abnormally high mortality and growth retardation in aquatic larval Trichoptera species (Rosi-Marshall et al. 2007; Tank et al. 2010). Increased larval mortality and delayed development have also been observed in many *Drosophila* species following ingestion of food contaminated with *Bt* products (Babin et al. 2020). Finally, *Bt* bioinsecticides are thought to cause gut disturbances in bees (Renzi et al. 2016) and behavioural changes in *Trichogramma*, a small endoparasitic wasp also used in biological control (Amichot et al. 2016).

22.7 Conclusion

The clear lack of environmental and health data on the adverse effects of *Bt* products used in agriculture poses a real challenge in terms of risk assessment. Although the amounts currently found in the environment are relatively low, they are expected to increase as biopesticide use becomes more widespread. Their potential long-term impacts must be anticipated in order to avoid repeating past mistakes associated with certain chemical pesticides. Many people are and will be exposed to these bioinsecticides, from professionals (in agriculture and forestry) to home gardeners handling these products and all species living in application areas. Ingestion by humans and animals of food treated with *Bt* bioinsecticides must also be considered. Although the involvement of *Bt*-treated foods in food poisoning appears to be sporadic thus far, consumption of such foods is on the rise. The issue of whether these foods should be allowed into medical establishments (hospitals, retirement homes) as well as any necessary precautions to take should also be examined. Indeed, *Bt* is an opportunistic bacterium that could take advantage of any opening, particularly in weakened, stressed, immunocompromised or older people – the archetype of people in such places. Additionally, spore-forming bacteria are a major concern in hospitals, as they are difficult to eradicate due to their resistance to chemical, thermal and antibiotic treatments (Setlow 2014). *Bt*, like *B. cereus*, is also able to form biofilms, particularly on medical equipment and devices (Kuroki et al. 2009). Limiting the introduction and dispersal of *Bt* spores into healthcare settings is therefore crucial. Finally, treatments with *Bt* bioinsecticides are sometimes combined with other treatments such as pyrethrins (pyrethrum-derived neurotoxins, see Chap. 13) that are allowed in organic farming. Co-exposure of organisms to *Bt*, in combination with other plant protection substances, should therefore also be investigated to assess any health and environmental risks.

In short, better assessment of the health and environmental risks related to exposure to *Bt* products is a major priority. The aim is to implement management and user protection measures and optimize the conditions of use of *Bt* products in order to limit their ingestion, environmental accumulation and impact on biodiversity. The knowledge gained could also be useful in setting up food-chain monitoring systems.

Chapter 23
Can Pests Develop Resistance to Biocontrol Products?

Marc Bardin and Myriam Siegwart

23.1 Many Biocontrol Agents on the Market, Variable Efficacy in the Field

Over the last 40 years, considerable research efforts have been devoted to developing biocontrol agents against plant pests (pathogens, insects, weeds), leading to the registration of an increasing number of biocontrol products on the plant protection market. However, when used in the field, these products regularly offer an inconsistent level of protection (Nicot et al. 2011b). This fluctuating efficacy is generally linked to microclimate variations (e.g. temperature, humidity, radiation) encountered in agricultural production conditions, the lack of environmental resilience of the biocontrol agents (e.g. survival, colonization capacity) or their intrinsic characteristics (e.g. variable production of metabolites or enzymes involved in control), or product quality (especially in the case of living organisms).

However, the inconsistent performance of biocontrol agents in the field can also be explained by the different levels of sensitivity of pests to these products. Biocontrol agents may become less effective due to the pre-existence of resistant individuals in natural pest populations. This loss of efficacy may also occur if pests are able to produce natural variants (or mutants) with reduced sensitivity to biocontrol agents that can be selected under the selection pressure of these plant protection products. The resistance of crop pests to biocontrol agents is still relatively unexplored. However, cases of resistance (or reduced susceptibility) to biocontrol products have been observed under commercial growing conditions, particularly in crop insect pests.

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23.2 Pest Adaptation to Plant Protection Methods

A plant protection method is said to be durable when it remains effective while being used on a large scale and over a long period of time. This durability depends on the selection pressure of the control method exerted on pest populations as well as on the pests' ability to adapt to the method.

