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Improving integrated pest management in horticulture

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Chapter 1

Advances in biopesticides for insect control in horticulture

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1 Introduction

Horticulture, from the production through to the sale of fruit, vegetables and flowers, is an industry that is crucial for food production and human wellbeing. The science of horticulture is critical to the prosperity and health of people and the environment. With an increasing population, it is estimated that food production will need to increase by 70% by the middle of the century to meet demand (Davies, 2015). Opportunities exist to develop new high-value and high-nutrition horticultural produce on the decreasing area of land available for food production. However, horticulture is a more intensive farming approach than that used for broad-acre crops such as wheat and corn, which means that poor sustainability practices will have more severe consequences for future generations, although the area impacted is less than for some other crops. This includes approaches toward invertebrate pest control, as insects and other invertebrates cause significant damage and production losses across the horticulture sector around the world.

The application of synthetic pesticides has been the main approach to controlling pest insects in horticulture. However, applications of certain

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pesticides on fruit crops, such as stone fruits, have pre-harvest intervals ranging between 7 and 28 days that preclude the use of treatments prior to fruit ripening, to minimise residues, during a period when the risk of pest outbreak is high (Hossain et al., 2006; Boston et al., 2020). Frequent use of broad-spectrum insecticides can also promote secondary pest outbreaks of insect pests such as mites, which are otherwise regulated by natural invertebrate predators (Epstein et al., 2000). Furthermore, the pest insect species of some horticultural crops occupy cryptic ecological niches. For example, *Carpophilus truncatus* beetles, pests in Australian almond orchards, live inside mummified nuts and in new nuts, so that insecticide sprays are often unable to reach them and are therefore ineffective (Boston et al., 2020). In the USA, pecan weevils are major pests that cause significant crop damage; the larvae and pupae occur inside the pecan nuts and in soil, so that control of the weevils using contact insecticides is restricted only to the adults (Mulder et al., 2012).

The development of insecticide resistance from the continual use of pesticides is also a key challenge. Development of new synthetic pesticides is now costly and difficult, partly due to the strict environmental and safety regulations involved when converting an active compound into a product (Qadri et al., 2020). For instance, a recent report estimated that in the USA, the development of a novel synthetic pesticide would cost in excess of $300 million and can take approximately 12 years (McDougall, 2016).

The withdrawal of pesticides, combined with health and environmental concerns and the rise in the resistance to current pesticides, is driving the need for new methods of control, including the growing use of biopesticides.

2 What are biopesticides?

Biopesticides have been loosely defined as biologically based pesticides (Glare, 2015). This can be quite a broad definition, including the incorporation of live organisms and their derivatives (e.g. metabolites), through to the use of semiochemicals in formulations. The use of plant extracts, for example, can fit into a broad definition of biopesticides, but authors often use the term to refer to a subgroup of biopesticides such as microbial-based biopesticides.

Kienwick (2007) limited the definition of biopesticides to only those based on microbes, while Copping and Menn (2000) included semiochemicals. The USA EPA defined biopesticides as ‘certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals’ (http://www.epa.gov/pesticides/biopesticides). However, biopesticides are divided into three groups – microbial-based, plant-incorporated protectants (e.g. Bt corn) and biochemical – which encompass formulations of naturally occurring substances including plant extracts and pheromones.

Several novel approaches to using microbes for the control of pest insects have expanded the basic definition of biopesticides. Endophytes, microbes which can live inside plants without causing disease, can often confer resistance to pests and pathogens (McKinnon et al., 2017) and can be classed as a new biopesticide (Glare et al., 2012). Other novel biopesticide approaches include the use of RNAi, which is the use of the antiviral immune system of insects for post-transcriptional gene silencing (Vogel et al., 2019).

It is more common for researchers to define the term 'biopesticide' based on their own area of expertise and focus. In this chapter, we will focus on microbial-based biopesticides for use against pest insects. Paradoxically, 'microbial' in the area of entomopathogens generally includes nematode entomopathogens, which are not microorganisms *per se*, although, as the most commonly studied entomopathogenic nematodes that use symbiotic bacteria as an agent for lethality, this is understandable.

The term 'biopesticide' has also been questioned from a viewpoint of public understanding. The term 'pesticide' has become associated with chemical insecticides and the connotation of broad-spectrum killing. As such, it can be viewed negatively, even for biopesticides. The term 'biorationals' has been used (e.g. Rosell et al., 2008) to encompass biopesticides and other biologically based approaches.

The history of biopesticides is as long as the history of agriculture. The use of plant extracts is probably the earliest example of biopesticides, dating from about some 4000 years ago, with regard to extracts of the neem tree (El-Wakeil, 2013). The use of microbes to control insects is relatively recent; the first demonstration of a disease caused by a microbial infection in insects was for silkworms infected by *Beauveria bassiana*, published in 1835 by Agostino Bassi. It was not until the nineteenth century, after the discovery that microbes cause disease, that the idea of using microbes to control insects was proposed by Pasteur, with the first practical attempts in Russia in the 1890s (Zimmermann et al., 1995). The product Sporéine, based on the bacterium *Bacillus thuringiensis* (Bt), is thought to be the first microbial-based insecticidal biopesticide that was commercialised.

Since that time, biopesticide use has increased incrementally, especially for those based on Bt. However, in the last decade, with the reduction in available synthetic pesticides and more concern around sustainable plant production, the use of biopesticides and the availability of products have increased dramatically. There are several areas where biopesticide development has advanced significantly, which will be reviewed below.

