Chapter 23
Can Pests Develop Resistance to Biocontrol Products?

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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.