The gradual decline in pesticide efficacy has been widely studied. The frequent and recurrent appearance of fungicidal resistance in fungal plant pathogens is well documented (REX Consortium 2013). The ability of insect pests to become resistant to insecticides and weeds to herbicides has also been studied extensively (Délye et al. 2013; Roush and Tabashnik 1990). Similarly, the durability of plant varietal resistances, especially those related to major resistance genes, is well documented in crop pests (REX Consortium 2016).

More anecdotally, cases of resistance (or reduced sensitivity) of pests to physical control methods have also been identified. For example, a pest belonging to the Lepidoptera order adapted its behaviour to be able to reproduce under nets, which were initially placed on trees to protect them from attacks by these insects (Siegwart et al. 2013). The plant pathogenic fungus *Botrytis cinerea* can gradually become accustomed to UV filter films (Nicot et al. 2001). In the absence of UV radiation, this fungus normally shows reduced sporulation, which can increase again after several successive generations under selection pressure.

Although many pests are known to develop resistance to conventional plant protection methods, only a few studies have explored their ability to circumvent the effects of biocontrol agents.

23.3 Proven Cases of Pest Resistance to Biocontrol Agents

Cases of resistance (or reduced sensitivity) to biocontrol agents have been observed in insect pests in agricultural systems for products ranging from microorganisms to plant or microbial extracts, semiochemicals and beneficial parasitoid insects (Siegwart et al. 2015; Tomasetto et al. 2017).

For example, several years after *Bacillus thuringiensis* (*Bt*), the most widely used microbial biocontrol agent in the world, was brought to market, resistance to its crystal protein was described in three species of crop insect pests (*Plutella xylostella*, *Trichoplusia ni* and *Plodia interpunctella*). At least 27 insect species are capable of developing resistance to *Bt* under laboratory conditions (Tabashnik 1994). The resistance of codling moth (*Cydia pomonella*) to a granulovirus-based biocontrol product is another textbook case. This resistance was detected in apple orchards in both France and Germany after more than 10 years of intensive use of commercial products developed using an identical entomopathogenic virus isolate (Asser-Kaiser et al. 2007; Sauphanor et al. 2006). This resistant codling moth population has a very high resistance factor: it takes 13,000 times more virus to kill a resistant insect population than it does to kill a susceptible insect population. Under lab conditions, repeated treatments with this virus on the resistant population of *C. pomonella* allowed for the selection for an even higher resistance, with a resistance factor of 60,000 times more than the susceptible population (Berling et al. 2009). That research team's work subsequently led to the selection of a new virus isolate that was effective on resistant insects. A new product resulting from the evolution of this virus has since been marketed without any new resistance having been detected in France.

Cases of insect pest resistance to bacterial extracts have also been described. Six insect species are for example resistant to spinosyns, insecticidal compounds produced by the bacterium *Saccharopolyspora spinosa* (Sparks et al. 2012). In the case of *Plutella xylostella*, this resistance appeared after only 2 years of intensive use of the product in Hawaii.

Mating disruption (see Chap. 17), another widely used insect pest control method, involves saturating the atmosphere with synthetic sex pheromones to cause confusion and prevent males and females from finding each other and mating. Natural pheromones are made up of a blend of volatile compounds (known as a pheromone bouquet). However, for various reasons (production costs etc.), synthetic pheromone blends used in agriculture have a much simpler composition, which can have consequences in terms of the insect's adaptive response. One well-documented case, described in Japan, is that of the smaller tea tortrix *Adoxophyes honmai*, which developed resistance to the use of pheromones after 10 years of field exposure (Tabata et al. 2007). The resistance mechanism in this case consists in the moths detecting molecules absent from the commercial product, but present in the natural pheromone bouquet, in order to be able to locate females despite the interference with certain chemical signals. This resistant strain can therefore mate with partners even in the presence of a high dose of the synthetic pheromone, thereby reducing its efficacy. Lab experiments have also shown that repeated treatments with this synthetic pheromone on a strain of the insect for 70 generations resulted in the selection of an even higher level of resistance.

In another recently documented case in New Zealand, resistance of a major grassland pest to a parasitoid insect was observed (Tomasetto et al. 2017). The authors described how the Argentinean stem weevil (*Listronotus bonariensis*) had evolved resistance to the introduced parasitoid *Microctonus hyperodae*, thus reducing its parasitic capacity by 44%. According to the authors, the loss of efficacy of the parasitoid insect observed in the field is linked to the selection of pre-existing resistant genotypes present at low frequencies in the original population. This resistance is thought to have appeared 7 years after the introduction of the parasitoid (i.e. after about 14 generations of the insect pest).

