Topics in Agricultural Entomology XIII

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Daniel Dalvan do Nascimento | Edimar Peterlini
Érica Ayumi Taguti | Joao Rafael Silva Soares
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Topics in agricultural entomology - XIII

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Revisão: Os autores

Dados Internacionais de Catalogação na Publicação (CIP)

T674 Topics in agricultural entomology - XIII / Joacir do Nascimento, Claudiane Martins da Rocha, Daniel Dalvan do Nascimento, et al. – Ponta Grossa - PR: Atena, 2022.

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Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-258-0544-3
DOI: https://doi.org/10.22533/at.ed.443220109

1. Agricultura. I. Nascimento, Joacir do (Organizador). II. Rocha, Claudiane Martins da (Organizadora). III. Nascimento, Daniel Dalvan do (Organizador). IV. Título.

CDD 338.1

Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166

Atena Editora
Ponta Grossa – Paraná – Brasil
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CIÊNCIAS AGRÁRIAS

Atena Editora
Ano 2022

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DECLARAÇÃO DA EDITORA

The authors are grateful to the São Paulo Research Foundation (FAPESP) for the grant 2022/04343-9

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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QUALITY CONTROL IN MASS REARING OF INSECTS

Matheus Moreira Dantas Pinto

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1 I INTRODUCTION TO INSECT REARING

Humans fear insects for numerous reasons, including disease transmission such as dengue, chikungunya, and zika by the mosquitoes *Aedes aegypti* (Diptera: Culicidae), Chagas disease by the bug *Triatoma infestans* (Hemiptera: Reduviidae); as well as fear of cockroaches (Blattodea: Blattidae) for carrying human pathogens; "painful" encounters with wasps (Hymenoptera: Vespidae); and finally, the numerous pest species that cause economic losses, particularly to the agricultural sector. Anyway, there are plenty of reasons to want insects to be killed but the question always arises: Why rear them?

Several problems caused by insects to humans have been solved using laboratory insect rearing methods (Parra, 1999), mainly for integrated pest management (IPM) programs (Schneider et al., 2018). Insect rearing has gained interest over the years to the point of being recognized as a profession by Dickerson and Leppla (1992).

Among the benefits that insect rearing has brought to humanity, the following can be cited:

- **Genetics:** genetic studies advanced greatly after scientists managed to rear in the laboratory insects of the genus *Drosophila* (Diptera: Drosophilidae) (Ørsted & Ørsted, 2019).
- **Textile industry:** from the lab rearing of silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), one of the global biggest industries was founded, with sericulture (silk production) reaching great proportions, using silk produced by this insect in manufacturing high-value fabrics (Watanabe et al., 2000).
- **Food industry:** availability of honey and its derivatives in the consumer market thanks to the rearing of *Apis melifera* (Hymenoptera: Apidae) (Klein et al., 2007); food products dyeing with carmine extracted from mealybugs of the genus *Dactylopius* (Hemiptera: Dactylopiidae) (Borges et al., 2012); use of *Tenebrio molitor*

(Coleoptera: Tenebrionidae) in human food and animal feed (Murefu et al., 2019; Hong et al., 2020).

- **Pest control:**

Population control: owning to insect mass rearing associated with genetic techniques, *A. aegypti* and *Ceratitis capitata* (Diptera: Tephritidae) could be controlled (Imperato & Raga, 2015). This can be achieved using the sterile insect technique (SIT), which consists of releasing large amounts of sterile males to copulate with wild females, resulting in no offspring (Krüger et al., 2020). Currently, Moscamed Brasil in Juazeiro, Bahia, leads the use of this technique in Brazil.

Chemical control: despite the impacts on the environment, the use of chemical products (agrochemicals) combined with insect mass rearing allowed the industry to meet the world demand for food, especially since the green revolution (Evenson & Gollin, 2003).

Biological control: consists of regulating plant and animal numbers by natural enemies, also known as biotic mortality agents, and can be generically of three types: classical, conservative, and augmentative (Huffaker, 2012).

2 I INFLUENCE OF ABIOTIC FACTORS ON INSECT DEVELOPMENT

Insect development is heavily affected by bioecological factors (González-Chang et al., 2019). Under extreme physical conditions, insects require physiological adaptations and other peculiarities for survival, thus generating adaptive responses (Savopoulou-Soultani et al., 2012).

Among the abiotic factors, temperature and humidity stand out as the most important for insect development, abundance, and distribution (Fisher; Rijal & Zalom, 2021). The temperature has a significant effect on insect community ecology, development time, survival, reproduction, and sex ratio, among others (De Bortoli et al., 2014; Bjorge et al., 2018).

Each insect population has an optimal temperature at which development is favored, as well as lower and upper limits for suitable growth (Azrag et al., 2017). Most insects develop faster when reared at higher temperatures, but often reach a smaller final body size (Semsar-Kazerouni; Siepel & Verberk, 2022).

Relative humidity and rainfall are also key abiotic factors for insect populations (Fisher; Rijal & Zalom, 2021). Associated with humidity, heat stress is usually responsible for reducing insect survival (Bubliy et al., 2012). In this sense, Khadka et al. (2020) observed significant reductions in *Halyomorpha halys* (Hemiptera: Pentatomidae) nymph hatching and survival due to exposure to low humidity. Tamiru et al. (2012) concluded that temperature and relative humidity affect developmental time, adult longevity, and fecundity of *Chilo partellus* (Lepidoptera: Crambidae).

Photoperiod is another important abiotic factor for insect development. It is also the most reliable for predicting seasonal changes, especially due to day length. Many insects use this factor to initiate migration and speed up development (Minter et al., 2018). Insects use day length to determine how long weather conditions remain favorable to complete their juvenile stage before the favorable growing season ends (Lopatina et al., 2011).

Photoperiod is also directly related to thermal responses to insect growth and development (Semsar-Kazerouni; Siepel & Verberk, 2022). Phoyoperiods shorter than the optimal one had delaying effects on both growth and development of *Lycaena phlaeas* (Lepidoptera: Lycaenidae), especially if associated with low temperatures (Semsar-Kazerouni; Siepel & Verberk, 2022). Therefore, studies on factors affecting the insect cycle (biotic and abiotic) are essential to achieve success for mass rearing, hence impacting positively pest management programs.

3 I GENETIC FACTORS INFLUENCING INSECT DEVELOPMENT

Insects have strong adaptive power. That is why they are one of the most abundant classes on the planet. Since environments can be altered by human actions, ecological changes may occur. This way, insects may respond by modifying their physiology or morphology. This phenomenon is characterized as phenotypic plasticity, which is the ability of a genotype to exhibit different phenotypes if exposed to environmental changes. It is, therefore, a genome reprogramming in response to the environment (Pigliucci, 2001; Sultan & Spencer, 2002), and extreme changes in their life history and behavior may also occur (Pigliucci, 2001). Polymorphism is an example of phenotypic plasticity and occurs in the wings of some insects; after being reared under different temperature conditions, these insects have plastic responses in terms of wing size and shape (Azevedo et al., 1998; Magistretti, 2006).

Another factor influencing insect development is gene flow. It is a mechanism for exchanging information or gene movement between individuals, populations, or species. It normally occurs through the dispersion of genetic variety, in this case, by the founder gene effect (Baker & Loxdale, 2003). Conversely, insect development variability can be lost by not introducing new genetic material into a population. Over time, this leads to a high degree of inbreeding (Hufbauer, 2002). Consequently, changes in insect size, offspring viability and fertility, mortality at immature and adult stages, as well as in their morphology, may occur, thus impairing the efficiency of biological control agents in the field (Cassel et al., 2001; Van Lenteren, 2009).

Some tools for detecting these factors are protein and nuclear and mitochondrial DNA analysis. The latter is the most used to assess gene flow, inbreeding degree, genetic structure, and natural selection intensity of populations (Hoy, 2003).

4 | ARTIFICIAL AND NATURAL DIETS: THE IMPORTANCE OF NUTRITION IN INSECT DEVELOPMENT

The success of an insect biofactory can be affected by the nutritive factor of the diet used, as it acts directly on the development of different insect life stages (Panizzi & Parra, 2009). Thus, nutritional issues must be evaluated with great caution both from a qualitative and quantitative point of view, always based on the nutritional requirements of the species under study, whether in a natural or artificial diet (Panizzi & Parra, 1991).

Studies on insect nutrition have been carried out since the last century (Uvarov, 1928). Still, only after 1960, the research on nutritional requirements was refined, and artificial diets began to be developed (Singh, 1977). Most of the essential nutrients to insects are available in their natural diet; however, some of them can be obtained from other sources, such as reserves accumulated in immature stages, synthesis from other nutrients that make up the diet or from the activity of symbiotic organisms (Hagen et al., 1984).

Food quality depends on its physical and chemical properties such as hardness and available form, as these characteristics directly influence the ability of organisms to ingest, digest, and absorb them (Parra; Panizzi & Haddad, 2009). Insects can find changes in food quality during different stages of their cycle. These changes may have several consequences such as a decrease or increase in body size for example (Reznick & Yang, 1993).

Food nutritional composition can influence different biological parameters of insects, acting positively or negatively on their longevity, fecundity, and development time (Rossetto, 1980). Within this context, amino acids and proteins are essential elements for good development and are often required at high concentrations in the diet (Parra, 2009).

Vitamins are required in small amounts in insect nutrition, but they act in several important metabolic processes, such as structural components of enzymes. Major vitamins are D (in fact is a steroid), A (retinol), E (alpha-tocopherol), and C (ascorbic acid); the C vitamin is almost constant in green plant tissues and used in most artificial diets fed to insects in the laboratory (Avé, 1995; Parra, 2009).

Other important groups for insect nutrition are carbohydrates, which work mostly as a primary energy source; sterols, which are needed for insect growth and reproduction; and water (Parra, 2009).

A natural diet has several forms and nutritional variations, depending on the conditions to which it is subjected. It also shows seasonality, challenging its use in insect rearing. Factors such as temperature, photoperiod, and humidity hinder natural food availability. Such scarcity leads insects to adapt by inducing events such as quiescence or diapause (Panizzi & Parra, 2009). Natural insect food from field or greenhouse can have contamination by microorganisms, which often makes it impossible to use in laboratory conditions (De Bortoli et al., 2015). The chemical composition of natural food substrates also changes as a function of seasonality, agronomic conditions of cultivations, and climatic conditions, significantly influencing insect development in rearing (Parra; Panizzi & Haddad, 2012).

Due to implications with natural diets, major studies were required to use artificial diets in insect mass rearing. Nonetheless, to maintain the rearing of certain species in the laboratory, artificial diets must meet basic parameters such as providing pre-imaginal development with survival greater than 75%; meeting nutritional requirements of insect species; maintaining reproductive capacity and vigor for several generations; being easy to prepare and with cost compatible with the objectives of the activity (Parra, 2012). Additionally, the use of artificial diets has the main advantages of obtaining individuals continuously and in number and quality for several generations, meeting objective work needs (Parra, 2009).

5 I QUALITY CONTROL IN MASS REARING

According to Prezotti (2002), quality control is essential in mass rearing since it identifies production problems, as well as lineage deterioration after several generations kept in the laboratory. After almost 30 years of the beginning of artificial diet development for insect rearing, the International Organization for Biological Control (IOBC) was founded to ensure quality control in insect mass rearing (Leppla & De Clercq, 2019).

To qualify an insect being mass-reared, Van Lenteren (1991) used the example of a natural enemy, stating: “a natural enemy, produced and released in the field, is expected to perform its role,” thus, control can compare whether the total quality is preserved in mass rearing in the laboratory.

Within the insect mass production system, there must be an operational procedure (Protocol) to be followed as a way of standardizing all production. There must also be monitoring from the beginning to the end of the production (production - process - product).

According to Leppla & Fisher (1989), production control is a guarantee of the execution of insect rearing and all related operations, following standard procedures for handling individuals, work routine, and insect development environment, in addition to checking and recording developed activities. On the other hand, process control consists of monitoring the entire insect development and potential biological losses through comparisons with pre-established standards. Finally, product control aims to ensure the final quality of the insect produced.

According to the purpose for which a species is produced, such as the form of release, intended crop, a pest to be controlled, local abiotic conditions (climate), among others, quality evaluation should be adapted. Therefore, biological, physiological, and ecological factors of each relationship involved in the object of study must be fully known (insect/natural enemy, for example) to establish quality assessment components. However, in general, the following are evaluated: fecundity, fertility, weight gain of larvae and pupae, percentage of emergence, sex ratio, mortality, longevity, flight capacity, and mating competitiveness (Bigler, 1992; Clarke & Mckensie, 1992). Specific temperature, relative humidity, and photoperiod conditions are recommended for each situation, in addition to specifying expiration dates of each shipment produced (on the packaging), quantity, and development phase, among other information. What must always exist, which is usually specific to each biofactory and species produced, are protocols for quality control of the product, as reported in Table 1 for natural enemies (Van Lenteren, 1992).

Other more accurate techniques that can be used to assess insect quality are electrophoresis, electroretinography, isoenzyme profiling, as well as DNA techniques such as RAPD (Random Amplified Polymorphic DNA) and microsatellites (Single Sequence Repeats - SSR) (Clarke & Mckensie, 1992). Quality control of biological products is a fundamental step in the production process, whether in laboratories, small and medium-sized biofactories, or large companies since it aims to evaluate bioproduct characteristics from different aspects and ensure its quality, safety, and effectiveness.

	Control agent	
Quality components:	Predators	Parasitoids
Number of individuals alive per container	X	
Number of live insects (immature form)		X
Number of adults emerged after a certain time		X
Sex Ratio: Minimum percentages of females may indicate inadequate rearing conditions	X	X
Fertility: Number of offspring produced during a period	X	
Fecundity: Efficiency in host control		X
Longevity: Minimum in days	X	X
Predation: Number of prey consumed during a period	X	
Adult Size: Hind tibia length		X
Pupa Size: Good indication of fecundity, longevity, and predation capacity		X
Longevity	X	X
Short-range flight: the ability to fly	X	
Long-range flight: predation capability	X	
Long-range flight: parasitism capability		X
Field performance: Locate and consume prey in the field	X	
Field performance: Foraging and parasitizing host in the field		X

Table 1 - Quality components according to standards established by the International Organization for Biological Control (IOBC) and partner companies for quality testing in rearing of various natural enemies (Van Lenteren, 1992).

6 I EXAMPLES OF MASS REARING OF INSECTS

Rearing of earwigs

Dermaptera comprises around 2,000 previously described species, belonging to 11 families (Haas, 2019). Popularly known as earwigs, they are omnivorous insects that use plant and/or animal resources for food/nutrition (Pasini; Parra & Lopes, 2007). *Euborellia annulipes* has been reared in the laboratory with an artificial diet since its discovery as a potential predator of Boll Weevil, *Anthonomus grandis* (Coleoptera: Curculionidae) in Paraíba (Lemos; Ramalho & Zanuncio, 2003).

E. annulipes rearing from the Laboratory of Biology and Insect Rearing (LBIR), nymphs are kept grouped in circular plastic containers (9 cm diameter × 15 cm height) in a total of 40 insects/container (Figure 1A). Adults are reared in rectangular plastic containers (13 cm × 20 cm × 7 cm) at a density of 36 individuals, a sex ratio of 3: 1 (Figure 1B), and under controlled conditions (25 ± 2 °C temperature, 70 ± 10% humidity, and 12:12

h photophase). Each container contains accordion-folded moistened toilet paper, about 2 cm wide, to shelter insects and maintain internal humidity (Figure 1C). The food provided consists of an artificial diet based on a starter feed for broilers - Premix (350 g), wheat bran (260 g), brewer's yeast (220 g), powdered milk (130 g), and nipagin (40 g) (Silva; Batista & Brito, 2009), arranged in 2 mL Eppendorf tubes (Figure 1D). Moisture and feeding are checked every two days. One of the biggest cares that must be taken in the rearing of this species, as for most dermapterans, is ensuring parental care preservation (Butnariu et al., 2013). Eggs must be kept or separated from rearing containers, together with mothers, always in a moist substrate, until the nymphs hatch (Figure 1D). Incubation of this species normally lasts from 15 to 30 days.

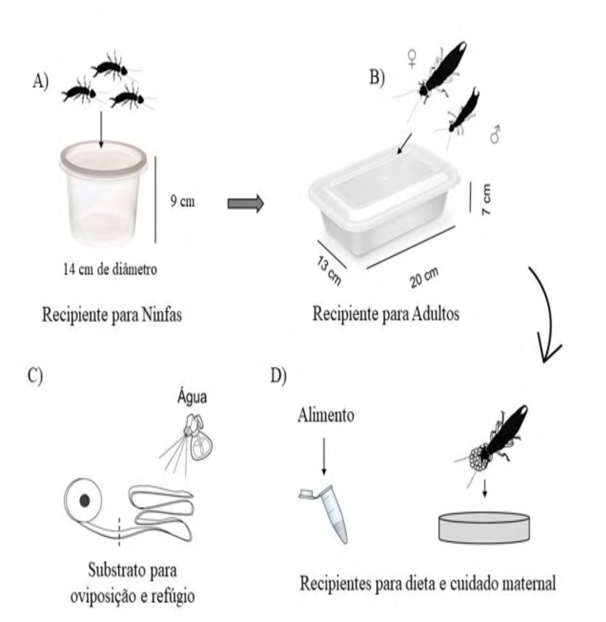

Figure 1 - Scheme of the rearing method for *Euborellia annulipes* under laboratory conditions. Containers used for rearing A) nymphs and B) adults; C) substrate for insect refuge and oviposition; and D) containers for providing food and maternal care (Source: Nunes, 2020).

