Topics in

Agricultural Entomology XIII

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Edimar Peterlini
Érica Ayumi Taguti
João Rafael Silva Soares
Matheus Cardoso de Castro
Sandy Sousa Fonsêca
Vinicius Ferraz Nascimento
Ricardo Antonio Polanczyk

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www.atenaeditora.com.br
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PREFACE

The Graduate Program in Agronomy (Agricultural Entomology) at the UNESP Faculty of Agricultural and Veterinary Sciences in Jaboticabal has always been characterized by its focus on Integrated Pest Management (IPM). Since its foundation, the program has graduated 287 students with a master's degree and 148 Ph.D. students. They are now active in various areas of the public or private sector and contribute to agriculture's economic and environmental sustainability.

This e-book entitled "Topics in Agricultural Entomology - XIII" was made possible through the immense effort of the Organizing Committee, formed by MSc and Ph.D. students from all research areas of our Graduate Program. In its 14 chapters, readers will find information on the most diverse areas of IPM, with a richness of information on both the fundamental and applied aspects of IPM.

As coordinator of the 2022 edition of the Winter Workshop on Agricultural Entomology, it is my pleasure to provide event attendees with an e-book of excellent content, demonstrating the importance of our research to society.

Prof. Ricardo Antônio Polanczyk

FCAV/UNESP

PPG Entomologia Agrícola Coordinator

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CAPÍTULO 5

TECHNOLOGICAL INNOVATIONS APPLIED TO INSECT PEST MANAGEMENT

Sandy Sousa Fonsêca

Ciro Pedro Guidotti Pinto

Ana Letícia Zéro dos Santos

Amanda Cristina Guimarães Sousa

Nicole de Paula Souza

Guilherme Duarte Rossi

1 I INTRODUCTION

Agricultural losses caused by pest insects have been estimated at up to 40% of global production (FAO, 2021). These losses occur due to the increase in populations of several pest insects above levels of economic damage (Pedigo; Hutchins & Higley, 1986). They can be minimized through the proper use of insect pest control methods (Oberemok et al., 2015; Sparks et al., 2020).

Therefore, pest insect control methods and tactics have been developed since the dawn of agriculture. Among the control methods available for use in Brazilian agriculture, chemical control, and genetically modified plants with *Bacillus thuringiensis* (Bt) genes (ISAAA, 2018) stand out. However, even with increasingly refined technologies, the improper use of chemical control can lead to several negative consequences for the environment (PISA et al., 2021). Additionally, both in the use of chemical control and Bt plants, the absence of insect resistance management (IRM) (Roush, 1993; Sparks et al., 2021) has accelerated the evolution of resistance in pest insect populations and hence control failures (Farias et al., 2015).

Therefore, developing new control methods is a dynamic process and must meet the requirements of production, market, environmental, and biosafety systems considering the best available technologies (Sparks, 2013). In this chapter, a brief description of the process of obtaining insecticide molecules and presentation of studies on technological innovations such as the use of arthropods as a source of insecticidal molecules and molecular techniques such as RNAi and genomic editing by CRISPR/Cas9 to control pest insects will be made.

2 I SEARCH AND DEVELOPMENT OF NEW INSECTICIDAL MOLECULES

New insecticidal molecules must be discovered and developed for crop pest management and hence high productivity (Godfray et al., 2010; Lamberth et al., 2013; Loso et al., 2017). Although there are many insecticides available on the market, the search for new efficient and safe molecules with different modes of action is relevant for three main reasons: (*i*) increasing resistant insect populations that invalidate modes of action and require new modes for their control, (*ii*) thorough regulatory factors for commercial approval, and (*iii*) increase in consumer demand (Gerwick & Sparks, 2014; Sparks & Nauen, 2015; Sparks et al., 2019a; Sparks & Lorsbach, 2017; Phillips, 2020).

The development of new insecticidal molecules is complex, and, over time, many companies have stopped acting in the sector, mainly due to the high time and capital investments (Sparks, 2013; Phillips, 2020). To optimize the discovery process, several methodologies have been developed or adapted (Loso et al., 2017). New approaches can be grouped into three main categories: (*i*) search for natural products; (*ii*) optimization of existing molecules, and (*iii*) search based on bioactive groups (Loso et al., 2017; Lorsbach et al., 2019; Sparks et al., 2019a).

Most of the currently available insecticidal molecules of natural origin are secondary metabolites of plants or microorganisms. Natural products can be used for insect control in the form of crude extracts and partially or completely purified molecules (Sparks; Hahn & Garizi, 2017; Sparks & Bryant, 2022). For instance, azadirachtin, nicotine, and pyrethrum are natural products of plant origin (Oberemok et al., 2015) and abamectin, milbemycin, and spinosyn are natural products of microbial origin (Kornis, 1995).