To our knowledge, most of the proven cases of pest resistance to biocontrol agents concern insect pests. The durability of biocontrol efficacy against plant pathogens (bacteria, fungi, oomycetes, nematodes, viruses) has not been widely studied and to our knowledge, field resistance to biocontrol products has not yet been described for these organisms (Bardin et al. 2015). Similarly, no cases of weed resistance to biocontrol products have been found in the field. The lack of documented cases of pathogen and weed resistance to biocontrol products may be explained by their still limited use in agriculture compared to the use of natural enemies (see Chaps. 11 and 13).

23.4 Risk of Crop Pests and Diseases Developing Resistance to Biocontrol Agents

Biocontrol durability can be linked to pest population characteristics, such as genetic diversity and the ability to evolve in response to selection pressure. It ultimately depends on classic evolutionary mechanisms such as mutation, recombination, migration and selection. These relationships have been the subject of many studies in other contexts, such as to assess the durability of varietal resistance to plant diseases. For example, McDonald and Linde (2002) hypothesized that pest populations with high evolutionary potential were more likely to overcome varietal resistance. The same hypothesis can be put forward with regard to pests developing resistance to biocontrol agents.

23.4.1 Estimating Diversity in the Level of Pest Resistance to Biocontrol Agents

Diverse levels of resistance to biocontrol agents within pest populations can reduce their efficacy in the field. Because of the a priori existence (even before the use of treatment products) of less sensitive phenotypes in a natural population, widespread biocontrol use could lead to rapid selection for resistance. Testing a sufficiently large number of samples of target pests for their level of resistance to biocontrol agents is therefore a first step in assessing the risk of resistance emerging. Monitoring the level of resistance in natural pest populations before and after treatment can also shed light on the distribution of resistance in the field, its impact and its evolution.

A rapid assessment of resistance to fungicides in large collections of plant pathogenic fungi isolates is, for example, widely practised by plant protection companies, based on the recommendations of the Fungicide Resistance Action Committee, in order to establish a baseline for these products (FRAC 2021). It is also practised in the medical field for the monitoring and management of antibiotic resistance in human pathogenic bacteria.

This type of study is emerging for biocontrol agents (Bardin et al. 2015; Siegwart et al. 2015). For example, geographical and temporal variability in the susceptibility of *Spodoptera frugiperda* to the Cry1F toxin in *Bt* has been described in Brazilian populations of this pest (Farias et al. 2014), while variations in susceptibility to infection by a baculovirus isolate have been described in natural populations of 12 insect species (Briese 1987; Abot et al. 1996). Different studies also exist for plant pathogens (Bardin et al. 2015). Very recently, a wide diversity of susceptibility to the fungus *Paraphaeosphaeria (Coniothyrium) minitans* has been observed in the polyphagous plant pathogenic fungus *Sclerotinia sclerotiorum* (Nicot et al. 2019). These studies, even partial and still recent, show a strong diversity in the susceptibility of plant pathogens to biocontrol agents, whatever their mode of action.

23.4.2 Assessing Pests' Adaptive Capacity to Biocontrol Agents

If the use of biocontrol agents were to become widespread in the field, resistance could emerge via increased selection pressure, which is what happened with pesticides. To assess this potential risk, repeated exposure under laboratory conditions of successive generations of a pest to a biocontrol agent or derivatives can serve as good indicators. This type of experiment is generally used to determine how durable antimicrobial agents (e.g. antibiotics) are against human pathogens (Cowen et al. 2002) or the ability of fungal plant pathogens to adapt to fungicides (Brent and Hollomon 1998). A few rare experimental pest evolution studies have also been carried out on biocontrol agents (Bardin et al. 2015; Siegwart et al. 2015).

For example, aphids treated for 40 generations with purified azadirachtin (the main active ingredient in neem oil, see Chap. 13) developed resistance to this compound (Feng and Isman 1995). However, aphids treated for 40 generations with crude neem oil did not develop resistance, suggesting the raw, more complex product is more durable compared to the purified active ingredient. Repeated treatments with baculoviruses on eight species of lepidopteran pests showed that resistance occurred in half of the cases, with the most severe in the potato tuber moth *Phthorimaea operculella* (Briese 1987).