Rearing of green lacewings

Green lacewings are predatory insects of the Neuroptera order and the Chrysopidae family. In Brazil, they are known as “lacewings”, due to the behavior of some species larvae to carrying debris and remains of their prey on their backs (Adams & Penny, 1987).

In the Laboratory of Biology and Insect Rearing (LBIR), *Chrysoperla externa* and *Ceraeochrysa cincta* are reared following a method adapted from Freitas (2001). Larvae are kept in Petri dishes with shredded paper as a refuge and in flat-bottomed test tubes (8.5 cm x 2.5 cm), to minimize cannibalism. Eggs of *Corcyra cephalonica* (Lepidoptera: Pyralidae) (*ad libitum*) are larval food substrates. Pupae are kept in flat-bottomed test tubes (8.5 cm x 2.5 cm) sealed with plastic PVC film, remaining until adult emergence.

Adults are kept in cylindrical PVC cages (20 cm x 20 cm) with an inner wall lined with bond paper (oviposition substrate). The cages were sealed at the bottom ends with plastic potted plant plates and at the top ends with voile fabric. Adults were fed honey and brewer's yeast-based diet at a ratio of 1: 1. Eggs were collected by removing the paper from the inside cages and, with the aid of a knife or scissors, they are removed by cutting their pedicels, as displayed in Figure 2.

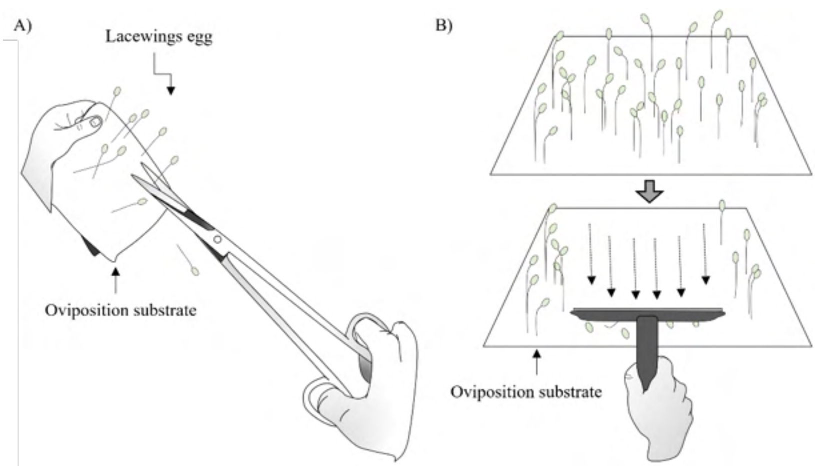

Figure 2 - A) Collection of eggs in voile fabric with the aid of scissors; B) Collection of eggs on bond paper with the aid of a knife blade.

Green lacewings are already commercially sold in several countries, as the products "Chrysopa®" by Koppert®, "Chrysopa-System®" by Biobest®, and "Chrysocontrol®" by Agrobío®, which contain *Chysoperla carnea*. In 2021, the first two biological products with crisopids (*C. externa*) were registered in Brazil, "Criso-Vit" from the Vittia group and "Crisopídeo Amipa" from the Minas Gerais Association of Cotton Producers – AMIPA (AGROLINK, 2021).

Rearings of *Diatraea saccharalis*, *Cotesia flavipes*, and *Trichogramma galloi*

Diatraea saccharalis (Lepidoptera: Pyralidae) is one of the most important sugarcane pests, having as main natural enemies the parasitoids *Cotesia flavipes* (Hymenoptera: Braconidae) and *Trichogramma galloi* (Hymenoptera: Trichogramatidae) (Parra, 2010)

In the case of *D. saccharalis*, laboratory rearing starts by assembling cylindrical PVC cages (20 cm high x 15 cm diameter) coated internally with bond paper and slightly moistened with distilled water. Therein, adults are kept for copulation and oviposition. Eggs deposited on the paper, which is replaced daily, undergo an aseptic process made with 0.05% sodium hypochlorite and 17% copper sulfate solutions.

After treatment, they are kept in a room at an average temperature of 20°C for incubation. Then, they are inserted into flasks with an artificial diet, which can be in flat-bottomed test tubes (2.0 cm x 8.0 cm) or glass jars (500 mL). The latter is closed with a mesh lid and the tubes with hydrophobic cotton, allowing internal aeration. On average, 100 caterpillars are kept in the glasses, and 25 in the tubes. These containers remain in an environment with an average temperature of 28ºC. After reaching the third instar, caterpillars are transferred to plastic plates (6.0 cm in diameter x 2.0 cm in height) also with an artificial diet, remaining until the pupal stage. Then, adults are sexed and inserted in new cages, followed by rearing. Only 5% of pupae are needed to maintain rearing, with the remaining being used to rear the parasitoid *C. flavipes* (Viel, 2009).

For *C. flavipes* rearing, after reaching the third instar, caterpillars are offered manually to parasitoid females to be parasitized. On average, five caterpillars are parasitized per plate with an artificial diet. Such a number may change according to the protocol of the biofactory. *C. flavipes* larvae remain in their host for 15 to 20 days, when they leave the caterpillar's body and form pupae surrounded by a cocoon of silk threads, which are called "pupa masses." About 5% of the population is kept in groups of 10 masses per plate to maintain *C. flavipes* rearing, while the other 95% is intended for field release to control *D. saccharalis* (Veiga et al., 2013).

Regarding the parasitoid *T. galloi*, rearing follows a method adapted from Valente et al. (2016) by the LBIR. It starts with adults kept in flat-bottomed test tubes (2.5 cm x 8.0 cm) where, with the aid of a needle, a small portion of honey is placed on the inner wall of each tube (food for adults). Eggs of *D. saccharalis* are added to the tubes and must remain exposed to the parasitoid for about 18 hours. Eggs already parasitized are transferred to flat-bottomed test tubes (2.5 cm x 8.0 cm), sealed with plastic PVC film. Therein, they remain until the new generation hatches, which occurs on average 10 days after parasitism.

7 | CURRENT SITUATION OF COMMERCIALIZATION OF BIOLOGICAL CONTROL AGENTS IN BRAZIL AND THE WORLD

There is a growing demand for biological products for pest management in Brazilian and global agriculture, mainly due to the need for more sustainable, economic, and social approaches (Baker; Greenb & Lokerb, 2020). A recent report by the United Nations (UN) dealt with the right to food, reporting how the use of agrochemicals in agriculture threatens human rights due to their impacts on health, the environment, and society.

Brazil is the leader in the adoption of organic products, with about R$ 1.7 billion in the 2020/2021 crop harvest, growing by 33% compared to the 2019/20 crop harvest (CROPLIFE BRASIL, 2021). Such growth is 30% higher than the global average (14.4%). The use of biological control agents in Brazil, involving macro and microorganisms, has been growing at a rate of 20% per year (Van Lenteren et al., 2018; ABCBIO, 2021), estimating that by 2030 there will be a turnover of about R$ 4 billion in the market of biological products, with an expected increase of 107% in sales (CROPLIFE BRASIL, 2021).

Brazilian legislation for biological product registration is one of the best in the world. Its change/update in 2010 increased significantly the number of registered products, from 26 in 2011 to 443 by March 2022 (AGROFIT, 2022).

Regarding economic aspects, the costs associated with release, control efficiency, and rearing of natural enemies for biological control have been the focus of many discussions (Baker; Greenb & Lokerb, 2020). Labor in the mass production of natural enemies represents 70-80% of total production cost (Parra, 2002).

In the Brazilian market, *Bacillus thuringiensis*-based pesticides are registered for biological control of various insect pests, including fruit trees (Do Nascimento et al., 2021). However, the major highlight of the Brazilian biological control program, which is a world reference, is the one carried out to combat *D. saccharalis*, with about half of the planted sugarcane, about four million hectares, being treated with releases of *C. flavipes* (Aya et al., 2017; Parra & Coelho-Júnior, 2019). Unlike other countries that employ biological control in small areas or greenhouses, the challenge in Brazil is to implement programs in large extensions of agricultural areas (Parra, 2014).

8 | FINAL CONSIDERATIONS

Based on what has been discussed throughout this chapter, we can conclude that insect rearing in the laboratory is as important as any other area of entomological study. More than a "profession," it is a fundamental science that supports Integrated Pest Management

(IPM), sustainability, and public health, as an important ally to solving world hunger.

We also observed that, like all science, insect rearing under laboratory conditions has its foundations or principles, which must always be well connected within all insect rearing protocols, as illustrated in Figure 3.

Among these principles, quality control is one of the most influential in insect mass rearing success in the laboratory, as such a process depends on a quality control protocol. This, in turn, directly affects obtaining insects in suitable quantity and quality so that they could perform the functions for which they are being produced in the different biofactories.

Figure 3 - “Insect Rearing Puzzle” adapted from the logo for the 2014 International Insect Rearing Workshop by Frank M. Davis (Schneider et al., 2018).

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CAPÍTULO 2

CONSERVATION PRACTICES FOR MAINTENANCE OF NATURAL ENEMIES IN AGROECOSYSTEMS

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1 I INTRODUCTION

Conventional agriculture has greatly impacted several agroecosystems due to improper management and constant, often indiscriminate, agrochemical applications. These products usually drastically reduce not only populations of insect pests but also their natural enemies, often causing imbalances and selective pressure for resistance in pest populations, also contributing to significant yield losses and negative impacts on the environment.

According to Michereff & Barros (2001), several environmental problems have occurred in conventional agriculture, including contamination of food, soil, water, and animals; intoxication of farmers; resistance of pathogens, insects, and invasive plants; biological imbalance, altering the cycling of nutrients and organic matter; elimination of beneficial organisms; and reduction of biodiversity.

Monocultures drastically interfere with the natural diversity of the environment, replacing a complex vegetative system with a few cultivated species. The use of agrochemicals from planting to harvest contribute to biological imbalances, where population outbreaks of insect pests compete with cultivated species, causing economic damage to the agricultural exploitation, environmental problems resulting from native vegetation destruction, biodiversity reduction, soil erosion, and contamination of natural resources and food.

According to Edwards (1989), agricultural systems must be redesigned and practices and conventional system inputs replaced, aiming at self-sustainability of agricultural production. Based on this premise, these methods should be used more often, which is not due to many institutional, economic, social, legal, and educational barriers (Zalom, 1993).

Thus, and according to Diniz et al. (2006), implementing alternative systems significantly reduces the risks of pollution and intoxication of operators and consumers. Notably, an integrated management involves conservationist practices, which are extremely important in agroecosystems, as integration between different management types has led to a reduction in agrochemical applications and environmental impacts, thus contributing to agriculture sustainability.

2 | SUSTAINABLE AGRICULTURE AS AN ALTERNATIVE TO THE TRADITIONAL MODEL

Agriculture became a practice more than 12 thousand years ago from means of subsistence and by advances such as adoption of chemical fertilization, crop rotation, mechanization, plant breeding, and transgenics. This agricultural practice intensified and modernized, starting to be referred to as “conventional agriculture” (Veiga, 1991; Costa Neto, 1999).

Although these advances have been revolutionary for the sector, as they have increased food supply to the population, they have had negative impacts on the environment, causing soil imbalance and narrowing down genetic biodiversity. Moreover, they increased susceptibility of cultivated varieties to attack by pests and diseases, leading to a dependence on agrochemicals, mainly insecticides and fungicides. The use of these products has promoted the emergence of chemical residues, contaminating the environment and human beings (Ehlers, 1994; Azevedo, 2018).

When conventional agriculture began to present problems due to the use of agrochemicals, biologist Raquel Carson played a very important role in raising awareness and changing behavior in relation to agricultural methods used, through the publication of her book “Primavera Silenciosa,” “Silent Spring” in English (Carson, 1962).

In the 1970s, many alternative systems emerged due to concerns about the negative impacts of conventional agriculture, including Organic Agriculture, Agroecology, Alternative Agriculture, Biodynamic Agriculture, Regenerative Agriculture, Natural Agriculture, Organic Agriculture, and Permaculture (Aubert, 1985; Carmo; Comitre & Dulley, 1988; Jesus, 1996; Zamberlam & Froncheti, 2007). However, these practices still had little adherence and financial support for adoption (Blobaum, 1984; Kramer, 1984; Hill, 1992).

After international meetings on environmental issues, the practice of more sustainable agriculture increased, which was positive for its political and economic reach (Ribeiro, 2001; De Passos, 2009). According to FAO (2001), sustainable agriculture acted efficiently in promoting crop production with available resources, offering food in quantity and quality to the population and preserving the environment.

According to Ehlers (1994):

> ...the notion of sustainable agriculture is still inaccurate and contradictory, ranging from the establishment of simple adjustments in the current production pattern to long-term goals that enable structural changes, not only in agricultural production but in society as a whole.

As Erhlers (1994), Weid (1994) compared conventional and sustainable systems, both authors observed imperfections in their literal states with a gradient between them, when combined, these practices result in a better production system that maintains productivity and preserves the environment.

Undoubtedly, the biggest challenge for agriculture is to combat food shortages. However, this problem cannot be solved without considering a sustainable management of natural resources and innovations in the sector, especially regarding pollution prevention (Da Veiga, 1994; Matten & Moon, 2020). In this sense, food production success becomes dependent on "sustainable innovations." Consumers want to purchase products of superior quality to those of conventional agriculture; therefore, a greater number of companies must adhere to this sustainable system (Hart, 1997; Hafezi & Zolfagharinia, 2018).

The search for sustainability in agriculture comes from the demand for production systems to adapt to processes that are less aggressive to the environment, farmers, and other human beings (Silva et al., 2013). Moreover, such an adhesion will result in healthier products in terms of toxic substances, and may even achieve lower production costs, as the use of external inputs is reduced (Canuto, 2021).

Recently, the Brazilian government (Ministry of Agriculture, Livestock and Supply - MAPA) has launched a sectoral plan for Adaptation to Climate Change and Low Carbon Emissions in Agriculture, aiming at a Sustainable Development (2020-2030), it is known as the ABC + Plan. This strategic view aims at productivity and sustainability of the domestic rural sector (agriculture and livestock), based on sustainable production technologies (MAPA, 2021).

In the Brazilian scenario, conventional agriculture is still predominant but has increasingly been adapted to a mix between conventional and sustainable practices, given technological advances in many social contexts of national agricultural production. In world agriculture, sustainability is inevitable, although it is an arduous task and requires medium and long-term structural changes, especially in the current agricultural context. Thus, work must be done to make the current scenario increasingly sustainable.

3 I CONSERVATIVE BIOLOGICAL CONTROL

Biological control is a major tool in Integrated Pest Management (IPM) programs. This tool can be applied either by maintaining existing natural enemies using selective products or releasing reared ones (Fernandes et al., 1999). In general, this method isefficient and compatible to be used in conjunction with other strategies, acting in harmony with the environment (Oliveira et al., 2004).

The arthropod complex in natural plant systems (herbivores [pests] and carnivores [natural enemies]) normally tend towards biological equilibrium. Such a situation can be obviously achieved without anthropic interference. Notably, biological control can and should be one of the alternatives to reduce impacts from pesticides applied in conventional farming system.

Based on agricultural aspects, biological control can be generically classified into three types: classic, augmentative, and conservative. The first refers to importation of exotic natural enemies to act on exotic or native pests; the second consists of inundative releases of parasitoids and predators, produced in biofactories; finally, the third is based on conservation and increase of natural enemies already present in the area (Ehler, 1998; Parra, 2000; EMBRAPA, 2006a; Abreu; Rovida & Conte, 2015).

Conservative biological control, then, refers to the population of naturally occurring enemies. In this model, one of the basic precepts of biological control must always be met, namely conservation. In this sense, populations of parasitoids and/or predators must be preserved through environmental manipulation, aiming at favorable conditions for survival, development, and behavioral and physiological performance of these organisms, since they are important/ essential for pest management programs (Barbosa, 1998).

A conservative biological control practice is one of the main tools of ecological management of insect pests. However, given the existing difficulty for interactions among plant populations, insect-pests, and natural enemies, this practice still has many challenges in terms of field applicability. Thus, it is essential to build conservative biological control strategies that are integrated with other practices carried out in a production system (Tylianakis & Binzer, 2014). This strategy requires more knowledge about the ecology of natural enemies present in agroecosystems. In addition, this method has numerous advantages such as ease of use, as well as being an alternative in pest management for sustainable crops, such as organic systems for example (Jonsson et al., 2008).

Natural biological control is based on taking care of natural enemies already present in farming areas, adopting practices to diversify agroecosystems (Landis et al., 2000). The agricultural practices, therefore, must influence populations of natural enemies within each agricultural system. In this sense, intercropping with companion plants can serve as a strategy, as it improves harmony and resistance to disturbances, in addition to enhancing environmental recovery (EMBRAPA, 2006b).

One way to increase the diversity of natural enemies in agroecosystems is adopting polyculture (plant diversity). This method consists of cultivation of a few plant species together, in intercropped or not (Altieri & Nicholls, 1999). According to Root (1973), abundance of natural enemies tends to be higher in diversified systems, since these increase the availability of foraging, refuge, and hibernation habitats (microhabitats). Such resources directly influence stability in phytophage population dynamics, favoring biology and dynamics of beneficial arthropods. Unlike monocrops, diversifying plant species in agroecosystems can directly influence the establishment of natural enemies of insect pests (Tschumi et al., 2015).

In diversified systems, biodiversity provides "ecological services" that go beyond production of food, fiber, energy, and income. This environmental characteristic promotes nutrient recycling, microclimate control, and regulation of water processes and abundance of undesirable organisms by predators, parasitoids, or pathogens (Aguiar-Menezes, 2004).