Improved efficacy and action spectrum of a product in use or under development characterize the optimization of pre-existing molecules (Seiber et al., 2014; Sparks, et al., 2019b). Examples include pyrethroids such as cypermethrin and deltamethrin to improve efficiency from the natural pyrethrum (Elliott, 1980) and the development of molecules as a high-efficiency mimic derived from spinosyn (Sparks et al., 2019b). Additionally, the natural compounds abamectin, milbemycin, and spinosyn were optimized to yield, respectively, the semisynthetic insecticides emamectin benzoate, lepimectin, and spinetoram (Jeanmart et al., 2016).

Search based on bioactive groups involves chemical and biochemical approaches. It is based on a biological hypothesis, followed by a molecular design, in which in-silico screening is used to enable high-performance search based on three-dimensional models or algorithms based on receptor protein active site (Loso et al., 2017; Sparks et al., 2019a).

3 I ARTHROPOD TOXINS AS INSECTICIDES

Arthropods compose a large clade in the animal kingdom, including insects, crustaceans, myriapods, and arachnids. A common feature among arthropod classes is venom production by various species or groups, such as spiders, centipedes, wasps, and small crustaceans (remipeds) (Daly & Wilson, 2018). Poisons are composed of various toxins produced in specific glands and when injected into or ingested by another organism, cause some negative effects (Schmidt, 2019). Venom glands can have different origins, for example, reproductive system modifications in bees and wasps (Cusumano et al., 2018; Pucca et al., 2019), adaptations of epidermis secretory glands in caterpillars (Villas-Boas et al., 2018), differentiation of the last abdominal segment in scorpions (Yigit & Benli, 2010), and specialization of chelicerae in spiders (Lüddecke et al., 2022).

Proteins and peptides are major components of arthropod venoms, but non-protein small molecules may also be present (King & Hardy, 2013; Daly & Wilson, 2018). Advances in proteomics and transcriptomics techniques have allowed extensive investigation of protein components of arthropod venoms (Xie et al., 2017). Therefore, the biotechnological use of these compounds has been widely discussed, tested, and applied (Fernandes-Pedrosa et al., 2013). In terms of agriculture, arthropod venoms are still poorly explored. Even so, in some places like California, the commercial products available have the mode of action based on the GS-omega/kappa-HXTX-Hv1A spider venom peptide (Sutton et al., 2020).

Many arthropod venom molecules have already been patented, with the main groups being scorpions, spiders, bees, and wasps. Among patents, scorpion venom has the highest number (7447), followed by wasp venom (7346), spider venom (2426), and bee venom (1624) (https://patents.google.com/). Major companies involved in patenting venom toxins are Monsanto Technology LLC (Bayer), Stine Seed Farm Inc., Pioneer Hi-Bred International, and Agrigenetics Inc., while major target crops are soybeans, corn, cotton, wheat, rice, and canola (Oliveira et al., 2021).

Insecticidal proteins and peptides from arthropods can be transgenically incorporated into the genome of plants of commercial interest. This technology may bring a reduction in the chemical insecticide application volume on crops and respective production cost reductions (Klümper & Qaim, 2014). Another way to use arthropod toxins is in the genetic transformation of entomopathogens to increase their efficiency (Lovett & St. Leger, 2018). In general, arthropod peptides and proteins are expected to be less toxic, less persistent, less aggressive to the environment, and more selective to non-target organisms than other synthetic insecticides (Czaja et al., 2015).

Arthropod-derived genes are still secondary when compared to other technologies (Oliveira et al., 2021). Despite the advantages of toxin-based biopesticides, groups of consumers have been against such technology (Gupta, 2015). Despite these controversial groups, arthropod toxins are widely used in medicine (Heinen & Veiga, 2011), and it will only be a matter of time to increase their use in agriculture.

4 I RNAI

Gene silencing by interfering RNA (RNAi) became widely known when described in the nematode *Caenorhabditis elegans* (Nemata: Rhabditidae) (Fire et al., 1991) and represents one of the main biotechnological advances in pest insect control (Dias et al., 2020; Yan et al., 2020). Through the use of RNAi, exogenous RNAs can directly affect specific functions that would be transcribed by messenger RNAs (mRNA) of a given organism (Zotti et al., 2017).

The RNAi mechanism is activated when double-stranded RNA (dsRNA) is absorbed by cells. After entering the cells, dsRNA is cleaved by the Dicer enzyme into small interfering RNA sequences (siRNA), which, through an RNA-induced silencing complex (RISC), function as a template to recognize and degrade complementary mRNA (Katoch et al., 2013). However, some factors such as the delivery and reception of RNAi by the target species can directly affect the efficiency of the method (Dias et al., 2020).