Only one documented example describes the ability of a plant pathogen, *B. cinerea*, to adapt to the effect of an antimicrobial compound synthesized by a microbial biocontrol agent, suggesting a potential risk of resistance developing in the field (Ajouz et al. 2010). However, the cost associated with this resistance is significant for the pathogen: reduced mycelial growth, loss of aggressiveness on the plant, and low sporulation capacity of resistant strains compared to wild susceptible strains (Ajouz et al. 2011). This would suggest a limited capacity for epidemic spread of resistant strains. Contrary to the situation observed in *B. cinerea*, no resistance was observed after treatments carried out during 15 successive generations in two biotrophic fungi (*Podosphaera xanthii* and *Pseudoperonospora cubensis*), as part of an experimental evolution study on melon leaves treated with a rhubarb root extract (Yang et al. 2008). This lack of evolution towards resistance of the tested pathogens could be due to a complex mode of action of the plant extract, known to have both a direct antimicrobial effect and an induced plant resistance effect.

23.4.3 Impact of the Mode of Action of Biocontrol Agents on Their Durability

Biocontrol durability is linked to the selection pressure exerted by the biocontrol product used, which depends on the areas treated and the amounts of product applied (e.g. dose, application rate). Durability may also depend on the mode of action involved in the effect of biocontrol agents on pests (see Chap. 11). Although all biocontrol agents create a priori selection pressure on target pest populations when applying treatments under field growing conditions, certain modes of action are likely more conducive to the development of resistance. This issue will need to be addressed by future research.

23.5 Conclusion

If biocontrol use becomes more widespread, the emergence of resistance to these products is a possibility. Although data are still too scarce to draw up general principles on precautions for the use of biocontrol agents in practice, this chapter underscores the need to properly manage these new products now to avoid repeating past mistakes made with synthetic pesticides and varietal resistance. It is therefore vital to improve our understanding of the risks of biocontrol agents becoming less effective in order to ensure a lasting effect of this plant protection method. We could use this knowledge to identify the pest characteristics likely to favour the selection of strains resistant to biocontrol agents on the one hand, and the characteristics of biocontrol agents that can easily lead to the selection of resistant isolates in natural pest populations on the other.

Major research efforts are still needed to gain detailed knowledge of the modes of action of biocontrol agents. This knowledge will allow optimizing their use in the field and should therefore foster their durability. For example, guidelines could be established for using complex formulations (mixtures of metabolites or microorganisms with different modes of action) or for alternating different products. This could also help ward off potential failures by guiding the screening procedure for new biocontrol agents towards more durable modes of action.

Acronyms

ABS	Access and benefit sharing
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
APHIS	Animal and Plant Health Inspection Service of the United States Department of Agriculture
AW-IPM	Area-wide integrated pest management
Bt	*Bacillus thuringiensis*
CBC	Classical biological control
CBGP	Centre for Biology and Population Management
CI	Cytoplasmic incompatibility
CLP	Cyclic lipopeptide
CNRS	French National Centre for Scientific Research
CTIFL	*Centre technique interprofessionnel des fruits et légumes*, organization for applied research in the French fruit and vegetable sector
DAPG	2,4-diacetylphloroglucinol
DGAL	French Directorate-General for Food
EAG	Electroantennography
EFSA	European Food Safety Authority
EPPO	European and Mediterranean Plant Protection Organization
EU	European Union
FRAC	Fungicide Resistance Action Committee
GC	Gas chromatograph
GR	Gustatory receptor
GRN	Gustatory receptor neuron
GSS	Genetic sexing strains
HIPV	Herbivore-induced plant volatiles
IBMA	International Biocontrol Manufacturers Association
IFOAM	International Federation of Organic Agriculture Movements

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IIT	Incompatible insect technique
INRAE	French National Research Institute for Agriculture, Food and Environment
INSA	National Institute of Applied Sciences of Lyon
IOBC	International Organization for Biological Control
IPM	Integrated pest management
IR	Ionotropic receptor
ISPM	International Standard for Phytosanitary Measures
ISR	Induced systemic resistance
LD_{50}	Median lethal dose
MRL	Maximum residue limit
MS	Mass spectrometer
NADES	Natural deep eutectic solvents
NRPS	Nonribosomal peptide synthetase
OBP	Odorant-binding protein
ODE	Odorant-degrading enzyme
OR	Odorant receptor
ORco	Odorant receptor co-receptor
ORN	Olfactory receptor neuron
PKS	Polyketide synthase
SIT	Sterile insect technique
SLOSS	Single large or several small
SPME	Solid-phase microextraction
SSR	Single sensillum recording
TFI	Treatment frequency index
USDA	United States Department of Agriculture
USDA-ARS	United States Department of Agriculture – Agricultural Research Service
VOC	Volatile organic compound