4 I PLANTS ATTRACTIVE TO NATURAL ENEMIES: BENEFITS AND STRATEGIES FOR MAINTENANCE AND INCREMENT

The success in introducing insect-attracting plants, also called "insectary plants," relies in the vital resources they provide such as shelter, mating, oviposition or hibernation sites, and food alternatives. These resources maintain the desirable insects in agrosystems, preventing their migration outside (Aguiar-Menezes & Silva, 2011; Naranjo et al., 2015; Wang et al., 2020).

Nectar and/or pollen, sometimes extrafloral nectar, represent alternative food resources for non-carnivorous life stages of parasitoids and certain predatory insects. These dietary items can also be a food supplement if prey is of inferior quality, or nutritional supplement when of superior quality (Portillo et al., 2012; He et al., 2021).

Floral resources act as vital sources for some beneficial insects, acting directly on their survival, fecundity, longevity, retention time, and immigration, positively influencing biological control (Aguiar-Menezes & Silva, 2011). They may also harbor non-pest phytophagous insects, which serve as alternative hosts or prey for entomophages, particularly when pests are at low population levels or absent (Souza et al., 2021).

Hinds & Barbercheckb (2020) demonstrated the importance of insectary plants for natural enemies, noting that buckwheat and cowpea increase longevity and fecundity of *Orius insidiosus* (Hemiptera: Anthocoridae). Irvin, Pierce & Hoddle (2021) proved the efficacy of buckwheat in increasing longevity of *Tamarixia radiata* (Hymenoptera: Eulophidae), as well as the number of mature eggs in ovaries. Another example of successful introduction of insectary plants was *Cnidium monnieri* (Apiaceae) in apple orchards, attracting natural enemies and providing efficient control of aphids (Cai et al., 2020).

Introducing or adding strips of insectary plants for beneficial insects between or around crop rows is one of the most feasible alternatives for farmers to increase population and diversity of biological control agents (Aguiar-Menezes & Silva, 2011). For instance, flies of the Syrphidae family benefit from attractive plant strips introduced in simple landscapes, with little plant complexity, since these predators can easily identify floral resources where there are not so many odors being emitted (Haenke et al., 2009).

Preliminary studies are crucial to identify the effect of attractive plants on natural enemies and protected crops (Winkler et al., 2010). One aspect to be considered is the distance between plant strips that benefit natural enemies in the field. Chaney (1998) mentioned that growing one row of alyssum [*Lobularia maritima* (Brassicaceae)] every twelve rows of lettuce [*Lactuca sativa* (Asteraceae)] benefits the parasitoid *Diaretiella rapae* (Hymenoptera: Braconidae).

Despite the increase in biodiversity and sheltering, the introduction of insectary plants into an agricultural environment can also interact negatively with beneficial insects (Gontijo, 2019). Not every plant or floral resource will attract natural enemies or ensure an effective biological control (Moore et al., 2019). Some plants and their floral resources can interact benefiting pests and reducing the effectiveness of their natural enemies (Moore et al., 2019) due to interactions among themselves (Lavandero et al., 2006; Jonsson et al., 2009).

Unsuitable shelters in crops can hamper biological control by undermining natural enemy movements, harboring pests, diverting predator attacks, mediating antagonistic interactions among insects (pests or not), and providing poor vital resources to support natural enemy populations. Such interactions depend on several factors, particularly plant traits, in addition to the physiology and behavior of arthropods involved. Understanding how these interactions occur is essential to plan and apply the most suitable shelters for natural enemies (Gontijo, 2019).

5 I CROP PRACTICES AND AGRICULTURAL LANDSCAPE MANAGEMENT: FEASIBLE AND LOW-COST STRATEGIES

One of the main current challenges for Brazilian and global agriculture is to ensure food security, combined with biological diversity conservation (Sunderland, 2011; Abranches, 2020). Over the last 50 years, the world population has grown unprecedentedly, and advanced techniques of food production have been developed, reducing the world's hunger. However, 30% of the world's population is still under-nourished (Uzêda et al., 2017; SOFI, 2021), demanding an increase in agricultural production by 60% to secure world's food supply until 2050 (SOFI, 2021).

However, increases in agricultural production have come at the expense of natural resources. The main tangible aspect has been significant changes in landscapes, converting natural areas into land used for production. This process makes the areas less complex and more homogeneous (Butcart et al., 2010), with 80% of the terrestrial surface being under anthropic alterations (Ellis & Ramankutty, 2008).

In this way, landscape can be broadly understood as a hybrid entity, building specific and interspecific natural, social, and cultural relationships (Uzêda et al., 2017). Agricultural landscape is highlighted for having numerous characteristics of its own, such as: intensity of crop management, extensive and routine soil management practices, in addition to wide and diversified use of inputs (Chabrerie et al., 2013), generally shaping local and regional landscapes.

Furthermore, the mosaic formed by current agricultural systems allows the formation of large and continuous homogeneous sites of land use system. These spaces are not very different from each other and are defined as monocultures. The dynamics of these places with their surroundings must be widely understood, defining appropriate management for agricultural production and conservation of diversity at the landscape level.

Despite being homogeneous, agricultural landscapes can favor the natural occurrence of organisms of interest, especially pollinators and natural predators (Landis et al., 2000; Langelloto & Denno, 2004). However, they must be properly managed to promote biodiversity corridors, reducing the effects of isolated fragments, depending on cultivation system, management, and land-use intensity (Gabriel et al., 2010).

Responses of organisms of agricultural interest, as well as others, occur on wide space-time scales (Benton et al., 2002). Therefore, biotic interactions, and mainly observed diversity patterns, are often conducted to multiple habitats and at different times (Tscharntke et al., 2005).

In this sense, extensive multiple-scale studies are needed (Gabriel et al., 2010), always considering agricultural landscapes of interest and local and regional mosaic. With this, potentialities of these areas can be identified, since food demand will continue to increase, and environmental issues will continue to be a trend.

In natural ecosystems, abundance of herbivores rarely increases to the point of causing noticeable damages such as massive loss of biomass and deleterious effects on plant reproduction (Sujii et al., 2010). This is largely due to the natural fluctuation of their populations and dynamism of naturally occurring ecological interactions (Townsend et al., 2006; Ricklefs & Relyea, 2016). These interferences result in beneficial or deleterious interactions at a specific (same species) or interspecific (different species) level (Ricklefs & Relyea, 2016).

In this sense, different forms of specific competition can be observed in production fields. Therein, plants of interest are specifically and minimally spaced, interfering little with each other during their growth and development. The current agroecosystem approach enables certain inferences consistent with well-established ecological theories, in which the more complex a habitat (landscape), the more resilient and productive it will be (Gliessman, 2005; Ricklefs & Relyea, 2016).

5.1 Crop intercropping and rotation

Intercropping and rotation systems are based on associations of two or more crop species, which have different life cycles and vegetative architectures. Examples of that can be corn and beans, corn and brachiaria, and soybeans and brachiaria, among others. Living mulch or cover crops can also be considered intercropping with species planted intentionally or grown spontaneously. These plants have special functions to the soil, such as reducing raindrop impacts; increasing soil water retention, porosity, and aeration; and decreasing temperature and humidity oscillations. Moreover, these roles of cover plants help to increase edaphic fauna and soil microbiological activity (Hartwig & Ammon, 2002).

Polycropping allows the maintenance of different plant groups simultaneously, making habitats more complex and heterogeneous. Such systems are often composed of vegetation mosaics that make it difficult for pests to locate resources. They also hinder weed establishment, which can negatively interfere with crop production (Root, 1973).

5.2 Agroecology or Agroforestry

Agroecology has gained a lot of prominence nowadays. This approach involves a way of producing food using few external inputs. In this system, human life and human and environmental health are valued in a broad perspective. Its differential is to include local and/or popular knowledge, which was historically built by the population (Gliessman, 2001;

Caporal, 2016; Reiniger et al., 2017). In turn, agroforests are adaptations of these concepts to forest environments, where there is a predominance of tree species, with concomitant exploitation of resources in natural environments.

6 I CHEMICAL PEST CONTROL: SEARCH FOR SELECTIVE PRODUCTS/ BOTANICAL ORIGIN INSECTICIDES

Food production has always been a great challenge for humanity. One of the main hurdles is the attack of insect pests, which have destroyed about a fifth of the world's total agricultural production annually (Hikal et al., 2017). In this scenario, insecticides have been the main strategy used for control, due to the ease of acquisition, high biological efficiency, low cost, and already established management and application programs.

A large part of the world market for insecticides is still dominated by synthetic products (organophosphates, carbamates, sulfonamides, pyrethroids, among other classes). However, these agrochemicals are widely reported as responsible for damages to both non-target organisms and the environment. Parallel to this, another important factor regarding synthetic insecticides is their persistence in the environment, which allows their accumulation at different food-chain trophic levels (Devine et al., 2008; Chowańsk et al., 2014). In recent decades, for example, many studies have shown the presence of agrochemical residues in food, soil, and water.

Much is discussed about economically viable and at the same time sustainable alternatives to synthetic insecticides. Some of these alternatives for controlling insect pests were presented throughout the chapter; yet insecticides of botanical origin are yet to be discussed. Looking back, botanical insecticides were once the main form of pest control in crops around the world and are still widely used nowadays. The number of studies on the use of substances of botanical origin to control pest arthropods has increased considerably in recent years, mainly due to the number of plant species that can be exploited and the ease of obtaining plant material.

Plants are rich sources of bioactive chemical compounds, which can be found in several species (Table 1). These compounds normally play a defense role in plants against pathogens and herbivores, in addition to modulating the relationship with pollinators, natural enemies, and seed spreaders. The compounds accumulate in small proportions in plant tissues and, from them are obtained powders, botanical extracts, and essential oils that can often be used as insecticides, repellents, and attractants in agriculture.

Extraction of essential oils requires elaborate equipment and techniques. Their applicability to agriculture depends almost entirely on the acquisition of those available in the market. At the same time, extracts and botanical powders can be produced by farmers themselves ("on farm"), using plants grown on their properties. Powders are obtained from dried and ground plant tissues, while extracts by contact of plant parts previously ground or not with solvents (water, ethanol, among others) (Santos et al., 2013). Compared to essential oils, extracts are less stable in the environment (mainly aqueous) and less concentrated in active ingredients. However, extracts need less sophisticated equipment to be prepared, in addition to less plant material for processing, thus reducing cost compared to essential oils.

When compared to synthetics, botanical insecticides (powders, extracts, and oils) are usually less toxic to non-target organisms, mammals, and plants; biodegradable; fast-degrading; and highly selective. Despite these advantages, plant origin insecticides cannot be considered harmless, as the toxic potential of a molecule varies with their chemical structure and not origin (Coats, 1994). Thus, all insecticides, whether synthetic or biological, must undergo tests for persistence in the environment and toxicity to non-target organisms.

After analyzing the advantages and risks of botanical insecticides, why are there still few products available on the market? In fact, many of the tested botanical insecticides have higher production costs than do the synthetic ones. This high cost comes from a lack of raw materials for production on a commercial scale. Moreover, the complex chemical characteristics of oils and extracts make it difficult to standardize formulations in terms of amounts of active ingredient with insecticidal properties. Thus, they cannot be launched on the market as regulatory bodies require proof of the concentrations of active ingredients (Shivkumara et al., 2019).

Botanical species	Main insecticidal compounds	References
Allium sativum	Methyl Allyl Disulfide, Diallyl Trisulfide	Ahmad et al. (2018)
Azadirachta indica	Azadirachtin	Tulashie et al. (2021)
Cinnamomum verum	Cinnamaldehyde, Eugenol	Marčić (2021)
Lonchocarpus negrensis	Rotenone	Doracenzi et al. (2021)
Melia azedarach	Isoxazole, Benzothiazoles	Khoshraftar et al. (2020)
Mentha piperita	Menthol, Menthone	Marčić (2021)
Nicotiana tabacum	Nicotine	Sarker and Lim (2018)
Piper nigrum	Piperamides	Scott et al. (2008)
Sophora flavescens	Oxymatrine, Matrine	Kim et al. (2009)
Syzygium aromaticum	Eugenol	Marčić (2021)
Tagetes erecta	α-terthienyl	Supriani and Wardini (2018)
Tanacetum cinerariifolium	Pyrethrum	Marčić (2021)
Thymus vulgaris	Thymol	Vite-Vallejo et al. (2018)

Table 1 - Some botanical species with insecticidal activity described in the literature

Botanical insecticides have a promising future in an increasingly demanding market for healthy food and sustainable production. Nevertheless, the focus of research has to be changed so that these insecticides could reach the consumer market, seeking technologies that allow the standardization and use of already known botanical insecticides, favoring their commercial production.

7 I FINAL CONSIDERATIONS

Conservation agronomic practices should be adopted to improve natural enemy richness and diversity in agroecosystems. The combined use of these strategies can increase resilience in these environments and decrease disturbances due to intense human interventions.

As discussed in this chapter, several methods can be used to maintain and attract natural enemies in agroecosystems. The techniques range from adoption of more sustainable production systems, against the so-called conventional agriculture, until pest control strategies deemed environmentally and socially safer.

With increasingly advanced studies and new research results published daily, besides an increasingly demanding consumer market for healthy and contaminant-free foods, conservation practices have become a cornerstone for current and future agricultural systems. The recognition of the importance of natural enemies and their contribution to insect pest population maintenance, as well as cost reductions agrochemical sprays, has led more farmers to use conservationist agronomic practices on their properties, highlighting the strong growth of the market of bioproducts acting against agricultural pests.

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CAPÍTULO 3

IMPLEMENTATION CHALLENGES OF INTEGRATED PEST MANAGEMENT PROGRAMS IN AGRICULTURAL SYSTEMS

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1 I INTRODUCTION

The agricultural sector is vital to the Brazilian economy due to income, jobs, raw materials, energy, fibers, and food production. The success of these production systems is related to obtaining high yields and carrying out proper and efficient management (Kureski; Moreira & Veiga, 2020; EMBRAPA, 2018). Phytosanitary problems are among the main factors that limit the achievement of high yields in these production systems. On average, these problems cause losses of about 30% in plant yield, and in extreme situations, these losses can reach up to 100% (Cerda et al., 2017; Picanço et al., 2007). Integrated pest management programs are the most efficient and sustainable way of phytosanitary management (Pedigo; Rice & Krell, 2021; Picanço et al., 2014). Thus, this book chapter will discuss the components, current scenario, obstacles, and innovations in integrated pest management programs.

2 I COMPONENTS OF INTEGRATED PEST MANAGEMENT PROGRAMS

Integrated pest management programs have three components: diagnosis (or assessment of agroecosystems), decision-making systems, and control methods (Pedigo; Rice & Krell, 2021; Picanço et al., 2014). The diagnosis identifies the pests, the factors favorable to their attack, and the critical control points. Identifying pests is essential for understanding their bioecology, appropriate decision-making systems, and control methods. Knowing the factors favorable to pests makes it possible to predict the risks of their occurrence in different regions and times of the year, plan decision-making, and use control methods (Pedigo; Rice & Krell, 2021; Bacci et al., 2019; Picanço et al., 2014). The main factors affecting pest attacks are climatic elements, natural enemies, pest biology, host plant, control methods, environment, and planting system. These factors affect the migration, colonization, dispersion, development, growth, mortality, survival, reproduction, and behavior of pests (Bacci et al., 2019; Felicio et al., 2019; Picanço et al., 2014). Critical control points are related to spatio-temporal distribution and pests' morphological and behavioral characteristics. These points indicate when, where and what details should be considered in pest control

(Felicio et al., 2019; Picanço et al., 2014).

Decision-making systems are composed of sampling plans and a decision-making index. A sampling plan is the detailed planning of how and when it will be evaluated plants in the field. In these plans, the densities of pests and natural enemies are determined. These determinations are used in pest control decision-making to prevent pests from causing economic damage. Sampling performed before and after applying control methods also allows the efficient evaluation of the control methods used (Pedigo; Rice & Krell, 2021; Picanço et al., 2014; Bacci et al., 2007).

Sampling plans can be conventional and sequential. Conventional plans are considered standard, have a fixed number of samples per field, are more straightforward, and in them the decision-making has two stages. The first step is carried out in the field, and the samples are evaluated in it. The second stage is in an office where calculations are made and the pest control decision is made. The sequential plans are validated by the conventional plans, and the number of samples per field is variable (it is usually 50 to 90% lower than the conventional one). The sequential plan is more complex, executed in a single step in the field when the samples are evaluated and decisions are made (Arcanjo et al., 2021; Bacci et al., 2007). The decision-making indices of management programs determine whether the pest density is low or high. When this density is equal to or greater than the decision-making index, the pest population is high, and control must be carried out. When this density is lower than the decision-making index, the population is low, and control should not be carried out. The two main decision-making indices of integrated pest management programs are the economic injury level and the economic threshold (Arcanjo et al., 2021; Bacci et al., 2007; Higley & Pedigo, 1996).

Control methods can be divided into two groups: preventive and curative. Methods for preventive use are cultural control (or manipulation of the growing environment), plant resistance, and conservative biological control. The methods curative used are chemical control, augmentative biological control, behavioral control, and genetic control. Methods should be selected using technical, economic, ecotoxicological, and social criteria. According to the technical criteria, methods allowed by legislation and which are efficient must be used. By economic criterion, control methods must provide high crop yields and reduce costs per unit produced. According to the ecotoxicological criterion, the methods used must enable the preservation of the environment and people's health. According to social criteria, the methods used must be suitable for the planting system, farmer, and consumer (Pedigo; Rice & Krell, 2021; Araújo et al., 2017; Picanço et al., 2014).