RNAi starts working soon after the delivery of specific dsRNAs to target insects. This delivery can be done via injection (experimental conditions) or feeding (field conditions). For insect pests to feed on dsRNAs, this genetic information must be produced in the laboratory and sprayed on plants or applied in such a way as to be absorbed by the roots of plants of interest (Christiaens et al., 2020). The delivery of dsRNAs can also be done by producing viruses or bacteria genetically modified to produce dsRNAs, which will be ingested by the target insects (Whitten et al., 2016), or by developing transgenic plants that express dsRNA (Christiaens et al., 2020).

However, after delivery to target insects, exogenous dsRNAs need adequate conditions to be functional. Factors such as insect nucleases, intestinal pH, non-specific effects, target gene or tissue, concentration, dsRNA resistance and especially the insect order of interest influence method efficiency (Jain et al., 2021).

Insect nucleases can degrade dsRNA in gut contents, especially when administered orally. Moreover, insect gut pH varies with orders and gut regions, affecting dsRNA stability (Arimat and Su et al., 2007; Katoch et al., 2013). As the RNAi mechanism is specific to a short nucleotide sequence, ingestion hinders dsRNA specific action since this route of ingestion can reduce the chances of finding genes with homologous sequences (Kulkarni et al., 2006). Therefore, gene region selection for dsRNA production should be careful (Katoch et al., 2013). Another important factor is the concentration of dsRNA available for pest insects, which directly depends on dsRNA size of a species and gene of interest (Bolognesi et al., 2012; Joga et al., 2016).

In insect pests of the order Coleoptera, so far, the one that has the highest susceptibility to RNAi-based control tactics, control efficiency can be above 90% (Rangasamy & Siegfried, 2012; Zhu et al., 2011). In less susceptible insects, such as those of the order Lepidoptera, this number is reduced to about 60% and silencing may be temporary (Huvenne & Smagghe, 2010; Li et al., 2013). Lepidoptera shows some refraction to dsRNA, mainly by its degradation in insect guts or absorption in degradation organelles (Yoon et al., 2017). In sap-sucking insects of the order Hemiptera, difficulty in reaching dsRNA is due to the insect's feeding habits. In this type of situation, hemipteran insects absorb dsRNA by feeding on citrus trees and vines exposed to dsRNA via spraying, root soaking, and trunk injections (Jain et al., 2021).

Even with its potential application in pest control, RNAi resistant populations can be selected, just as what happens with Bt technology (Tabashnik; Brévault & Carrière, 2013). The RNAi technology is not restricted to the genetic transformation of plants or microorganisms and can be used by non-transgenic methods (Cagliari et al., 2019).

5 I CRISPR/CAS9

Locus with repeated nucleotide sequences joined and equally spaced was identified in *Escherichia coli* (Enterobacteriales: Enterobacteriaceae) (Ishino et al., 1987). It was reported in other bacteria in later studies, with this locus being defined as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) (Jansen et al., 2002). In addition, a set of genes, composed mainly of nucleases and polymerases, located close to this region was identified and named Cas genes (CRISPR-associated genes) (Balbino et al., 2016; Lins et al., 2018).

The "CRISPR-Cas" complex originally belongs to the bacterial defense mechanism against infections by bacteriophages (bacteria-infecting viruses) (Balbino et al., 2016). When a bacteriophage infects a bacterium, the viral DNA is cleaved by restriction enzymes and integrated into the CRISPR locus, generating spaced sequences (Makarova et al., 2011; Balbino et al., 2016). In case of reinfection, RNA molecules, together with Cas proteins, recognize and then eliminate this nucleotide sequence, thus protecting the organism (Makarova et al., 2011; Kolli et al, 2018).

CRISPR-Cas9 gene editing is a technique based on this adaptive immune system of bacteria. In this method, endonuclease (Cas9) is directed to the target DNA through a single guide RNA (sgRNA) fragment, which has a complementary target DNA sequence (Albino et al., 2016; Mitsonubu et al., 2017; Lins et al, 2018). Cleavage by endonuclease occurs through interaction domain formation in Cas9 structure, resulting from its interaction with sgRNA, which allows interaction of Cas9/sgRNA complex with the target DNA, leading to a simultaneous separation of DNA strands (Jinek et al., 2012; Mitsonubu et al., 2017).

Today, one of the main uses of CRISPR-cas9 in Entomology is gene function tests in insects (Bi et al., 2016). In *Spodoptera litura* (Lepidoptera: Noctuidae), the technique demonstrated homeotic gene deactivation effects on this species. Homeotic genes are responsible for identifying body segments. Thus, they are interesting targets for genetic knockout application, because when deactivated or incorrectly expressed, they can compromise insect development.