Glossary

Allee effect (and Allee threshold): a positive effect of density on one or more components of fitness (component Allee effect), which can be translated into a positive effect of density on the population growth rate (demographic Allee effect). The demographic Allee effect is described as strong if the rate of increase becomes negative below a threshold density. This threshold is the Allee threshold; any population that drops below the Allee threshold will be deterministically driven to extinction.

Allele: a variant of a gene, characterized by a specific DNA sequence. In diploid individuals, each gene has two alleles: one from the father and one from the mother, with one on each homologous chromosome. Alleles can be identical (homozygous) or different (heterozygous).

Allelochemical: a chemical involved in communication between different species. A volatile chemical, alone or combined, that is produced and emitted into the environment by a living organism and that is likely to reach another organism of a different species, triggering a behavioural response from the latter. In the case of **kairomones**, the detection of the chemical signal is beneficial to the recipient – for example, herbivores can recognize their host plant by its scent. In the case of **allomones**, the detection of the chemical signal brings a benefit to the emitter – for example, flowering plants that attract pollinators without offering rewards. Signals that benefit both the emitter and the recipient are called **synomones**.

Allelopathy: the phenomenon by which microorganisms and plants produce biochemicals that affect (positively or negatively) the development, growth, survival or reproduction of other organisms.

Area-wide integrated pest management: a management strategy that includes a set of available tools and appropriate measures that allow the control of pest organisms on the scale of a geographical area.

Augmentative biological control: a type of biological control in which pest populations are regulated by introduced individuals or their descendants. In contrast to classical biological control, the introduced biological control agents come from indigenous populations.

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Banker plant: a non-crop plant used to provide alternative food or prey to a natural enemy to support its survival and reproduction when pest populations are low in the crop (see also **companion plant**, **cover crop**).

Beneficial organism, beneficial: a broad term used to describe organisms that provide ecosystem services in an agronomic context. They are useful in agriculture because they enhance soil quality, pollinate crops and control other organisms that damage crops: weeds, pathogens and plant-eating pests. When used for biological control, they are referred to as biological control agents or natural enemies.

Biological control, biocontrol: the use of living organisms to reduce the abundance or impact of pests. There are three general strategies: classical (or importation), augmentative (which includes inundation and inoculation) and conservation.

Biological control agent, biocontrol agent (synonym: natural enemy): an organism (arthropod, pathogen, microorganism, virus, nematode) used to control populations of a target pest or weed. Biological control agents used to manage plant diseases are known as **antagonists**.

Biological pesticide, biopesticide: a pesticide derived from a biological source, such as an entomopathogenic organism, a plant extract, etc.

Business model: a strategy developed by a company or programme to create a relevant and competitive offer while guaranteeing viability and profitability over time.

Classical biological control (synonyms: importation or introduction biological control): a type of biological control in which pest populations are regulated for long-term periods by the descendants of introduced exotic organisms that have become established and spread.

Commercial insectary (synonyms: biocontrol manufacturer, biocontrol company): a factory that produces biological control agents. By extension, a company producing and marketing said biological control agents.

Companion plant: a plant grown near another plant (crop) to attract or repel pests as part of a pest control strategy or to provide alternative habitats to support beneficial insect and natural enemy populations (see also **banker plant**, **cover crop**).

Conservation biological control: a type of biological control consisting of maintaining pest insect population levels below a harmful threshold by combining methods that act directly on these populations (e.g. olfactory disturbance of the host plant location) and methods that foster the development or activity of native natural enemies.

Cover crop: a plant sown in a field to provide a service, such as to attract or repel a pest, compete with weeds, regulate soil nitrogen, etc., but which is not harvested (see also **banker plant**, **companion plant**).

Culturomics: a method used to isolate microorganisms through the use of various media and culture conditions.

Cytoplasmic incompatibility: the reproductive incompatibility caused by factors in the cytoplasm, often associated with microorganisms such as *Wolbachia* bacteria.