3 I CURRENT SCENARIO OF THE AGRICULTURAL SECTOR AND PEST CONTROL IN BRAZIL

Currently, Brazil is the second-largest food exporter in the world. Agricultural and forestry products are the main items of our exports, and this sector is an excellent generator of jobs and income in the country. However, until the 1960s, Brazil was a food importer (EMBRAPA, 2018). Given this favorable current scenario, a question arises: what factors contributed to this positive change? Between these decades, changes in the public and private sectors led to these advances. Among these changes are expanding the Brazilian university system, graduate courses, state research companies, Embrapa, public and private extension services, private companies, agronomic prescriptions, no-tillage, expansion of the agricultural frontier, the program to collect empty pesticide containers, patenting and genetically modified varieties.

In pest, disease, and weed control, the global pesticide market moved US$84.5 billion in 2019 and is expected to reach US$130.7 billion in 2023. Brazil is the world's largest consumer of pesticides, and in 2021 this market moved 13.3 billion dollars. Among the three main groups of pesticides (fungicides, herbicides, and insecticides), the most commercialized (in weight or volume) are herbicides. However, due to their unit value in most years, insecticides are generally the ones with the highest market value. Sales of natural products represent about 6% of the pesticide market. In Brazil, in recent years, the market for natural products to control pests, diseases, and weeds has expanded by more than 70%. At the moment, more than 170 natural products are registered in Brazil to control pests, diseases, and weeds (MAPA, 2022; Researchandmarkets, 2022). In 2021, the global seed market was 62 billion dollars and was expected to reach 86.8 billion dollars in 2026, with a large part of these seeds being pest-resistant varieties (Marketsandmarkets, 2021).

4 I OBSTACLES TO THE IMPLEMENTATION OF INTEGRATED PEST MANAGEMENT PROGRAMS

The failure of integrated pest management programs is due to failures in research and the non-adoption of these programs by farmers. The research failures are due to the topics addressed not being relevant or due to the conduct of the studies having been carried out improperly (Peshin, 2013; Coutts & Christiansen, 2003). These researches should address diagnosis, decision-making, and control methods, as these are the components of integrated pest management programs. These studies must represent the crops where they will be used, and they must also consider the users and systems where they will be implemented. In selecting the components of these programs, technical, economic, ecotoxicological, and social criteria must be adopted (Picanço et al., 2022; Pedigo; Rice &

Krell, 2021; Picanço et al., 2014). In order to carry out these studies, it is important to have adequate infrastructure, a trained team, and resources. These surveys are usually carried out jointly by the public and private bodies. Another critical factor is the scientific training of the team responsible for these studies. This training is carried out by the Scientific Initiation programs and the Postgraduate Courses.

The adoption of integrated pest management programs by farmers depends on carrying out adequate technology transfer. The critical points for the success of the technology transfer process of the integrated pest management programs are efficient communication, simplicity of the process, economic part as a key point of the studies, actions to encourage technology transfer and extension, opinion-makers as critical customers, promoting quality over quantity and increasing interaction between producers and the community (Peshin, 2013; Coutts & Christiansen, 2003).

Another important aspect of technology adoption is that people are more likely to adopt innovations associated with products than processes. Thus, the procedures to be adopted must be related to products to enable greater adoption of technologies generated by farmers (Peshin, 2013). In this context, professionals in the resale of agricultural products in Brazil play a significant role in adopting technologies by farmers. This happens because these professionals are in daily contact with the farmers, and they have credibility with this public.

5 I INNOVATIONS IN INTEGRATED PEST MANAGEMENT PROGRAMS

Currently, the most demanding and enlightened consumers have significant influence over the technologies used in the production process. This influence can go beyond country borders. This happens when these consumers influence the selection of technologies to be adopted to produce products imported by their countries (Burnier; Spers & Barcellos, 2021; Basso et al., 2018; Picanço et al., 2016). In the agricultural sector, this influence has resulted in the production process requirements for certification. This certification establishes norms for using technical and ecotoxicological criteria to preserve the environment and human health and comply with local and international legislation. These production systems require the use of integrated pest management programs. Examples of this influence are the certification processes of planted forests, production of animal products, coffee, and fruit plantations in Brazil. In addition, the banning of organochlorine insecticides and organophosphate insecticides in various parts of the planet also influences these consumers (Burnier; Spers & Barcellos, 2021; Basso et al., 2018; Picanço et al., 2016; Edwards & Laurance, 2012).

In the coming years, there will be innovations in all components of integrated pest management programs. In these researches, new tools will be used that will help develop these studies. In the diagnosis, new problems with pests will be detected. This will occur due to climate change on the planet, changes in the production system, and biological invasions. With the increase in air temperature and the dry period of the year, the pests that live in the canopy of plants will increase their populations, especially the leaf-sucking and leaf-mining pests. With the increase in international trade and migration of people, new pests will be introduced in countries. Changes in production systems with the intensification of plantations in time and space, use of inputs, and the increase in crop yields will be favorable to having greater problems with pests (Chakravarthy, 2020; Chen et al., 2020; Gao & Reitz, 2017; Picanço et al., 2016).

Innovations in decision-making will occur both in the sampling plans and the adopted indices. In the samplings, sensors with artificial intelligence technology will evaluate the pest populations in the fields. Expert systems will make faster, more accurate, and more cost-effective control decisions using management zones. Innovations in control methods will include efficient products suitable for the user and the production system with less environmental impact. These products will have a higher unit cost, but they will have a lower cost per unit produced in the crops (Shah & Razaq, 2021; Chakravarthy, 2020; Chen et al., 2020; Picanço et al., 2016).

Another critical point in the innovations of integrated pest management programs will be the teaching and extension activities in this area of knowledge. It is vital to carry out remote and face-to-face teaching activities in high school, undergraduate, and graduate technical courses. In addition, it is also essential to carry out extension activities with rural workers, farmers, and technicians. We thank the National Council for Scientific and Technological Development (CNPq), the Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES) Finance Code 001, and the Minas Gerais State Foundation for Research Assistance (FAPEMIG) for the financial support of the activities that produced the knowledge contained in this book chapter.

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CAPÍTULO 4

LANDSCAPE STRUCTURE AND INSECT PEST MANAGEMENT

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1 | INTRODUCTION

Landscape, which refers to the spatial arrangement of natural, semi-natural, and anthropic elements, as well as their interactions, can provide a series of ecosystem services, such as soil nutrient cycling, plant pollination, water conservation, and natural control of pests (Metzger, 2001; Power, 2010). These services are essential for humans and the maintenance of biodiversity (Woltz; Isaacs & Landis, 2012; Duarte et al., 2018). However, the landscape configuration can influence the availability and interaction between these multiple services (Rieb & Bennett, 2020).

In the agricultural context, the arrangement of landscape elements highly influences the ecosystem services provided to the crops of interest, especially in the population dynamics of insect pests and their natural enemies (Jonsson et al., 2010; Zamberletti et al., 2021). With the expansion of agriculture, natural habitats have changed or even reduced (Figure 1). Therefore, the indiscriminate alteration of the environment can cause the loss of diversity of species that act in pest control (Bianchi; Booij & Tscharntke, 2006).

Figure 1 - Typical landscape of Central Brazil used for raising livestock with agricultural, natural, and semi-natural elements. Source: O.A. Fernandes

Natural habitats near crops are essential for providing shelter and food to maintain populations of predators and parasitoids (Cronin & Reeve, 2005; Bianchi; Booij & Tscharntke, 2006). Although beneficial to natural enemies, natural vegetation fragments can also benefit pest species in some contexts (Blitzer et al., 2012). As a result, several studies have evaluated how the proportions of different landscape elements can affect pest abundance and their biological control at various spatial and temporal scales (Veres et al., 2013; Haan; Zhang & Landis, 2020).

This chapter discusses the interactions between natural and agricultural habitats, the role and influence of landscapes on different types of insects, and how it is possible to manage the environment to keep the ecosystem services provided by the landscape.

2 I COMPOSITION AND CONFIGURATION OF NATURAL AND AGRICULTURAL LANDSCAPES

The concept of landscape has been discussed since the 19th century, when geography was established as a science (Schier, 2003; Silveira, 2009; Barbosa & Gonçalves, 2014). The discussion on this topic presented multiple divergent approaches influenced by social and philosophical trends that occurred at the time (Luchiari, 2001; Detoni, 2012). Landscape can be defined as a set formed by heterogeneous units that interact with each other or even a system of natural and semi-natural formations that produce services and natural resources (Metzger, 2001; Rodriguez; Silva & Cavalcanti, 2022).

The study of landscapes considers the relationships between the various components for their formation, as structural, functional, and dynamic-evolutionary processes. In general, the study of landscapes requires the evaluation of a set of physical or cultural elements, which are interconnected in space and time (Sauer, 1998). The most important features to consider in a landscape are its structure, composition, and function (Noss, 1983). Structure refers to the arrangement of units, the distribution of elements, and their relationships, while composition describes the variety of elements or species within a region. Finally, the function encompasses all processes, from cycling materials to defining the ecological role played by each species (Forman & Godron, 1986; Walz, 2011; Vidal & Mascarenhas, 2019).

As landscape comprises a heterogeneous set of interactive units, each unit will have a different structure and composition (Opdam et al., 1993). The landscape structure can be characterized by three types: horizontal, vertical, and functional. The horizontal structure is the organization and spatial distribution of individuals on the surface of a territory. The vertical structure is formed by the interrelation of the components considering their participation or function, such as dominant, intermediate, or dominated species. The functional structure is expressed by the sequence of permanent processes that are interrelated in the exchange of energy, matter, and information in which the landscape operates (Rodriguez; Silva & Cavalcanti, 2022). Therefore, the structure of a landscape is determined by its type of use, size, arrangement, shape, and the distribution of individual elements constituting it (Walz, 2011). Thus, a landscape structure can be defined by understanding its elements, parameters, and indices. These components change their composition due to natural biological processes, as well as modifications due to anthropic action. Such modifications alter the functioning of a landscape, affecting its structure and, consequently, the pattern distribution of species (Vidal & Silva, 2021).

It is also noteworthy that the changes in the natural environment resulting from human activities are not easily dissociated from studies of the ecology of natural landscapes, besides impacting ecological processes leading to the loss of biodiversity (Tannier et al., 2016). The natural landscape can be understood as a mosaic formed essentially by natural elements, and modified according to their processes, without human interference (Shafer; Hamilton & Schmidt, 1969). On the other hand, cultural, humanized, or semi-natural landscapes are those that relate biological and geographical elements to elements derived from human action (Tricart, 1982; Thomine et al., 2022).

To study the fragmented natural structures and their composition, the concepts of fragment, corridor, and matrix were created (Siqueira; Castro & Faria, 2013). Fragments can be defined as non-linear surfaces that differ in appearance from their surroundings (Forman; Godron, 1986 & Casimiro, 2009). The corridors, on the other hand, are the elements that promote the connection between other ecosystems in the landscape; however, they can also represent physical barriers that prevent the movement of species (Marsh, 2005). Finally, the matrix represents the type of ecosystem that occupies the largest area, thus being the most extensive and the most connected with the other elements (Casimiro, 2009).

One of the main changes in natural landscapes is due to agriculture (Tilman et al., 2011; Tanentzap et al., 2015). The traditional agroecosystem is characterized by growing a plant species on a large scale, with low genetic variability, and generally with a short life cycle (annual or semi perennial). Agricultural intensification and expansion reduce the number of natural habitats around crop fields and, consequently, the complexity of the ecosystem (Rieb & Bennett, 2020). Landscape simplification modifies the amount and diversity of land cover types (compositional heterogeneity), including natural and semi-natural habitats (Gagic; Paull & Schellhorn, 2018; Blassioli-Moraes et al., 2022).

Landscape homogeneity also alters the spatial arrangement of cultivated and uncultivated areas, resulting in low connectivity (Blitzer et al., 2012; Gámez-Virués et al., 2015). Habitat loss and fragmentation reduce the compositional and configurational heterogeneity of the landscape, leading to biotic homogenization, species loss, and reduction of ecosystem services (Tscharntke et al., 2012). These factors contribute to a concentration of resources, favoring herbivorous organisms.

The need for sustainable agricultural practices, especially the reduction of the use of pesticides, guided the search for agroecosystem management options that improve biodiversity and ecosystem services (Duru et al., 2015). Thus, the services provided by the landscape are a field of study that considers the spatial configuration and the influence of elements external to the ecosystem (Bastian et al., 2014). These biodiversity-based management options can be complex, as they require prior knowledge, the integration of agricultural practices, and ecological processes that can occur at different spatial and temporal scales (Turner, 2005; Médiène et al., 2011; Petit; Deytieux & Cordeau, 2021). Therefore, knowing the influence of these ecological processes on phytophagous or carnivorous arthropods is the first step towards the correct inclusion of landscape components in management programs, and maximum benefit from the services provided.

3 I INFLUENCE OF LANDSCAPE ON INSECT PESTS

Habitat structure has the potential to influence the number of a species in a location, as it can affect the reproduction, dispersal, and mortality of such a species, including its natural enemies (Veres et al., 2013). In agricultural fields, the presence of natural and semi-natural areas can affect the development and damage caused by insect pest species (Poveda et al., 2012; Rice et al., 2017). However, the influence of landscape structure on pests is often contradictory or taxon-specific (Jonsson et al., 2010; Karp et al., 2018). Thus, understanding the relationships between the source and sink of insects in fragments close to crops is of paramount importance to improve pest management, and predict the occurrence of insect infestations (Goethe et al., 2022).

The low diversity of agricultural habitats is usually considered to benefit the population increase of insect pests and a disadvantage to natural enemies. Trophic interaction (intraguild predation), agricultural practices, crop susceptibility, or abiotic conditions are some reasons for such a scenario (Tscharntke et al., 2016). However, increasing landscape diversity can be detrimental to pest populations (Karp et al., 2018; Paredes et al., 2021). The predation intensity of the soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), increases with a greater diversity of the landscape and/or soil cover (Gardiner et al., 2009; Noma et al., 2010; Mitchell et al., 2022). Similarly, populations of tobacco thrips, *Thrips tabaci* (Thysanoptera: Thripidae), are also influenced by habitat in leek crops, where lower populations of this insect are found when there is greater habitat diversity surrounding these crops (Den Belder et al., 2002).

Although there are positive examples of pest reduction with the presence of semi-natural habitats in the cultivation area, there are also cases in which these environments may be suitable for the development of insect pests and, consequently, the growth of their populations (Landis; Wratten & Gurr, 2000; Blitzer et al., 2012; Tscharntke et al., 2016). Five hypotheses can explain this situation: (1) there are more important resources in crops than in non-agricultural habitats for natural enemies; (2) there are no effective natural enemies in the region; (3) natural/semi-natural areas are more suitable for pests than natural enemies; (4) absence of configuration, composition, or proximity in uncultivated habitats to supply the demand of natural enemies in biological control, and (5) agriculture neutralizes the installation of natural enemies (Tscharntke et al., 2016). As an example, more abundant populations of *Oulema* spp. (Coleoptera: Chrysomelidae) and *Sitobion avenae* (Hemiptera: Aphididae) were found in landscapes with greater amounts of semi-natural habitat (Redlich; Martin; & Steffan-Dewenter, 2021). Non-agricultural habitats were positively correlated with aphid populations in wheat crops, as they assist in the life cycle, serving as a source of alternative host plants and shelter for aphid colonization (Yang et al., 2019).

However, it is important to note that different pest groups and species within the same cropping system show varying responses to landscape composition (Perez-Alvarez; Nault & Poveda, 2018). Landscape simplification in maize, for example, favors the Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Crambidae), a specialist pest, but negatively affects the small corn borer, *Conogethes punctiferalis* (Lepidoptera: Crambidae), a polyphagous pest in the crop (Dong et al., 2020). The abundance of some thrips species (Thysanoptera: Thripidae) was positively related to the proportion of forest in alfalfa crops, while other species of the same family were negatively affected (Madeira et al., 2022). It has been found that polyphagous leafhoppers (Hemiptera: Cicadellidae) can move more between patches because they are more likely to have long wings, while many specialist leafhoppers species have limited dispersal abilities due to their short wings. Furthermore, species richness of polyphagous leafhoppers is affected by connectivity, landscape composition, and fragment size, while specialist leafhoppers are not affected by this influence (Rösch et al., 2013).

Another issue to be considered is that the landscape structure can affect the movement and dispersion of pests (Goodwin & Fahrig, 2002). The presence of more diversified vegetation increases the complexity of the habitat and can negatively affect the location and use of the insect pest host plant (Mazzi & Dorn, 2012; Togni et al., 2021). Finding and choosing a host in environments with many plant species can be a difficult task, especially for polyphagous insects (Silva & Clarke, 2020). Semi-natural vegetation can limit the migration and diffusion of volatiles (pheromones and allelochemicals), which reduces, for example, the possibility of finding mates at low population densities, and recolonization of the cultivation area (Ricci et al., 2009; Blassioli-Moraes et al., 2022). In addition, the increase in time spent on dispersal results in costs, such as increased mortality from predation, and decreased resources for fecundity and survival (Mazzi & Dorn, 2012). Therefore, pest responses to landscape change must be linked to the ability of the species to disperse, and to changes in landscape structure (Goodwin & Fahrig, 2002).

Thus, it is possible to verify that natural habitats can simultaneously benefit or even disadvantage nearby crops depending on cropping systems and geographic regions (Thies; Roschewitz & Tscharntke, 2005; Tscharntke et al., 2016; Karp et al., 2018). However, it should be noted that there are a limited number of studies on the influence of landscape structure on pests. Furthermore, the existing studies on insect pests encompass mainly the orders Lepidoptera, Coleoptera, and Hemiptera (Worner & Gevrey, 2006; Letourneau et al., 2011).