Sex determination-linked genes have been explored by CRISPR/Cas9 for different lepidopterans; changes in gene cascade related to sex have resulted in alterations in the insect reproductive process. Examples of them are fertility changes in both males and females, reduction in the number and malformation of eggs, and impairment of spermatogenesis (Chen et al., 2019; Fujinaga et al., 2019; Fujii et al., 2020; Zhu et al., 2021).

In addition, mating and reproduction were also affected when pheromone-related genes were edited, thus reducing the pest insect population (Chang et al., 2017; Cao et al., 2020; Jiang-Jie et al., 2021). Thus, manipulation of pest-insect reproductive aspects through CRISPR/Cas9 can be used to develop control tactics (Smagghe, Zotti & Retnakaran, 2019).

In addition to insect pest gene editing, CRISPR/Cas9 may be useful for gene editing in plants of economic interest (Lu et al., 2018). Besides conferring resistance against insect pests, the technique can generate edited non-transgenic plants, as gene edits from CRISPR/CAS9 are evaluated by regulatory agencies on a case-by-case basis (ISAAA 2021).

To end this chapter, we would like to emphasize that new molecule development is a continuous task, as agricultural environments are under constant changes and transformations. The discovery of new molecules with all desirable requirements is a complex but achievable task, mainly due to technological advances. Remarkably, solutions will never be definitive, thus resistance to insecticides must always be managed for insect pests, preserving the efficiency of available molecules.

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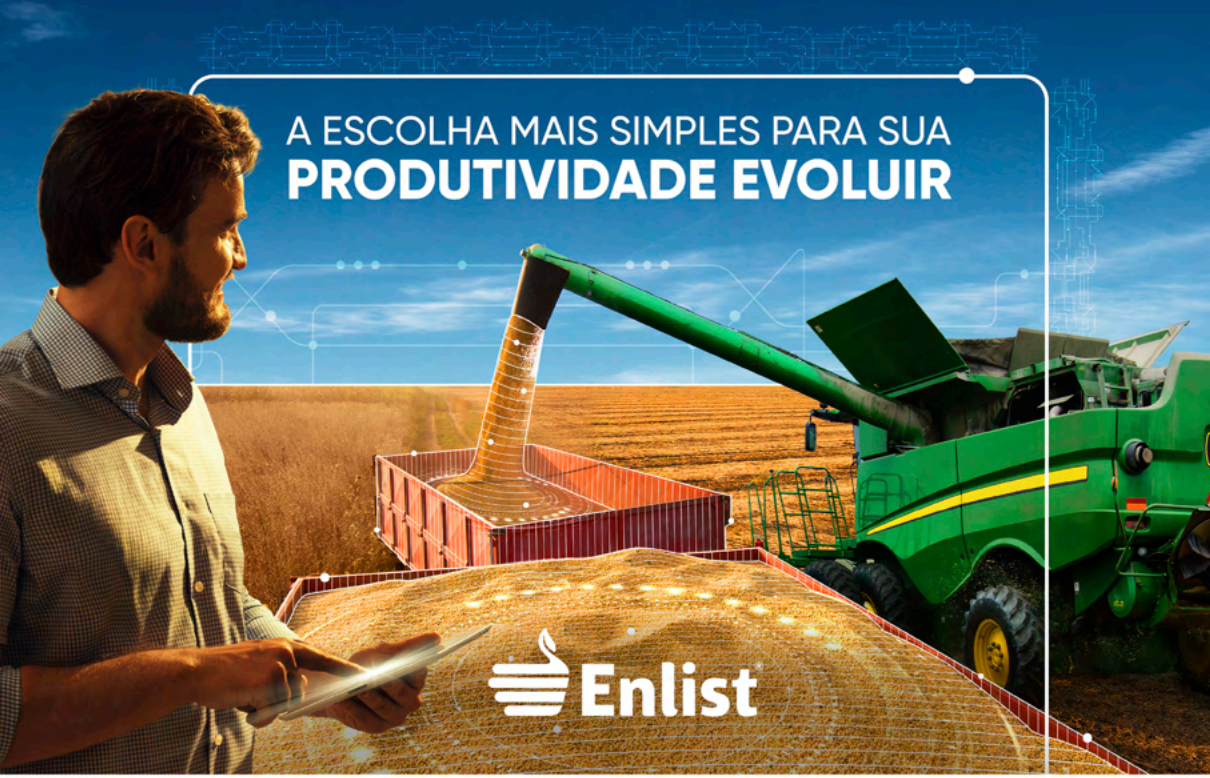

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