Density dependence: positive or negative effects of population density on fitness components (mating success, larval survival, etc.) and/or on the rate of population growth. A positive density dependence is usually due to cooperative interactions, while negative density dependence is due to competitive interactions.

Deterministic: describes a perfectly identified process that produces the same results each time it is repeated, without any degree of uncertainty. The same initial state always produces the same final state. Some mathematical models of population dynamics are deterministic (generally opposed to stochastic models that include chance events).

DNA barcoding: a molecular characterization method for species-level identification using a small number of DNA markers (e.g. a portion of the mitochondrial cytochrome c oxidase subunit I gene in animals).

Durability: see **sustainability**.

Ecosystem services: the benefits to humans rendered by certain species, communities or biological processes.

Ecophyto Plan: an environmental plan implemented in 2008 by the French government with the aim of reducing the use of plant protection products in the country by half within 10 years. This plan was followed up by the Ecophyto II plan, published in 2015, which reaffirmed the original aim of reducing plant protection product use by half in ten years (extending the deadline to 2025), strengthened some of its key provisions, and introduced additional measures. In 2018, the French government released its Ecophyto II+ plan to reinforce the commitments undertaken for the Ecophyto II plan and integrate further actions to end the use of glyphosate.

Effective population size: the number of individuals that participate genetically in the reproduction of the population and determine its genetic diversity. Generally, the effective population size is considerably smaller than the census size, either because not all individuals participate in reproduction or for other reasons that cause the population under study to deviate from a theoretically ideal population size.

Endoparasitoid: see **parasitoid**.

Endophyte: an organism (often microscopic) which spends part or all of its life within the tissues of a plant and which has a beneficial or no effect on the fitness of the plant.

Endosymbiotic bacteria: bacteria that have established a close association with a eukaryote, such as *Wolbachia*, living within the cytoplasm of insect cells.

Entomopathogen: a microorganism that is pathogenic to an insect.

Entomophagous: refers to an organism that consumes (by predation, parasitism, pathogenicity) insects, or even, by extension, other arthropods such as mites.

Entomovectoring: the use of an insect (pollinator or sterile insect) as a vector to spread a substance used in biological control of plant pests and diseases (typically a powdered substance containing a virus, bacteria or fungus).

Extended biocontrol: the use of living organisms or their products to reduce the abundance or impact of pests. This includes the use of macroorganisms and microorganisms as pest natural enemies, sterile males, the plant microbiome, plant-derived pesticides, and semiochemicals. This excludes mineral substances (such as sulphur) that may be considered natural but are not derived from living organisms.

Exotic (synonym: alien): not native to a geographical range.

Exotic or alien biocontrol agent: an invertebrate organism (insect, mite or nematode) not indigenous to a given geographical range.

Fitness: an individual's ability to survive and reproduce in a particular environment (see also **phenotype**).

Functional trait: a measurable characteristic of an individual that affects individual performance (see also **fitness**). This type of trait can be morphological, physiological or phenological.

Genetic sexing: the use of genetic differences to separate males and females (e.g. sorting or eliminating females to produce only males).

Guild: a group of species that exploit the same resources.

Gustatory receptors (GR): receptors responsible for detecting non-volatile cues, such as sweet and bitter flavours, as well as CO_2 and some non-volatile pheromones detected by contact. Insect GRs are proteins with seven transmembrane domains expressed in taste neurons.

Holobiont: a theory that considers that the host and its associated microorganisms constitute the unit under selection in an evolutionary context.

Hologenome: the genome of the holobiont, which is the combination of host and microbe genomes.

Horizontal integration: the grouping of identical activities under a single management authority with a view to becoming more competitive.

Horizontal transmission: acquisition of a microorganism by a plant from the local environment (e.g. atmosphere, soil).

Hyperparasitism: generally refers to parasitoids that parasitize other parasitoids, which are then called hyperparasitoids, or secondary parasitoids. In microbial control, hyperparasitism refers to microbes that parasitize other microbes, which are then called hyperparasites.

Indigenous: native to a geographical range.

Inoculation biological control: a type of augmentative biological control in which pest populations are regulated for an extended period, but not permanently, by the descendants of the introduced individuals. This method is often implemented in greenhouses that are emptied between growing cycles.

Integrated pest management: a crop protection strategy combining different approaches, such as biological control, the use of resistant crop varieties, preventive treatment, etc.