Although there are divergences among existing studies, evidence suggests that landscapes with sufficient levels of non-agricultural vegetation are more diverse and have a greater provision of ecosystem services (Haan et al., 2021). However, the influence of semi-natural habitats cannot be generalized, as arthropods can be affected in different ways (Kheirodin; Carcamo & Costamagna, 2020).

4 I INFLUENCE OF LANDSCAPE ON NATURAL ENEMIES

Cultivated areas with little vegetation diversity and disturbed frequently are usually unsuitable for some beneficial insects (Landis; Wratten & Gurr, 2000; Haan; Zhang & Landis, 2020). Therefore, the presence of more stable non-agricultural areas (natural or semi-natural) near cultivated areas may favor the presence of these individuals (Bianchi; Booij & Tscharntke, 2006; Haan; Zhang & Landis, 2020). As for phytophagous insects, natural and semi-natural habitats can provide different resources, such as alternative foods (nectar, pollen, prey, and hosts), shelter, or a microclimate in which natural enemies can hibernate or seek refuge (Landis; Wratten & Gurr, 2000; Bianchi; Booij & Tscharntke, 2006; Haan; Zhang & Landis, 2020). In addition, organisms that require resources from various types of land cover can benefit from landscape complementation and move between different types of habitats (Veres et al., 2013; Haan; Zhang & Landis, 2020). Therefore, the interaction between natural and semi-natural habitats with agricultural fields can benefit the presence of natural enemies and lead to more effective pest control (Tscharntke et al., 2007; Woltz; Isaacs & Landis, 2012; Rusch et al., 2016).

Several studies demonstrate the benefits of the presence of uncultivated areas on natural enemies. The increase in landscape complexity can augment the response of natural enemies (abundance, richness, diversity, and direct effects on pest reduction) by about 25% (Duarte et al., 2018). Egg predation rates of the cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae), are reported to be positively correlated with structurally complex landscapes, and negatively correlated with a horticultural area (Bianchi et al., 2005). The abundance of nymphs of *Lygus lineolaris* (Hemiptera: Miridae), an important strawberry pest in the USA, was negatively associated with increasing proportions of semi-natural habitats in the landscape, resulting from increased parasitism rates (Grab et al., 2018).

In tropical and subtropical regions, forest fragments can also be important sources of natural enemies. The forest fragments of the Atlantic Forest (Brazil) are important habitats for predatory and omnivorous ant species that are involved in the regulation of herbivores in sugarcane cultivation (Santos; Bischoff & Fernandes, 2018). In the Chaco Serrano region (Argentina), it was found that as the forest cover in the landscape decreases, fewer species of natural enemies are found moving between native forest fragments and soybean plantations. In addition, natural enemies (especially the orders Coleoptera and Hymenoptera) move more frequently from the forest to the crop field (González et al., 2016). More species and specimens of natural enemies were also found, as well as a higher rate of biological control of green belly stink bug eggs, *Diceraeuss furcatus* (Hemiptera: Pentatomidae), in places with high forest cover. In these locations, biological control by predators and parasitoids was 20% higher (González; Salvo & Valladares, 2017).

Although there is evidence that the responses of natural enemies (abundance, diversity, predation, parasitism) to landscape complexity are positive, these effects may vary between different insect groups and species (Thies; Steffan-Dewenter & Tscharntke, 2003; Woltz; Isaacs & Landis, 2012; Medeiros et al., 2019). In soybean crops in Canada, aphid regulation has increased as the landscape has been simplified (in other words, landscape complexity has decreased). In addition, there was a trend of reduction in the regulation of aphids with the abundance and diversity of their predators (Mitchell; Bennett & Gonzalez, 2014). Therefore, it is observed that even if the landscape complexity in agroecosystems is more important for natural enemies than for pests, the positive response of natural enemies does not necessarily translate into pest control (Chaplin-Kramer et al., 2011). Landscapes heavily disturbed by agricultural practices and insecticide applications can become unsuitable for their specialized organisms, and do not allow the establishment of pest enemy populations. In addition, intraguild predation and greater predation of alternative prey that may not be considered pests may occur (Veres et al., 2013).

In some cases crop damage does not decrease with an increasing diversity of natural enemies, and even with increased pest control, actual crop damage is not necessarily smaller (Bianchi; Booij & Tscharntke, 2006; Chaplin-Kramer et al., 2011). Instead, the diversity of enemies increases due to the abundance of pests in the environment (Martin et al., 2016; Madeira et al., 2022). Thus, to demonstrate benefits to farmers, it is necessary to consider pest control, as well as crop yield, and reduction of insecticide spraying (Macfadyen et al., 2015; Gagic; Paull & Schellhorn, 2018).

It is important to consider that different species of natural enemies generally respond differently to landscape variables (Pfister et al., 2017; Karp et al., 2018; Jowett et al., 2019). The response of generalist natural enemies occurs on larger spatial scales than for specialist individuals (Chaplin-Kramer et al., 2011). Furthermore, for natural enemies that depend only on resources within agricultural fields, proximity to semi-natural habitats may be irrelevant or even harmful (Haan; Zhang & Landis, 2020).

It is also worth noting that natural enemies must present a rapid numerical response to herbivore density to contribute to pest control. For this, they must be present in the area, act easily nearby, or colonize at a greater distance (Tscharntke et al., 2007). Initially, the influence of landscape structure on pest suppression by natural enemies focused on the effects of landscape composition (number of different habitats). However, more recent studies demonstrate that landscape configuration (habitat size and shape, amount of shared edge, and connectivity) is an important factor for pest suppression (Haan; Zhang & Landis, 2020).

In general, pest suppression is expected to be greater in fine-grained agricultural landscapes (small patches and higher edge density) because, in smaller fields, enemies that emerge from the edges of fields or nearby semi-natural habitats can reach the edges and the interiors of crop fields more easily (Haan; Zhang & Landis, 2020). Martin et al. (2016) demonstrated that landscape configuration (edge density) positively influences the abundance and species richness of natural enemies such as parasitoids (Hymenoptera), hoverflies (Diptera: Syrphidae), predatory wasps (Hymenoptera: Vespidae), and rove beetles (Coleoptera: Staphylinidae). However, different landscape parameters also influence different groups of natural enemies in different ways. Fragmentation in rice fields negatively influences the richness and abundance of parasitoids but favors the abundance of the ladybird *Micraspis* spp. (Coleoptera: Coccinellidae) (Dominik et al., 2018). In coffee-growing areas, species richness, and abundance of wasps (Hymenoptera: Vespidae) increased with the expansion of the forest cover in multiple spatial extents, while richness and abundance of hoverflies (Diptera: Syrphidae) were not affected (Medeiros et al., 2019). However, even with these variations between the different groups, of the parameters tested, the landscape configuration had the most stable and consistently positive effects on natural enemies and reaffirms the importance of fine-grained landscapes that facilitate the movement of insects between habitats (Martin et al., 2016).

When considering the agricultural context, landscape management seeks to ensure sustainable biological control that can reorganize itself after disturbances (Tscharntke et al., 2007). Keeping non-agricultural vegetation in cultivated areas contributes to promoting biodiversity and favoring pest suppressive landscapes that can reduce the need for insecticides (Veres et al., 2013). It can be noted that the complexity of the landscape affects natural enemies positively as non-agricultural habitats act as reservoirs for biodiversity in agricultural landscapes (Bianchi; Booij & Tscharntke, 2006; Chaplin-Kramer et al., 2011; Rusch et al., 2016; Garratt et al., 2017). However, the size of the natural fragment, its shape, composition, amount of shared edge, the distance between habitats, characteristics of the area, and the organism in question are characteristics that influence the results of this interaction (Holland et al., 2016; Tscharntke et al., 2016; Haan; Zhang & Landis, 2020). Thus, landscape management strategies to improve natural pest control should differ depending on their specificities (Chaplin-Kramer et al., 2011).

5 I CONCLUSIONS AND FUTURE PERSPECTIVES

Knowledge about the influence of the landscape on insect pests and natural enemies has advanced, but mainly in temperate regions. It was found that this response is variable so the landscape may or may not favor pest populations. In a recent review, Pinto et al. (2022) compiled studies involving mortality factors in both temperate and tropical regions. This assessment indicated that abiotic factors are important in causing mortality of herbivorous insects, but that predators exert greater mortality in tropical than temperate environments. As predators are usually generalists, there is a need to improve the understanding of landscape influence in tropical regions, as predators can benefit from the landscape structure. Although most research on the ecology of landscapes and agroecosystems has been carried out in temperate areas (Veres et al., 2013), information regarding tropical regions is also emerging (Gagic; Paull & Schellhorn, 2018; Togni et al., 2021).

Despite the divergence of results, the preservation and restoration of semi-natural habitats in agricultural areas increase the abundance and richness of natural enemies, in addition to improving pest control services. Thus, landscape management can be important to maintain or even expand ecosystem services. The simple preservation of biodiversity could already be considered an important condition, even if biological control does not drastically reduce the populations of pest arthropods. However, this requires the cooperation of many stakeholders, who often have different interests.

Decision-making in Integrated Pest Management programs is mainly based on the monitoring and adoption of pest control products, or genetically modified plants that express insecticidal proteins as control tools. Thus, only pest dynamics are considered, while natural enemies are neglected. Therefore, a better understanding of the contribution of ecosystem services, including natural biological control, could be crucial to improving such pest management programs. In Brazilian conditions, these studies are already being carried out (Togni et al., 2021), and there is an increase in the number of researchers interested in this topic. Such concern is an important step forward for the sustainable production of food, fiber, and bioenergy, besides contributing to achieving the goals established within the United Nations (UN) Sustainable Development Goals.

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CAPÍTULO 5

TECHNOLOGICAL INNOVATIONS APPLIED TO INSECT PEST MANAGEMENT

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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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CAPÍTULO 6

GOOD PRACTICES IN AGRICULTURAL SPRAYING FOR PEST MANAGEMENT

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1 I INTRODUCTION

Phytosanitary products must be applied when it comes to large-scale production. Phytosanitary applications should aim the effective control of arthropods and pathogens affecting crops and the reduction of production losses. In this sense, quality spray applications are essential to allow a successful operation.

The application technology of pesticides consists of using techniques and scientific knowledge for the correct placement of active ingredients at a target in the proper amount, economically, and with minimal environmental contamination (Matuo, 1990). As it is an interdisciplinary field, it encompasses several factors that, if disregarded, an efficient and economic deposition of a product is impaired (Contiero; Biffe & Catapan, 2018).

Most pesticides are applied via liquid application, by spraying the solution through droplets that produce greater coverage, and therefore, a greater chance of reaching the target. The efficiency of proper product deposits can decrease resistance development in pest populations, as a uniform product distribution makes pests get in contact with lethal doses of the product, decreasing the selection of resistant organisms (Volpe et al., 2012).

Thus, the objective of this chapter is to present important aspects related to the application technology of phytosanitary products aimed at pest control.

2 I THE IMPORTANCE OF SPRAY NOZZLES

Spray nozzles are the main components in sprayers, as they produce and distribute droplets that carry the active ingredient to the target. They, therefore, have a great influence on phytosanitary management quality and efficiency, such as pest arthropod control. These components are also responsible for breaking up and distributing insecticide spray solutions, determining the flow rate, size, and uniformity of droplets (Fernandes et al., 2007; Camara et al., 2008).

Poor choice of spray nozzles will hardly be fixed by changing working pressure, application flow, spray bar height, nozzle spacing, application speed, or even using adjuvants in the solution.

Such a mistake may cause economic losses to farmers, low insect control efficiency, contamination of non-target organisms, and damage to the environment. Therefore, application success starts by selecting a proper spray nozzle to be used for a given situation.

Determining factors for a proper spray nozzle selection are desired droplet size, application speed and rate, weather conditions during application, working pressure, and formulation of the phytosanitary products composing spray solution (Miller; Butler Ellis, 2000). In addition to these, biological factors related to the crop are also important, such as the phenological stage and target to be reached, which considers the preferred location of the target insect.

Droplets are formed by pressure-aided passing of spray liquid through a small outlet orifice in spray nozzles, with sufficient speed and energy to spread the liquid. In this process, a thin liquid sheet is formed, which disintegrates into different droplet sizes. Thus, the droplet spectrum constitutes the amplitude among the droplet sizes produced by nozzles during spraying (Matthews; Bateman & Miller, 2014).

Droplet spectrum is determined by a set of factors such as uniformity coefficient (Relative Span), which indicates uniformity in droplet size; volumetric median diameter (VMD), which is the droplet diameter that 50% of the sprayed liquid volume consists of smaller droplets than it; and droplet volume percentage of droplets smaller than 100 μm, which stands for drift-susceptible droplets. A droplet diameter is considered appropriate when it provides maximum pest control, with a minimum amount of pesticide and ecosystem contamination (Himel, 1969).

Overall, droplets larger than 400 μm have a low capacity to penetrate the plant canopy and increase product runoff from plants. Although they may provide reduced surface coverage, these droplets are less likely to be lost by drift and evaporation. Conversely, droplets smaller than 200 μm provide increased surface coverage, but also have higher drift and evaporation risks (Figueiredo et al., 2007).

For insect control by contact insecticides, droplet sizes between Fine (136 to 177 μm) and Medium (177 to 218 μm) are recommended (Asabe, 2013). As contact products require a large surface coverage, Fine droplets increase the chances of active ingredients reaching lower plant strata (Cunha; Marques & Alvezs, 2016). On the other hand, systemic insecticides demand Medium and Coarse droplets. The systemic action of products requires less surface coverage, thus reducing the risk of drift and evaporation.

There are several spray nozzle models available on the market, which have been developed specifically to meet application needs. The nozzles mostly used by farmers are those that use hydraulic energy to form droplets. The main hydraulic nozzle models are those of flat and conical spray jets.

Flat fan spray nozzles, produce single plane spray jets and are recommended for a variety of applications, especially for pre-emergent herbicide and systemic post-emergence herbicide applications. There are models recommended for soil insecticide application, which vary with the target to be reached, applying droplets of larger size classes, such as Extremely Coarse and Ultra-coarse (Román, et al., 2009).

3 I DRIFT CONTROL

An effective insect control application must consider the maximum reduction of spray losses as possible. Drift reduction, besides improving spraying effectiveness, reduces the risk of environmental contamination and intoxication of sensitive crops, humans, and animals. Incidents of pollinator mortality, for example, have been reported due to pesticides drift, directly affecting pollination-dependent crops, with yield losses of up to 24% (Garibaldi et al., 2016).

Spray drift can be defined as the volume of spray solution droplets diverted from the target area by wind action (Matthews; Miller & Bateman, 2014). The spray solution amount drifting off the target can also reach the soil, which is a result of high application volumes or large droplets applied in the same direction (Al Heidary et al., 2014; Zhang; Luo & Goh, 2018).

Drift control strategies include correct sprayer setup, use of proper spray nozzle and working pressure to produce ideal droplet sizes, proper boom or flight altitude (in case of aerial spraying), proper spray jet direction, selection of optimal sprayed band, application speed control, use of adjuvants and other technologies such as electrostatic sprayers, pulse width modulation (PWM), and air-assisted equipment, in addition to spraying under suitable weather conditions.

Overall, the smaller the droplet size, the greater the drift potential by evaporation and wind drag (Al Heidary et al., 2014; Miranda et al., 2010). This way, to reduce losses by drift and evaporation of products at the time of application, larger droplets and evaporation reducers are used. However, in such cases, droplet potential for coverage and penetration into the crop canopy can be reduced (Garcerá et al., 2017).

Boom height and nozzle spacing are also part of the sprayer configuration and may directly impact spray liquid distribution uniformity and hence its drift (Benez et al., 2016). The higher the boom height, the greater the drift potential. Higher boom heights tend to increase droplet exposure to wind, thus increasing the risk of evaporation and off-target drag (Al Heidary et al., 2014). In aerial sprays, the distance between nozzles and targets can reach several meters away; therefore, flight height should be properly selected. By decreasing flight height from 6.7 to 2.4 m, for example, reductions of up to 2.5 times in drift can be observed (Matthews; Miller & Bateman, 2014).

Directed spray applications can also improve drift control. Band applications with the use of inter-row directed spraying nozzles, for example, have been effective to control pests such as the root spittlebug, *Mahanarva fimbriolata* (Hemiptera: Cercopidae) in sugarcane (Peixoto et al, 2009). Directed applications sprays have been effective in fruit trees and generated great savings in application volume, without adversely affecting leaf coverage and significantly reducing drift losses (Carvalho, 2014; Andrade; Ferreira & Martinelli, 2014).

Effective spray swath must also be determined regardless of spraying mode, as it directly affects distribution uniformity over the target. In the case of aerial sprays, air turbulence generated by aircraft movement, especially at the wingtip, directly impacts distribution profile, effective deposition range, and drift potential. Effective spray swath smaller than 75% of the aircraft wingspan are recommended to reduce the vortex effect on droplets, thus reducing the risk of drifts (Teske et al., 1998, Matthews; Miller; & Bateman, 2014). Another alternative for decision making for drift reduction includes decreasing working speed. Reductions from 12 to 6 km h^{-1}, for example, reduced drift by up to 77% (Van De Zande et al., 2004).

The addition of adjuvants to the spray solution is another alternative to minimize drift potential. The use of lecithin in applications with very fine droplets, for example, significantly reduces the potential for drift (Griesang et al., 2017). Such changes in droplet size, which allow reducing the volume with smaller droplets, more prone to being dragged by the wind, can also be related to adjuvant inclusion into agrochemical formulations (Costa et al., 2017).