3 Biopesticide use in horticulture

The global market for biopesticides has an estimated value of $3 billion USD and is expected to increase by 15% in the next few years, outpacing market growth of novel synthetic pesticides by 10-fold (Damalas and Koutroubas, 2018). Although biopesticide sales have been increasing year on year, they still make up only a small percentage of the insecticide market worldwide. When looking at the current, most exploited, microbes used as biopesticides in horticulture, there is an indication of where some of the key challenges lie.

Microbial-based biopesticides for insect control have been based mainly on bacterial, nematode, viral and fungal entomopathogens. Although there have been products based on protozoa and microsporidia, the lack of rapid-kill and *in vitro* production methods has greatly limited their development. For viruses and nematodes, *in vitro* production limitations have restricted biopesticide development to only a few examples, such as nucleopolyhedroviruses (Nguyen et al., 2016) and the nematodes in *Heterorhabditis* and *Steinernema* which are associated with the entomopathogenic bacteria in the genera *Xenorhabdus* and *Photorhabdus* (Shapiro-Ilan et al., 2012).

Fungi in the genera *Beauveria* and *Metarhizium* are the most exploited entomopathogenic fungi that are currently formulated into biopesticides (Quesada-Moraga et al., 2020). Strains of these genera are the most common to infect insects naturally, with host ranges in the hundreds of species across a range of insect orders. These species are largely asexual, with just conidia produced in bulk on cadavers. They can also be grown easily on simple media, including grains (Grzywacz et al., 2014), which makes mass production possible. In general, strains of *Metarhizium* and *Beauveria* are safe for non-target organisms, such as predators and parasites, when applied in the field. This does not mean these other beneficial agents may not be infected in the laboratory in some cases, but these other organisms are far more resistant to infection, meaning they have a little impact in the field. The compatibility of these beneficial fungi with other environmentally sensitive control approaches has been exploited in integrated pest management (IPM) programmes (Glare et al., 2020b).

Insect-killing bacteria are known from diverse taxonomic groups, although only a few groups have been exploited as commercially available biopesticides (Jurat-Fuentes and Jackson, 2012), and even fewer used in horticulture. Spore-forming bacteria in the genera *Bacillus, Paenibacillus, Lysinibacillus* and *Brevibacillus* are the most commonly used for insect-control products, the majority of which target caterpillars or Diptera. Among the Proteobacteria, species of *Serratia, Pseudomonas, Chromobacterium* and *Burkholderia* have been used. Bt strains and subspecies have been the mainstay of biopesticides used in horticulture for over 70 years, with the first commercialised Bt sold in 1938, and still accounting for around 60–70% of all microbial-based biopesticide sales (Olson, 2015). Some bacteria act through infection, but in many cases, including Bt, most target mortality is due to toxins. The use of toxins, as opposed to microbes with an infectious mode of action, results in very different commercial products. For example, in the case of Bt, in which there is no requirement for live cells in the final product. This greatly simplifies formulation and storage.

Entomopathogenic nematodes can be found in several major groups, but the most exploited for biopesticides are in the Heterorhabditidae and Steinernematidae families. These nematodes have symbiotic bacteria involved in the infection cycle. The two groups are not particularly closely related but do share common characteristics, since they are obligate parasites, horizontally transmitted, have an infective juvenile stage and are always associated with bacteria that are involved in infection (Koppenhőfer et al., 2020).

This group of nematodes has been the subject of commercial development, with 13 species now developed. Commercialisation has been aided by the often broad host range of the main nematode families and the development of *in vitro* mass-production methods (Shapiro-Ilan et al., 2012). Although the range of hosts can be broad in the laboratory, with over 200 species for *S. carpocapsae* across 10 insect orders (Koppenhőfer et al., 2020), for many species, the host range has not been investigated in the field. Infective juveniles are able to disperse actively and passively, which can greatly aid effectiveness.

Entomopathogenic viruses are known from 15 families, but most viruses used in biopesticides have been from the family Baculoviridae, containing the nucleopolyhedroviruses (NPV) and granuloviruses (GV), which target Lepidoptera (Srinivasan et al., 2019). These viruses have several attractive characteristics, principally that the infective virons are encased in a proteinaceous coat, ensuring more robust particles in the environment and suitability for formulation into biopesticides. The known viruses in these groups are host-specific, sometimes only known to infect a single lepidopteran species, meaning they are safe for non-target organisms. However, growing these viruses outside living cells is not currently possible, which greatly increases the cost of production and limits their use as biopesticides in many areas.

Some successful biopesticides have been developed based on baculoviruses. In Africa, commercial formulations of the *Maruca vitrata* Mavi NPV have been used in Benin, Niger and Nigeria (Srinivasan et al., 2019). NPV formulations have also been used against *Spodoptera*, *Plutella xylostella, Mamestra brassicae* and *Helicoverpa armigera,* including in citrus orchards in South Africa (Moore and Kirkman, 2010). In the Canary Islands, a *Chrysodeixis chalcites* NPV is under development as a commercial biopesticide for the pest of banana crops, *Chrysodeixis chalcites* (Bernal et al., 2018).

4 Key challenges in the successful use of biopesticides in integrated pest management programmes

There is a common list of challenges that have been reported to limit the uptake of microbial-based biopesticides. Some are based on the limitations of using

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