Integrative systematics: a discipline that aims to organize the relationships between organisms according to a standardized hierarchy (species < genus < family < order < class < etc.) by drawing from complementary sources of information (see **integrative taxonomy**).

Integrative taxonomy: a discipline that aims to characterize and delimit living species using complementary sources of information, including morphological, behavioural and molecular characterization.

Intraguild predation: predation between species belonging to the same ecological guild, i.e. between those species consuming a common resource. For example, a lady beetle and a parasitoid, which are both aphidophagous, prey on aphids, but lady beetles can also consume aphids parasitized by a parasitoid, therefore making it an intraguild predator. When an individual of one species consumes another individual of the same species, this is called cannibalism.

Introgression: the acquisition by a certain species or population of genes or alleles from another group of organisms as a result of their backcrossing.

Inundation biological control: a type of augmentative biological control in which pest populations are regulated *solely by the released* biological control agents (in contrast to inoculation biological control, where pest populations are regulated by the *descendants* of the released biological control agents).

Kairomone: see **allelochemical**.

Mesocosm: an experimental system used to study populations of median size and complexity under controlled conditions. As opposed to a microcosm, which offers a much smaller and simpler environment.

Metabarcoding: a method based on the sequencing and amplification of genomic regions in order to estimate the taxonomic diversity of the microbiota.

Metabolite: a substance synthesized by a living organism and which contributes to that organism's own functioning or its interactions with the outside world.

Metacommunity: a set of local communities that are linked together by species dispersal processes and which interact with each other.

Metagenomics: a technique based on the random sequencing of DNA extracted from an environmental sample. This technique makes it possible to infer the taxonomic and functional diversity of the microbiota.

Microbiota: all the microorganisms living in a habitat.

Mummy: a generic term for insects parasitized by a parasitoid, giving them a characteristic swollen appearance.

Mycorrhizal symbiosis: a mutually beneficial association between a fungus and a plant root leading to the formation of a mycorrhiza, where the trophic exchanges between the two partners occur.

Natural pest control: this refers to the predation or parasitism processes involved in the biological control of pests or phytophagous insect species attacking crops.

Network connectance: the proportion of interactions taking place in an ecological network, calculated by establishing the ratio between the number of interactions actually observed and the total number of possible interactions.

Niche: this term indicates the position occupied by an organism, population or species in an ecosystem, and includes all the conditions and resources necessary to maintain the individuals.

Non-target effect: a negative impact caused by a biological control agent on a species other than the target pest for which it is used.

Non-target species: any species other than the pest targeted by the biological control agents or biological control product.

Nurse plant: a plant that can promote the growth of neighbouring plants.

Odorant receptors (OR): receptors responsible for detecting volatile organic compounds. Insect ORs are proteins with seven transmembrane domains expressed in olfactory receptor neurons.

Oophagous: describes an insect that feeds on the eggs of another insect (by predation or parasitism).

Ootheca: a group of insect eggs laid at the same time. Also referred to as an egg mass, cluster or patch.

Pathogen: a microorganism causing disease in the infected host.

Parasitism: when individuals of a species (the parasites) develop at the expense of individuals of another (the hosts), whose development will then be impacted, although they may not necessarily be killed.

Parasitoid: an insect that is a parasite to another species (host) during its preimaginal development, before becoming a free-living adult. A parasitoid lays its eggs in or on an insect (egg, larva or adult stage). An endoparasitoid is an insect that lays its eggs inside its host. Unlike with a true parasite, the development of a parasitoid eventually leads to the death of the host, and unlike with predators, a parasitoid completes its immature development from a single individual host.

Parthenogenesis: the development of an organism from an unfertilized egg; reproduction without fertilization.

Pathobiota: set of potentially pathogenic microorganisms in a host organism.

Passivation: the process of creating a new product or the processing of an object to ensure that it reacts in a predictable, stable and sustainable way regardless of the conditions of use.

Pest (synonym: harmful organism): any organism that lives and grows where it is not wanted. In an agricultural context, pests include insects, pathogens and weeds that can damage crops and lead to yield losses.

Pesticide: a substance used to control or destroy an insect pest, disease or other harmful organism. The term is sometimes used interchangeably with plant protection product, but pesticides also encompass products employed for non-plant uses such as biocides (see also plant protection product).

Phenotype: an individual's observable traits in a given environment (see also **fitness**).