Regarding the adoption of different technologies with drift reduction potential, one can mention electrostatic systems. The transfer of electrical charges to droplets formed in spraying induces an electric field that generates a force of attraction between droplet and plant (Chaim & Wadt, 2015). This attractive force increases droplet speed and deposition. Another technology, known as PWM (Pulse Width Modulation), makes it possible to increase or decrease nozzle flow without changing working pressure. It, therefore, causes no droplet size changes, thereby better controlling the risk of drift. In addition, air-assisted spray boom is another technology that allows drift reduction by increasing droplet transport speed using a wind sheet.

Among the weather conditions that can affect drift during agricultural spraying, wind speed is the most relevant (Nuyttens et al., 2005). According to FAO (1998), wind speed limits for pesticides applications are up to 10 km h^{-1}. However, in most farming areas in Brazil, wind may exceed this limit, thus, other methods need to be adopted to enable applications outside the recommended conditions. Accordingly, using larger droplets as well as all other techniques discussed here (spray nozzle, boom/flight height, directed spray application, application swath, application speed, adjuvants and others) should be considered to reduce effects from wind or other meteorological conditions (relative humidity and temperature), aiming to reduce drift and effectiveness of phytosanitary control.

4 I SPRAY SOLUTION PREPARATION

One of the several factors influencing the quality of agricultural sprays is incorrect spray solution preparations, which includes water quality for product dilution. Water physical quality in terms of the number of suspended sediments has been much discussed nowadays. Such a factor can cause several damages to sprays such as clogging of filters and nozzles, reducing the longevity of sprayers and pumps, hence impairing applications (Ramos; Araújo, 2006; Contiero; Biffe & Catapan, 2018). Studies on the physical-chemical properties of water have shown that some factors such as hardness, pH, and suspended sediments, interfere with the integrity of phytosanitary products, negatively affecting the effects of active ingredients and expected control (Queiroz; Martins & Cunha, 2008).

Apart from the physical factor, chemical water quality has been another factor interfering with the action of crop protection products. Water hardness, which refers to concentrations of alkaline-earth cations (Ca^{+2}, Mg^{+2}, Sr^{+2}, and Ba^{+2}), can negatively affect the efficiency of spray solutions by generating product incompatibility. This process includes the occurrence of precipitates and flocculation, which can clog filters and spray nozzles. An example of this is the presence of Fe^{+3} and Al^{+3}, which can react with phytosanitary products and reduce their effectiveness (Ramos & Araujo, 2006; Petter et al., 2013).

Another factor that may be associated with the incompatibility of spray mixtures is the pH, which indicates potential changes in the chemical characteristics of mixtures (Ramos & Araújo, 2006). The interference of pH with spray efficiency varies with an active ingredient that requires a different pH range. Higher pH values in spray solutions can accelerate degradation by alkaline hydrolysis. In this case, the pH of agrochemical mixtures in the tank must be below 7.0, which delays hydrolysis, maintaining the composition of molecules for a longer time. Thus, attention must be paid to the appropriate pH range of each product (Cunha; Alves & Marques, 2017).

Another factor to consider is electrical conductivity in spray solutions. This water property refers to ions and their concentrations and valences, which vary according to the products used and/or their mixtures (Cunha; Alves & Marques, 2017). Furthermore, this parameter can also alter the biological action of agrochemicals (Carlson & Burnside, 1984).

A practice that has been commonly used not only in Brazil but also in other countries, is spraying solution preparation with more than one product, to widen the spraying action spectrum, reducing the number of applications and hence costs (Guimarães, 2014; Oliveira, 2014 & Krause, 2014). To control pests, diseases, and weeds all at once, and even to nourish plants together, farmers mix several products. This practice, however, can generate problems for applications. The preparation using more than one product results in interactions between them, promoting physical/chemical or biological changes in spray solutions, which may not promote the expected effects (Damalas, 2004).

Gazziero (2015) surveyed farmers and asked about issues arising from tank mixtures; they listed excess foam, poor homogenization, decantation, hydraulic clogging, and increased crop phytotoxicity, among other problems. Moreover, these tank mixtures can generate challenges due to product loss and consequent environmental risks (Vale et al. 2019).

In addition to mixing various chemical and biological products, another key factor for preparing solutions is the sequence in which products are added to the sprayer tank. Proper order of addition can avoid problems related to phytotoxicity and control ineffectiveness (Cessa et al., 2013). When mixing different formulations, the most suitable addition sequence should be followed, thus avoiding potential problems. As an example, water-dispersible granule formulations (WG) are added first because their specific surface is smaller than those based on powder (WP). Thus, the order of mixtures is based on each formulation's affinity with water. In short, first insoluble products are added, then suspensions and emulsions, and finally, soluble formulations (Costa & Polanczyk 2019).

Still, several are the exceptions to that rule as there is a wide range of formulations available, which are individually designed, as well as variations in their uses in terms of the amount of water for dilution. Therefore, the most recommended is to prepare mixtures thoroughly and on a small scale for each farm, ensuring solution stability. Another crucial recommendation is to apply the prepared spray solution over a pilot area to verify its control effectiveness.

Among the precautions for the preparation of spray solutions, stirring is a key factor for homogenization and must be carried out continuously from the addition of the first product until the end of spraying in the field (Costa & Polanczyk, 2019). It is also recommended pre-dilution in a smaller container, to be later poured into the spray tank. This pre-mix container must allow a proportion of 1 kg or L of product to at least 3 liters of water (Miguela & Cunha, 2010).

A good alternative that has been used to mitigate incompatibilities in spray solutions is the use of agricultural adjuvants. These can modify the physicochemical properties of spray solutions. Thus, such additives make the products of a mixture compatible, hence improving solution characteristics such as viscosity, surface tension, contact angle, pH, electrical conductivity, and droplet retention and deposition (Prado et al., 2016; Cunha et al., 2017).

5 I USE OF ADJUVANTS

Adjuvants are substances added to phytosanitary formulations or spray solutions. They can increase or change the efficiency of products against their targets and physicochemical properties of spray solutions, minimizing potential problems in applications. Among their functions are product compatibility in-tank mixing, drift reduction, increased target coverage and wetting, better product efficiency, greater droplet spreading on leaves, and faster absorption of the active ingredient, all of which often allow operational performance gains.

To properly select adjuvants as a function of application needs, their specificities should be well known, which are: foam reducers, volatilization, evaporation and drift, dispersants, emulsifiers, spreaders, wetting agents, adhesives, acidifiers, and buffers (Hunsche et al., 2006; Kraemer et al, 2009, Griesang et al., 2017). In other words, adjuvants must be selected according to their functions. Notably, a single product will hardly be able to perform well all necessary corrections for an efficient and safe application (Tu & Randall, 2003). Moreover, the effectiveness of active ingredients cannot be impaired either by mixing active ingredients in a tank or by adding adjuvants to the solution.

Adjuvants can change physicochemical properties inherent to spray solutions, depending on their chemical composition and formulation (Iost & Raetano, 2010). Thus, some authors have reported adjuvants acting to break water surface tension (Iost & Raetano, 2010; Barrêto, 2011), change volumetric median diameter (Mota, 2011; Checheto & Antuniassi 2012), modify pH and electrical conductivity in *Bacillus thuringiensis*-based solutions (Santos et al., 2019), and reduce the percentage of drift-susceptible droplets (<100 μm) (Matthews, 2000), thus contributing to the correct placement of the product on the target (Ferreira et al., 2003).

One of the primary and relevant functions of adjuvants is to reduce spray solution surface tension, and hence the contact angle between droplets and the applied surface. Accordingly, the spread and coverage of droplets over the desired target tend to increase. Furthermore, solution pH and electrical conductivity can alter the degradation and leaf absorption of phytosanitary products. However, crop leaves may have different affinities with adjuvants, making its recommendation difficult (Cunha, 2017).

Adjuvants are classified into two groups according to their role in phytosanitary products and spray solutions. The first group comprises the activators, which increase the quality of active ingredients and can be further classified as surfactants (reduce the surface tension of solutions to spread droplets on leaf surfaces); oils (increase the active ingredient penetration into leaves by diluting leaf waxy layer, besides reducing evaporation); and silicone derivatives (work as adhesive agents, reducing evaporation and run-off losses, as well as foam formation) (Kissmann, 1998). The second group includes the modifiers (also known as special-purpose adjuvants), which alter the physicochemical properties of solutions/formulations and can be further classified as humectants (reduce the surface tension of solutions and increase the contact of active ingredients with water); compatibilizers (avoid the chemical interaction between products, thus reducing incompatibilities); solution conditioners (used in water with lots of salts [cations]); pH buffering (balance pH and improve compatibility between products); defoaming agents (reduce the foaming during preparation, improving dilution and reducing spillage); drift control agents (increase the droplet sizes and decrease the number of droplets susceptible to drift); and thickening agents (increase the solution viscosity, most used in aerial applications) (Kissmann, 1998). Thus, in short, it can be concluded that a proper adjuvant selection is vital to ensure a good performance of the phytosanitary products and spray safety.

The quality of application of phytosanitary products must undoubtedly be maintained to obtain the expected economic results, with lower environmental risks. The information and technologies discussed in this chapter, if well implemented, are capable of contributing to this objective.

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CAPÍTULO 7

RESISTANCE OF CITRUS PEST MITES TO ACARICIDES

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1 | INTRODUCTION

Citrus growers primarily use chemical control to keep pest populations below the economic threshold. However, the inappropriate use of pesticides results in undesirable side effects, such as population outbreaks of secondary pests and the evolution of resistance in populations. The evolution of pest resistance to pesticides has been considered one of the highest threats to the implementation of integrated pest management (IPM) programs. The citrus crop is home to numerous arthropods, including several species of pest mites. Citrus mites are basically controlled worldwide with chemical acaricide applications (Van Leeuwen et al., 2010). However, the continuous use of the same active ingredient can increase the frequency of resistant individuals and compromise the efficiency of products (Omoto & Alves, 2004). Failures in the control of mites after acaricide applications are frequent in the Brazilian citriculture and have been reported for several decades. In this scenario, this chapter addresses the main cases of pest mite resistance in citrus to pesticides published in scientific journals.

2 | THE CITRUS LEPROSIS MITE (*Brevipalpus* spp.)

Transmission of the Citrus leprosis virus (CiLV-C), which causes citrus leprosis, one of the most destructive diseases in citrus, was attributed exclusively to the mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae) until 2015 (Beard et al., 2015; Tassi et al., 2017). An extensive taxonomic review allowed concluding that *B. phoenicis* was a species complex. New species have been described and other species have been recovered since then (Beard et al., 2015). Population surveys of *Brevipalpus* mites showed that *Brevipalpus yothersi* Baker (Acari: Tenuipalpidae) is the predominant species in commercial citrus crops in the State of São Paulo, Brazil, and not the species *B. phoenicis* (Mineiro et al., 2015).

Acaricide applications in orchards of the State of São Paulo and the Triângulo Mineiro region in the State of Minas Gerais, considered the Brazilian citrus belt, are the main method used to control the mite vector of leprosis virus (Miranda et al., 2017). However, the increase in the frequency of resistant individuals in mite populations has been common due to high selection pressure, resulting in decreased control efficiency. Reductions in the efficiency of acaricides lead to an increase in the number of applications, greater environmental contamination, and a reduction in natural enemies and beneficial insects (Omoto & Alves, 2004).

However, in addition to the high selection pressure, certain biological and ecological factors of *Brevipalpus* spp. have contributed to accelerating the evolution of resistance. For instance, the predominant form of reproduction is thelytokous parthenogenesis, in which unfertilized eggs give rise to females. Therefore, the offspring had the same genetic makeup as the parents (Helle et al., 1980). Another important factor is the low dispersion capacity of this mite compared to other mites, which makes it difficult to reduce the frequency of resistant individuals through mixing between populations (Alves et al., 2005).

The resistance of mites *Brevipalpus* spp. has been reported for some active ingredients, such as dicofol, hexythiazox, propargite, lime-sulfur solution, and spirodiclofen (Omoto et al., 2000; Campos & Omoto, 2002; Franco, 2002; Casarin, 2010; Rocha et al., 2021).

Extensive monitoring of populations of *B. phoenicis* from commercial orchards in the State of São Paulo was carried out for the acaricide dicofol. This study revealed variability in susceptibility to this acaricide at diagnostic concentrations of 100 and 320 mg dicofol/L water (Omoto et al., 2000). A 57-fold resistance ratio has been estimated in the dicofol-resistant strain (Omoto et al., 2000). In addition, positive cross-resistance between dicofol and bromopropylate, negative cross-resistance between dicofol and fenpyroximate, and no cross-resistance with fenbutatin oxide and propargite have been observed (Alves et al., 1999).

The frequency of resistance of *B. phoenicis* to the acaricides propargite and hexythiazox was also variable among populations. Survival percentages ranged from 0.0 to 88.3% for propargite and 30 to 94% for hexythiazox (Franco, 2002; Campos & Omoto, 2002). The high estimated resistance ratio for the hexythiazox-resistant strain was higher than 10,000 times (Campos & Omoto, 2002). On the other hand, a resistance ratio of 5.69 times was found for the lime-sulfur solution, and cross-resistance between lime-sulfur solution and sulfur was confirmed (Casarin, 2010).

More recently, the resistance of *B. yothersi* to the acaricide spirodiclofen was detected (Rocha et al., 2021). This study showed variability in the population responses to the diagnostic concentration of the acaricide, and a resistance ratio of 10.6 times was estimated for the resistant strain (Rocha et al., 2021).

3 I THE CITRUS RUST MITE *Phyllocoptruta oleivora*

Small in size, but capable of damaging citrus leaves and fruits, the citrus rust mite *Phyllocoptruta oleivora* (Acari: Eriophyidae) is considered a key pest in Brazilian citriculture. The presence of the citrus rust mite can be observed throughout the year, but the largest populations are observed in hot periods with high relative humidity.

Citrus rust mite control is carried out in Brazil with intensive applications of sulfur and abamectin, which contributed to the selection of resistant individuals (Omoto & Alves, 2004). Populations of *P. oleivora* resistant to the acaricides dicofol and abamectin have been detected in the United States (Florida) (Bergh et al., 1999, Omoto & Alves, 2004). However, research on the resistance of this mite to acaricides has not yet been carried out in Brazil.

4 I THE CITRUS RED MITE *Panonychus citri*

The mite *Panonychus citri* (Acari: Tetranychidae), popularly known as the citrus red mite, is considered in several parts of the world as one of the main species of pest mites in citrus. High levels of infestation can be observed in a short period due to its rapid development and reduced time between generations. In Brazil, the red mite occurs mainly in the dry periods of the year, especially in autumn-winter.

Panonychus citri causes damage to leaves, branches, and fruits although its infestations occur preferentially on leaves, which can lead to high leaf drop. The red mite is one of the most notorious pest mites for its ability to rapidly increase the frequency of acaricide-resistant individuals. It is due to their high reproductive aptitude associated with the selection pressure of numerous acaricide applications (Niu et al., 2011; Pan et al., 2020).

Studies on the resistance of *P. citri* to acaricides have been carried out in several countries, including China, Japan, New Zealand, Taiwan, Turkey, and the United States of America (Gotoh et al., 2004; Hu et al., 2010; Doker & Kazak 2012; Ouyang et al., 2012; Mota-Sanchez & Wise, 2019). Cases of *P. citri* resistance to organophosphates, pyrethroids, organochlorines, keto-enols, and bifenazate have been reported (Chen et al., 2009; Hu et al., 2010; Niu et al., 2011; Van Leeuwen et al., 2011).

There are no reports of resistance related to *P. citri* populations in Brazil. However, population levels of the red mite have increased considerably in recent years due to the intensification in the use of insecticides to control *Diaphorina citri* (Hemiptera: Liviidae) in citrus orchards in Brazil, requiring frequent spraying of acaricides for its control (Yamamoto & Zanardi, 2013; Ribeiro et al., 2014).

5 I RESISTANCE MANAGEMENT

Resistance is an evolutionary phenomenon that depends on complex interactions of three major groups of factors: genetic factors, biological factors, and operational factors. Operational factors are largely under human control, which provides a basis for resistance management (Georghiou & Taylor, 1986).

Among the operational factors, moderation and multiple attack are the most used strategies for resistance management in citrus from a practical point of view (Omoto et al., 2008). Management strategies by moderation seek to reduce selection pressure. It can be achieved by reducing the use of chemical acaricides. In this sense, adequate pest monitoring in the field is essential to guide applications (Omoto et al., 2008). The use of other control methods such as natural and biological products can also contribute to this regard. In the multiple attack management strategy, acaricides are used in rotation or as a mixture (Nauen et al., 2001; Omoto et al., 2008). However, attention should be paid to the use of different modes of action and the absence of cross-resistance between products.

Furthermore, the adaptive disadvantage of resistant individuals compared to susceptible individuals in the absence of selection pressure may contribute to resistance management programs. This fitness cost may delay the increase in the number of resistant individuals in the population and contribute to the return of the susceptibility condition (Roush & McKenzie, 1987; Alves, 2004; Kliot & Ghanim, 2012).

The citrus leprosis mite showed an fitness cost for the acaricide dicofol, with lower fecundity and lower longevity of individuals of the resistant strain (Alves, 1999). A reduction in adult longevity, days of oviposition, and fecundity in the resistant strain was observed for spirodiclofen (Rocha et al., 2021). On the other hand, resistance to hexythiazox was stable under laboratory conditions, but unstable under field conditions (Campos & Omoto, 2006). No fitness cost associated with resistance was observed for propargite and lime-sulfur solution (Franco et al., 2007; Casarin, 2010).

Research on monitoring the resistance of these mites to acaricides, as well as cross-resistance relationships and resistance stability are critical to the success of resistance management strategies. Currently, few products accepted in orange juice importing countries from Brazil are available in the market to control these mites (Fundecitrus, 2022). Therefore, mite control strategies must be implemented aiming at the preservation of existing acaricide products (Omoto et al., 2008). Thus, the adoption of resistance management strategies that aim to delay its evolution is fundamental to guarantee the efficiency and preserve the useful life of acaricides, as the research for the development of new products is long and expensive.

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CAPÍTULO 8

CHALLENGES IN INSECT PEST MANAGEMENT IN SUGARCANE CROP

Aimée Regali Seleghim

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Dagmara Gomes Ramalho

1 I INTRODUCTION

The sugar and ethanol world market moved US$3.61 millions in 2019, with an estimated increase of 85% (US$6.70 millions) by 2027, being in this market Brazil stands out for being the world's largest producer of sugarcane (*Saccharum* spp.) (Poaceae). According to the monitoring of the Brazilian sugarcane harvest by CONAB (2022), sugarcane production in the 2021/22 season totaled 585.2 millions tons, representing a volume of raw material 10.6% lower than the 2020/21 season. This reduction is due to a 3.5% decrease in cultivated area and, above all, adverse weather effects of drought during crop production cycle and low temperatures recorded in June and July 2021.

For Brazil to remain the world's largest producer of sugarcane, permanent studies on solutions about factors that impact and reduce sugarcane yields are required, with one of the bottlenecks being ethanol production costs, of which 70% is from sugarcane production (Santos et al., 2018).

Agrochemical costs, machinery, fleet, maintenance, new technologies and innovation, labor, harvesting (straw), management and monitoring (precision agriculture), transport and logistics, water use, finance, storage, agricultural planning, safety, personal protective equipment (PPE), topography, residues (e.g., vinasse), technical expertise and outdating in rural extension, droughts, fires, and frosts are just some of the challenges faced by farmers, in addition to issues related to pest control.

The exposure of crops to insect pest attacks have promoted losses of around 60 million tons, which also have significantly reduced industrial production (Parra et al., 2010; Oliveira et al., 2014). Moreover, owning to different climatic conditions, changes in sugarcane management, among other challenges mentioned above, many crops have undergone even higher losses due to pest insect infestations.

2 I SUGAR CANE PESTS AND MANAGEMENT APPROACHES

About 85 insect species have been recognized as damage-causing factors to sugarcane crops in Brazil. Among them, some are considered important or primary pests and, in some cases, of regional or national scope. The importance of one or another insect pest species depends on several factors, the most relevant being: growing area (edaphoclimatic conditions), agricultural year, and techniques adopted for crop practice and management. The main pests for sugarcane, also called key pests, are sugarcane borer (Lepidoptera: Crambidae), root spittlebug (*Mahanarva* spp.) (Hemiptera: Cercopidae), and weevil, *Sphenophorus levis* (Coleoptera: Curculionidae), with each of them posing different challenges to production. Besides them, other pests attack stalk base or ratoon, such as: sugarcane rhizome borer, *Migdolus fryanus* (Coleoptera: Vesperidae), sugarcane hairy borer, *Hyponeuma taltula* (Lepidoptera: Erebidae), lesser cornstalk borer, *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae), subterranean termite, *Heterotermes tenuis* (Isoptera: Rhinotermitidae), mound building termite, *Cornitermes cumulans* (Isoptera: Termitidae), and the burrower bug, *Scaptocoris castanea* (Hemiptera: Cydnidae).

Plant shoot pests can also be related, namely: small sugarcane borer, *Diatraea flavipennella* (Lepidoptera: Crambidae), giant sugarcane borer, *Telchin licus* (Lepidoptera: Castniidae), leaf-cutter ants, *Atta* spp. (Hymenoptera: Formicidae), leaf spittlebug, *Mahanarva posticata* (Hemiptera: Cercopidae), and West Indian cane weevil, *Metamasius hemipterus* (Coleoptera: Curculionidae) (Leslie, 2007; CTC, 2013; Seleghim, 2020).

Challenges in *D. saccharalis* control are related to increased use of delta and pheromone traps to monitor adult movement patterns. This modality poses an operational challenge (in adapting yield to pest timing) and, due to the lack of a synthetic option in the market for natural pheromones, as well as difficulty in expanding, modeling and/or automating pest monitoring. Infestation modeling studies have brought great advances to the use of such technologies; however, pest and varietal management programs should be rethought due to the "borer-rot" complex, including the use of pest resistant Bt cultivars (Allen & Singh, 2016; Carbognin, 2019; Franco et al., 2021). Finally, *D. saccharalis* control is still being done by the parasitoids *Cotesia flavipes* (Hymenoptera: Braconidae) and *Trichogramma galloi* (Hymenoptera: Trichogrammatidae), which are reared in biofactories and released in the field. In this case, a great challenge is automation of laboratory production, which aims at improving production efficiency and quality of produced individuals, as well as reducing production costs. Also, the release of these parasitoids has represented a significant challenge for the sector, particularly via drones.

Remarkably, a great challenge for researchers in the field remains to obtain artificial diets for insect production in the laboratory in quantity and quality. Such conditions are needed for insects to be able to efficiently perform what is expected from them. Among the actions, one must highlight the improvement of borer diet and a diet that can efficiently produce *S. levis* and *H. taltula* in biofactories.

For root spittlebug and sugarcane weevils, challenges are mainly related to their control, since these species develop in the soil (Dinardo-Miranda, 2008). Allied to this is the fact that these pests require highly difficult monitoring and sampling and their presence is only noticed after attack symptoms become visible. Moreover, soil insect pests have been practically controlled by agrochemicals and in a very intensive way. In this sense, Arrigoni (2007) already pointed out the monitoring and proper use of agrochemicals as challenges to minimize negative environmental impacts. Currently, methods of controlling these pests have not advanced much, with chemical control still being the most used. However, new control forms alternative to the chemical have been studied, such as the use of entomopathogenic nematodes (Silva, 2020).

Another insect that has been worrying part of the sugar-alcohol sector is the *H. taltula*. This pest, according to Arrigoni (2007), was already a concern almost 20 years ago and seemed to become a primary pest in sugarcane plantations. The absence of bioecological studies that could guide control strategies was also a major concern, which today is still scarce.

Studies are also needed to increase understanding of potential effects of climate change and other factors on pests and their natural enemies, which can affect both animals and sugarcane itself. In this sense, De Bortoli et al. (2017) and Martins (2018) found important information to advance studies and develop new solutions for pest control.

Apart from treated points, there are several other challenges for sugarcane pest management and control, including: low efficiency of control measures available; pest resistance to pesticides; biological control compatibility with other agricultural inputs; production and logistics of biological inputs (micro and macro) to meet the market demand; control measures suited to irrigated, rainfed, drip fertigation, no-tillage or minimal planting systems in the face of challenges posed by shoot and in particular soil pests; new cultivars with different pest tolerance and resistance levels; and finally newly emerged pests. All this can be coupled with the little information in the literature on pests and mainly population dynamics at extreme temperatures and humidity (in parallel with drought, frost, and similar conditions).

Farmers who produce sugarcane and use biological control should pay attention to research recommendations, including: quality and age of individuals to be released in the field, form of transport and distribution, time of release in the field, and use of selective agrochemicals in the case of joint use with chemical control.

Biological control issues must be addressed and solved under a systemic view to provide efficiency and positive results. Currently many solutions are available but only a few have connected complete and/or integrated practices for agricultural management and its various realities. Despite these solutions, little is known about pest biology or agroecology, or even how to time applications with storage and agricultural planning. Among the solutions, the use of drones has been proposed to spray products, release biological agents, and crop imaging; yet, little is known about its legality and correct use, as well as the reliability or accuracy of the data collected. Added to this, the relationships of pests with sugarcane farming, equipment restrictions, and aspects such as rainfall and connectivity are still poorly understood. In this sense, the urge to fulfill this unexplored market has made many companies forget a few important stages of biological control development, and solutions end up being launched unfinished, with some or many “buts”, doubts, and low reliability. Fortunately, some companies understand this hindrance and have developed solutions jointly with customers and research institutions, seeking to resolve doubts and uncertainties in the face of the existing challenges, which is what the sector needs.

In general, challenges vary with crop species, cropping systems (perennial, semi-perennial, conventional, organic, mechanical planting, manual, ratoon, etc.), and amount of investment available. The latter varies with management type and final objective, especially those directly or indirectly related to pest control. End goals determine the line of work, the sugar and ethanol sector often tend to seek sustainable solutions that ensure profitable production over several crop cycles with less damage to the environment, besides greater cost-benefits. However, this is not always an option or possibility in the face of all scenarios that farmers or large companies and groups experience. Moreover, there is a “rooted” tendency towards more intensive use of agrochemicals, which is more behavioral than related to access to information.

According to Diógenes & Silva (2020), the conventional agriculture spread worldwide applies principles that do not respect major principles of nature. As a result, there are many invariably negative consequences for the environment. In this regard, Altieri (1999) stated that conventional agronomists have used dominant assumptions of modern science when it comes to “doing agriculture”, such as disregarding that one crop or lack of it can interfere with another. These professionals also often study physical soil properties separately from the biological ones and from the life that sustains them. Universalism is also noted when they propose, for example, plowing land using the same North American equipment and techniques, disregarding the different conditions in tropical countries. Another widely used assumption is objectivism, which assumes that agricultural production can be understood objectively, without considering the farmers and their way of thinking, the social systems, and agroecosystems surrounding plantation areas. For Altieri (1999), conventional agronomists based on such principles develop technologies for plant nutrition and pest management in isolation, assuming that they can be transferred to farmers as new technologies since they believe that they may fit into any agricultural system.

Regarding pest control, sustainable solutions beyond the conventional agriculture paradigm include concepts of Integrated and/or Ecological Pest Management (IPM and/or EPM). These practices are based on strategic decisions after monitoring pests, aiming to develop control measures that typically combine more than one approach (e.g., chemical, biological, and cultural controls). For an Ecological Pest Management, biological, cultural, and ecological management practices based on applied agroecology are especially preferred.

In any case, challenges in their base and essence are very similar between conventional systems and those that use biological control. According to Parra (2019), it should be considered that agriculture in Brazil is different than in any other part of the world, with two to three annual harvests in certain regions. When stating that biological control in the Netherlands or Spain covers around 80 to 90% of plantations, it should be remembered that, in these countries, most of the cultivation is done in greenhouses. Therefore, like what has been done in Brazilian agriculture that has made it a leader in Tropical Agriculture in the last 40 years, a biological control model specific to tropical regions must be developed, especially for open fields. And, of course, there are great challenges ahead, considering the type of agriculture, including the logistics of storage and transport across Brazil's long territorial extension; legislation problems (legislation for agrochemicals); sampling for release of biological control agents; release techniques over large areas; how to properly manage areas with transgenics (currently occupying more than 50 million hectares in Brazil); availability of biological control agents to farmers; automated insect mass rearing techniques for macro-organisms; formulations for microorganisms, among others (Parra, 2014).

Thus, it can be understood that the pest control challenges in sugarcane crops are not exactly one-off or specific to each system/objective. They usually come from a lack of specific and broad knowledge about the entire production chain, causes and consequences, costs, logistics, and reality of farmers, thus requiring wide-range and efficient visions and solutions. Lastly, pest control challenges in sugarcane farming are numerous, complex, and are not just for farmers, but for the entire system, from academia, large groups, and companies, as well as the government, and the solution may come from the union of these components in actions and programs.

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CAPÍTULO 9

SELECTIVITY OF INSECTICIDES AND BIOINSECTICIDES TO COMMERCIALLY USED PARASITOIDS OF *Diatraea saccharalis* ON SUGARCANE

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1 I INTRODUCTION

Brazil is considered the world's largest producer of sugarcane, *Saccharum* spp. (Poales: Poaceae), with a production of approximately 654.8 million tons and 8.62 million hectares of harvested area in the 2020/2021 growing season, representing an increase of 1.8 and 2.1%, respectively, relative to the previous year (CONAB, 2021).

This high production can be affected by the infestation of pests such as *Diatraea saccharalis* (Lepidoptera: Crambidae), *Hyponeuma taltula* (Lepidoptera: Noctuidae), *Mahanarva fimbriolata* (Hemiptera: Cercopidae), *Sphenophorus levis* (Coleoptera: Curculionidae), *Migdolus fryanus* (Coleoptera: Vesperidae), *Aclerda takahashii* (Hemiptera: Aclerdidae), *Saccharicoccus sacchari* (Hemiptera: Pseudococcidae), *Telchin licus* (Lepidoptera: Castniidae), and *Heterotermes tenuis* (Isoptera: Rhinotermitidae), with *D. saccharalis* being one of the main pests of the crop (Machado; Habib, 2006; Zenker et al., 2007; Dinardo-Miranda et al., 2007, 2012, 2013a; Pavlu & Molin, 2016; Monteiro; Peronti & Martinelli, 2022).

The sugarcane borer, *D. saccharalis*, causes direct damage in its larval stage such as the opening of galleries and causes the death of buds, generating great losses in production and low sugarcane quality. Thus, the insect requires efficient control measures for population reduction (Botelho & Monteiro, 2011; Dinardo-Miranda et al., 2013b).

Among these measures, integrated pest management (IPM) should be used to reduce the population of insect pests (Van Lenteren et al., 2018). According to Kogan (1998), IPM is the decision-making about which control strategies will be used, together or separately, considering the cost-benefit and social and environmental impacts. IPM uses chemical and biological control. The biological considers the macrobiological (parasitoids and predators) and microbiological (viruses, bacteria, fungi, and nematodes) (Van Lenteren et al., 2018; Marrone, 2019).

One of the alternatives in biological control is the use of natural enemies, especially *Cotesia flavipes* (Hymenoptera: Braconidae) and *Trichogramma galloi* (Hymenoptera: Trichogrammatidae) to control the sugarcane borer *D. saccharalis* at two different stages of development. The parasitoid *C. flavipes* acts while the sugarcane borer is at the larval stage and *T. galloi* acts in the eggs, that is, preventing the sugarcane borer from developing and completing its cycle (Vacari et al., 2012; Parra; & Coelho Junior., 2019; Kassab et al., 2020). These natural enemies can be used alone or associated (Botelho et al., 1999).

Another method of biological control is based on entomopathogenic fungi, which represent a total of 80% in Brazil, especially *Metarhizium anisopliae* and *Beauveria bassiana* (Ascomycota: Hypocreales) (Mascarin et al., 2019). Both are used in sugarcane mills to control the sugarcane borer and other pests that attack the crop, such as leafhoppers. Their use has increased by 20% annually (Destéfano et al., 2004; Van Lenteren et al., 2018), showing to be important in IPM due to their selectivity, specificity, efficiency, low toxicity to natural enemies, and causing little environmental impact (Lacey et al., 2015).

Insecticides used for chemical control in sugarcane cultivation can affect the action of other organisms, such as entomopathogenic fungi and natural enemies, causing toxicity (Botelho & Monteiro, 2011). In many cases, chemical control is applied with biological control, making it essential to use selective products that act on insect pests and not on natural enemies (Degrande et al., 2002).

There are some methods to assess the selectivity of insecticides to natural enemies for these products, such as spraying the eggs before offering them to the parasitoid, spraying the eggs after offering them to the parasitoid, direct contact with surfaces containing the product, or transgenerational generation, which is the female's ability to hatch from treated eggs (Foerster, 2002).

Selectivity is the ability of a product to control the target pest without causing impacts on natural enemies, thus allowing its survival and reproduction, both in the same environment (Foerster, 2002). Different from selectivity, specificity is related to the variety of hosts that a parasitoid can attack, which can be generalists when using a large number of host species and different taxa, or specialists, when they have few host species, for instance, *T. galloi* (Querino & Zucchi, 2012; Laumann & Sampaio, 2020).

Selectivity of products is important in sugarcane, allowing knowing its correct property and formulation to control the insect pest, associated to the correct form of application, cultivation conditions, and the environment of the target pest (Foerster, 2002), without affecting the natural enemies present.

2 I METHODS TO ASSESS SELECTIVITY

The International Organization for Biological Control (IOBC) coordinates international activities and standardizes selectivity methods to form a database with various information to show which products to use in IPM. For this, test protocols were developed, including laboratory, semi-field, and field tests (Hassan et al, 1994).

Many selectivity studies have been carried out with egg parasitoids to assess their potential, such as *T. galloi* to control *D. saccharalis* (Parra & Zucchi, 2004). Some methods can be used for this purpose, such as:

2.1 Pre-parasitism spraying

Diatraea saccharalis eggs are sprayed/immersed in the product and then offered to *T. galloi*, then checking whether the eggs have been parasitized (Goulart et al., 2008; Potrich et al., 2009; Taguti, 2021).

2.2 Post-parasitism spraying

Diatraea saccharalis eggs are offered to the parasitoid *T. galloi* and then sprayed/immersed in the product, observing the emergence and longevity of the parasitoid (Potrich et al., 2009).

2.3 Exposure to treated surfaces

The product is applied to the plant or glass surfaces and, after drying, *T. galloi* is placed in contact with the contaminated surface and its survival is evaluated (Taguti, 2021).

2.4 Transgenerational effect

The caterpillar of *D. saccharalis* is contaminated with the product and follows its phase until the formation of a couple and oviposition. The eggs from the contaminated female are offered to *T. galloi* and, subsequently, assessed whether or not were parasitized and if the parasitoid will develop (Costa et al., 2014; Santos, 2021).