Phenotypic plasticity: the ability of an organism to adapt, without genetic changes, to different biotic or abiotic conditions in its environment (see also phenotype).

Pheromone: a chemical signal used to communicate between individuals of the same species.

Phyllosphere: the aboveground parts of a plant.

Phytophagous: describes an animal that feeds on plants; such animals are commonly known as herbivores.

Plant protection products: pesticides used to protect crops or other useful plants, often in the agriculture, forestry or horticulture sectors (see also pesticide).

Polyphagous: describes an organism that feeds on various types of food.

Population bottleneck: a sudden drop in the size of a population generally resulting in a decrease in genetic diversity, particularly in terms of allelic richness.

Predator: an animal that preys on one or more species. For instance, lady beetles are predators of aphids.

Preimaginal development (or immature development): in arthropods, the life stages (eggs, larvae) that precede the adult stage (also called the imago).

Rhizosphere: area of the soil subject to the influence of plant roots.

Semiochemical: a substance emitted by a living organism and perceived by other organisms, which constitutes a chemical signal. The chemical signals used to communicate between individuals of the same species are called **pheromones**. The chemical cues involved in communication between different species are called **allelochemicals**. They are involved in many interactions between insects and plants.

Sexual competitiveness: in the context of the sterile insect technique (SIT), the ability of reared individuals to inseminate wild counterparts; commonly used to define the quality of sterile males.

Socio-technical agencement: a set of heterogeneous elements that form a system because they have been arranged together: laws, institutions, conventions, groups of actors, technologies, physical artefacts, etc.

Species complex: a group of similar species that are closely related and very difficult, if not impossible, to distinguish by morphology alone.

Specificity: the degree of specialization of a biological control agent with regard to a target organism. Host specificity testing is used to evaluate the specificity of a biocontrol agent and to assess its host range, particularly with regard to its food preferences (in the case of predators and phytophagous insects) and its physiological suitability (in the case of parasitoids).

Stakeholder: an entity, whether an institution, company, person, association or group of people, which holds a stake (with regard to security, legal, sustainability, reputation or other issues) specific to the situation in question.

Sterile insect technique (SIT): a pest control method where mass-reared sterilized males are released in large numbers into the agricultural landscape, where they mate with wild females; the females then lay sterile eggs, producing embryos that die in early development.

Stochastic: describes a process that can be thoroughly characterized but which includes some uncertainty, and therefore produces a potentially different result each time it is repeated. Some simulation-based models of population dynamics are stochastic. As opposed to deterministic.

Sustainability (durability): a pest control method is said to be sustainable when it remains effective while being used on a large scale and over a long period of time. It is therefore difficult to measure, except after the fact; however, sustainability is the end goal when designing a control method to avoid an overly rapid adaptation of the pest population.

Synomones: see **allelochemical**.

Tastant: a chemical that provokes a taste sensation. A non-volatile chemical signal detected upon contact with an animal's sensory structures; an animal's sense of taste enables it to assess the quality of a food source, among other things.

Thelytoky: a mode of reproduction characterized by the possibility of a female giving birth to viable female offspring without a male partner.

Turnaround time (synonym: intercropping period): the period of time between two successive crops planted in a specific field.

Value chain: a set of relevant activities relating to a firm or industry's own field (known as primary activities) or relating to peripheral activities (known as secondary activities) in order to analyse the firm's margin structure.

Vertical integration: the grouping of successive activities within a value chain under a single management authority.

Vertical transmission: transmission of a microorganism from the mother plant to its offspring.

Volatile organic compound (VOC): an organic substance with physicochemical properties that allow it to be volatilized and carried in the air. Most pheromones and allelochemicals are VOCs. The most abundant VOCs in the atmosphere are those emitted by plants and microorganisms.

Weed: any plant that grows where it is unwanted. In an agricultural context, these may be referred to specifically as crop or arable weeds. The term weed illustrates context-dependent anthropocentrism underlying any definition of a wanted or unwanted living organism. For instance, while some may weed crops, others may crop weeds…

X-rays and gamma rays: high-frequency electromagnetic radiation, produced by electron acceleration for X-rays and by the radioactive decay of radioisotopes (cobalt-60 or caesium-137) for gamma rays. The rays pass through a material and are stopped to varying degrees depending on the different components they encounter. Depending on their power, they are used for medical imaging and treatments.

Zoophytophagous: describes an organism that can consume animal and plant food sources.

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