3 | PARASITOIDS USED IN SUGARCANE

3.1 Cotesia flavipes

Cotesia flavipes is a parasitoid native to the Indo-Australian plate, belonging to the eastern and Australian zoogeographic regions (Overholt et al., 1997). The wasp parasitizes different species of caterpillars of the families Crambidae, Noctuidae, and Pyralidae (Lepidoptera), which feed on plant tissues of Cyperaceae, Poaceae, and Typhaceae (Poales) plants (Overholt et al., 1997; Fujie et al., 2018).

The insect, dispersed worldwide, was introduced in Brazil in the 1970s from specimens from Trinidad and Tobago to control the sugarcane borer *D. saccharalis* and is currently used in 3.5 million hectares to control this pest (Pinto et al., 2022). In sugarcane, there is also a record of parasitism of the braconid on the sugarcane borer *Diatraea flavipennella* (Lepidoptera: Crambidae) (Barbosa et al., 2020).

Adult females of the insect, which are approximately four millimeters long, lay approximately 40 eggs in the host's body cavity (Fujie et al., 2018; Pinto et al., 2022). The larvae start feeding the hemolymph, the host's body fluids, after three or four days (Barbosa et al., 2020; Pinto et al., 2022). The larvae, which have passed through three instars, leave the host's body at approximately 14 days to weave a cocoon and become a pupa, which will be located within the plant tissues of the host plant of the pest organism (Barbosa et al., 2020; Pinto et al., 2022). The adult will emerge within six days (Overholt et al., 1997; Fujie et al., 2018; Barbosa et al., 2020; Pinto et al., 2022).

3.2 Trichogramma spp.

There are more than 210 *Trichogramma* spp. parasitoids distributed worldwide, of which 28 occur in South America and all of them in Brazil (Pinto, 2006). These parasitoids, some of unknown origin, are mostly from the Nearctic zoogeographic region, native to the United States and Mexico, such as the species *Trichogramma pretiosum* and *T. galloi* (Hymenoptera: Trichogrammatidae) (Zucchi & Monteiro, 1997). This group of wasps parasitizes eggs of insects of the orders Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, and Thysanoptera, which are present in several crops (Monnerat et al., 2007; Dalvi et al., 2014; Amaro et al., 2015).

The control of sugarcane pest insects by the parasitism of *D. saccharalis* eggs, using trichogrammatids, began in 1925 in Louisiana, the United States of America, and Barbados (Hinds & Spence, 1929). In Brazil, the first releases of wasps occurred in 1983 in the sugarcane crop in the states of Sergipe and Rio de Janeiro (Querino & Zucchi, 2002). Wasps are released annually in more than four million hectares of sugarcane to control sugarcane pests. In addition to the sugarcane borer *D. saccharalis* and *D. flavipennella*, there is a record of parasitism of the cotton leafworm *Alabama argillacea* (Lepidoptera: Noctuidae) eggs by *T. galloi* (Zucchi et al., 2010).

Adult females lay their eggs in eggs of insects of the orders Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, and Thysanoptera (Monnerat et al., 2007; Dalvi et al., 2014; Amaro et al., 2015). The characteristic of a parasitized egg is its darkening (Dalvi et al., 2014; Valente et al., 2016). The larva inside the egg will go through three instars and adults emerge from the pupa and leave the parasitized egg at the end of the cycle (Milanez et al., 2018). Two to three adult individuals come from each egg (Oliveira et al., 2017).

4 I INSECTICIDES AND BIOINSECTICIDES USED IN SUGARCANE

Integrated pest management (IPM) is one of the most concise ways to control the sugarcane borer, using mainly biological and cultural control and resistant varieties. Chemical control is also used in more challenging cases (Cruz, 2007) due to the habit of the larval stage of the borer to remain inside the stalk, which makes its reach more difficult through insecticides. Thus, insecticides should be applied when the larvae are in the 1st and 2nd instar, before entering the stalk (Matioli, 2019).

Control methods must be used concomitantly as an IPM premise to better act in pest control (Van Lenteren et al., 2018). Thus, special attention should be given to the strategy of associated methods, such as biological and chemical controls, due to the presence of the insecticide, which may negatively influence parasitoid performance. A total of 53 insecticides are registered for sugarcane to control sugarcane borer, among them ten active ingredients (chlorantraniliprole, chlorfluazuron, diflubenzuron, fipronil, flubendiamide, lufenuron, novaluron, tebufenozide, teflubenzuron and triflumuron) and three insecticides derived from the association of two active ingredients (chlorantraniliprole + lambda-cyhalothrin, lambda-cyhalothrin + thiamethoxam and methoxyfenozide + spinetoram), belonging to eight chemical groups (MAPA, 2022).

5 I SELECTIVITY OF PHYTOSANITARY PRODUCTS TO SUGARCANE PARASITOIDS

The selectivity of phytosanitary products to natural enemies, such as parasitoids of agricultural pests, is the one that selects a product that poses a low risk to the agricultural environment (Gazzoni, 1994). In other words, it is the use of a product that targets the pest and does not affect the pest parasitoid, soil, and water.

Methods that assess selectivity can often classify whether the product is suitable or not to be used safely against natural enemies (Benvenga et al., 2016). Some studies have investigated the selectivity of insecticides and parasitoids. These studies are based on different aspects, some of them evaluated through applications to the host and subsequent evaluation in the parasitoid, and others by residual contact in leaves; both can affect the parasitoid performance and its biological parameters (Antigo et al., 2013; Costa et al., 2014; Fonseca et al., 2015; Matioli et al., 2019).

Matioli et al. (2019) tested seven insecticides used to control the sugarcane borer. Chlorantraniliprole + lambda-cyhalothrin and lambda-cyhalothrin + thiamethoxam led to 100% mortality to the parasitoid *C. flavipes* in direct contact with leaf discs of sugarcane, being classified as harmful. However, chlorantraniliprole, chlorfluazuron, triflumuron and novaluron were harmless to the parasitoid, with mortality lower than 25%, while tebufenozide was completely harmless.

Parasitoids evaluated by residual contact left by insecticides were investigated; unlike triflumuron, fipronil negatively affected survival, longevity, and growth of *C. flavipes*. *Cotesia flavipes* successfully parasitized larvae fed diets treated with lufenuron, but delayed biological development was observed (Fonseca et al., 2015).

Eggs parasitized by *T. galloi* were submerged in insecticide solutions: tebufenozide did not affect the development (egg to pupa) of *T. galloi*, but lufenuron showed high toxicity. Likewise, these eggs submerged before being parasitized had the same results. Both insecticides did not affect parasitism, but lufenuron reduced adult emergence (Cônsoli et al., 2001). In addition to these insecticides, parasitized eggs sprayed with fipronil, triflumuron, and lambda-cyhalothrin + thiamethoxam caused negative effects on the egg, larva, pre-pupal, and pupal stages of the parasitoid *T. galloi* (Costa et al., 2014).

Importantly, several insecticides are available in the agricultural market to control the sugarcane borer, and, among them, there are those that, in addition to control, are also compatible with other forms of control. Studies carried out on the selectivity of parasitoids used in the sugarcane crop have shown that the insecticides chlorantraniliprole, chlorfluazuron, triflumuron and novaluron have low mortality for *C. flavipes* and tebufenozide is totally innocuous for *C. flavipes* and *T. galloi*.

Among the bioinsecticides used to control sugarcane pests, those based on the entomopathogenic fungi *B. bassiana* and *M. anisopliae* have been used to control the sugarcane borer and other pests, such as *M. fimbriolata* (Destéfano et al., 2004).

According to Rossoni et al. (2014), the fungi *B. bassiana* (IBCB 66 and ESALQ PL63) and *M. anisopliae* (PL43, IBCB 425, and E9) demonstrate selectivity to females of *C. flavipes*. Similarly, Santos et al. (2022) observed that *B. bassiana* (IBCB 425) and *M. anisopliae* (IBCB 66) are compatible and safe for *C. flavipes* and Hayashida et al. (2012) observed that *M. anisopliae* (UFGD 05, IBCB 348, and IBCB 425) was selective for this parasitoid.

The parasitism rate, emergence, and longevity of *T. galloi* were reduced when postures were immersed in a bioinsecticide solution with three different strains of *M. anisopliae* (IPA159E). The strains (IPA 211 and IPA 139E) did not affect the parasitism and only *M. anisopliae* (IPA 211) affected the emergence rate and longevity (Broglio-Micheletti et al., 2006).

Taguti (2021) evaluated *B. bassiana* and *M. anisopliae* in adults of *T. galloi* and eggs of the host *D. saccharalis* parasitized before and after. *Metarhizium anisopliae* and *B.*

bassiana did not affect the parasitism rate, emergence rate, and longevity of the parasitoid. On the other hand, *M. anisopliae* stood out with a higher parasitism rate relative to the fungus *B. bassiana* (Santos, 2021).

The compatibility between chemical and biological control agents present obstacles to be investigated and certainly solved. However, these relationships become more complex because they influence another controlling agent, the parasitoids (Santos, 2021). This interaction can often harm in some way, affecting biological parameters and even affecting the behavior of parasitoids, repelling or attracting them (Smaniotto et al., 2013; Luckman et al., 2014). Therefore, *B. bassiana* and *M. anisopliae* showed selectivity to *C. flavipes* and *T. galloi*.

6 I FINAL CONSIDERATIONS

Several authors have studied the selectivity of pesticides to natural enemies, but few studies have been dedicated to exploring the selectivity to hymenopteran parasitoids used for the biological control of sugarcane pests. Therefore, studies focused on this area are important to clarify information on the joint use of chemical and biological controls, so that there is no depreciation of the efficiency of the used parasitoids.

These control methods should be used together with other control methods as a premise of IPM to promote higher efficiency on the pests, which cause economic losses to sugarcane fields. Further studies on the use of biological and chemical controls are required. There is no information on other parasitoids and insecticides that can be used in the sugarcane crop. Moreover, the information on *T. galloi* and insecticides is scarcer than information to *C. flavipes* even though the braconid has been used commercially for more time for the sugarcane borer than the trichogrammatid (Botelho, 1992). The sugarcane market is quite innovative and technological. Thus, it is interesting that the focus of new studies is always on the selectivity of insecticides together with bioinsecticides, showing all parasitoids that can be used to control pests in the sugarcane crop.

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CAPÍTULO 10

INTEGRATED MANAGEMENT STRATEGIES FOR KEY PESTS OF COFFEE CROP

Bruno Henrique Sardinha de Souza

1 I INTRODUCTION

Brazil is the largest coffee producer and exporter worldwide. In the 2022 crop season, coffee production was estimated at more than 55 million 60-kg bags, for both *Coffea arabica* and *C. canephora* species; of this amount, almost half comes from crops grown in the state of Minas Gerais (CONAB, 2022). Despite Brazil's prominence in the coffee production international scenario, losses due to insect pests represent one of the main problems both in quantitative and qualitative terms. Among the main coffee-growing regions in the country, coffee leaf miner and coffee berry borer stand out as key pests.

2 I KEY COFFEE PESTS: COFFEE LEAF MINER AND COFFEE BERRY BORER

Coffee leaf miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), is the main insect pest of coffee plants in Brazil due to its widespread occurrence in producing regions, causing significant economic losses, especially in the Cerrado areas of Minas Gerais state (Mendonça et al., 2016). Coffee leaf miner damage to coffee crop is due to photosynthetic area losses by larvae feeding in the leaf mesophyll tissue, creating mines and galleries as distinctive injury. Under severe infestations, pest attack can lead to premature leaf fall, further impacting photosynthesis and hence plant yield. In regions favorable to coffee leaf miner infestations, such as under environmental conditions of high temperature and low relative humidity, and where crop cultivation is carried out mechanically and with larger row spacing, high population densities may occur, decreasing up to 80% coffee production in the following crop season (Souza et al., 1998).

Coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae), is another key pest of coffee plantations. It is regarded as the second most important pest in arabica coffee (*C. arabica*) and the main pest in robusta coffee (*C. canephora*) in Brazil. In global terms, the coffee berry borer stands out as the main pest of the crop and is currently present in nearly all countries, but Nepal and Papua New Guinea (CABI, 2022).

Coffee berry borer infestations in coffee plantations start about 90 days after the main flowering in green berries, which is characterized as its transit or flight period. Females penetrate the berry by boring a hole in the disk region until reaching the seed. Under ideal humidity conditions in the fruit (40-60%), females lay eggs in the galleries formed by their feeding, with larvae completing development in the endosperm. These injuries can lead to premature fruit abscission, in addition to quantitative damage by grain weight losses and qualitative damage to coffee beverage characteristics (Souza et al., 2014).

3 I CONTROL METHODS USED FOR KEY COFFEE PESTS

Key pests of coffee plantations, *L. coffeella* and *H. hampei*, are mainly controlled by chemical insecticide products. Applied insecticides belong to various chemical groups and have different mechanisms of action. In the case of coffee leaf miners, insecticides are applied both by spraying and via drench, while for coffee berry borer by only spraying. About 150 commercial products are currently registered for coffee leaf miner control, including several active ingredients belonging to organophosphates, carbamates, pyrethroids, neonicotinoids, spinosyns, butenolides, diamides, benzoylphenyl-ureas, avermectins, and juvenoids groups. Commercial mixtures between some of these insecticides or even between insecticides and fungicides are also available. In addition to insecticides, a sex pheromone-based product is also registered for use in delta traps for insect population monitoring (MAPA, 2022).

On the other hand, few insecticides are officially registered for coffee berry borer, totaling 29 commercial products, in addition to another 15 products based on *Beauveria bassiana* and one more kairomone (methanol: ethanol) used for monitoring using specific traps for coffee berry borer. Of the 29 chemical insecticides registered, 14 are based on the organophosphate chlorpyrifos, representing almost half of the products. Insecticides of the chemical groups of diamides, metaflumizone, spinosyns, oxadiazines, avermectins, and pyrethroids, formulated singly or in commercial mixtures, complete the list (MAPA, 2022).

The use of insecticides is complemented by cultural practices, such as thorough fruit-harvesting in plants followed by reharvesting and sweeping of fruits remaining on the ground, which are the main foci of pest infestations (Silva et al., 2010). For coffee leaf miner, the resistant cultivar ‘Siriema AS1’ was developed, which is a hybrid originated from a cross between *C. arabica* x *C. racemosa* (Carvalho et al., 2013; Matiello et al., 2015). However, more work needs to be done under high insect infestation to confirm the efficiency of the host plant resistance. Green lacewings have also been used by some farmers, whose larvae are predators of coffee leaf miner larvae.

Due to the high cost of the modern insecticides, frequent use of highly toxic insecticide chemical groups, and pest resistance to some active ingredients, currently, few are the options available for the management of these two key pests of coffee plantations. Thus, research aimed at developing strategies and applying efficient and sustainable technologies in practice for their integrated management should be encouraged.

4 I INTEGRATED PEST MANAGEMENT

The occurrence of high coffee leaf miner and coffee berry borer population densities in the main coffee-growing regions of Brazil and the potential cases of resistance to active ingredients of the most used insecticides make inefficient the use of only one control method, such as only insecticide applications. There are reports, for example, that coffee leaf miner populations resistant to chlorantraniliprole in areas of intense coffee production can reach 85%, being up to 94% in Bahia state, with resistance levels ranging from 10 to 40-fold (Leite et al., 2020). Several cases of resistance in *L. coffeella* populations to organophosphates were also identified (Fragoso et al., 2002; 2003), which is one of the most used insecticide groups in coffee crop.

Integrated Pest Management (IPM) programs should be developed and implemented mainly at regional scale to increase pest control efficiency, as well as to reduce production costs and toxic residues in agroecosystems and final produce. IPM is a planning and monitoring system for strategic deployment of pest control tactics, keeping insect pest populations below economic injury levels, while maintaining productivity and quality of agricultural produce, in which pest control decision-making is based on cost-benefit analysis based on economic, ecological, toxicological, and social principles. IPM has a multidisciplinary approach, involving several areas of knowledge such as Entomology, Plant Physiology, Ecology, Plant Nutrition, Chemistry, Statistics, Toxicology, and Economics, among others (Souza, 2020).

IPM programs for key coffee pests should be always optimized to reduce damage, benefiting both commodity coffee yield and specialty coffee production and quality. The Laboratory of Plant Resistance and Integrated Pest Management (LARP-MIP) at the Federal University of Lavras (UFLA) has conducted several studies to develop efficient and sustainable strategies for IPM-Coffee. Research has achieved promising results in terms of coffee leaf miner and coffee berry borer population reductions. The studies are performed by undergraduate students in Agronomy and Master's and PhD students in Entomology. The results are published in monographs, dissertations, theses, and scientific and extension publications. In the next section, the main results of these studies on integrated management strategies of coffee leaf miner and coffee berry borer are summarized.

5 I INTEGRATED MANAGEMENT STRATEGIES OF COFFEE LEAF MINER AND COFFEE BERRY BORER

Plant Resistance is one of the cornerstones of IPM and consists of growing cultivars or hybrids of plants with chemical and morphological traits that defend them against insect pest oviposition, feeding, and development. Plants can express these defense mechanisms constitutively or induced, i.e., when manifested constantly or only after a pest attack, respectively. They can also be direct when these plant traits directly affect insect biological performance, or indirect, by producing volatile organic compounds or extrafloral nectaries that attract natural enemies, favoring both plant growth for acting as biological control agents and pest population reductions (Smith; Clement, 2012).

Of all Brazilian coffee plantations, 90% are grown with IAC Mundo Novo and IAC Catuaí cultivars (Giomo, 2015; Gomes; Galdino, 2017). There is a lack of information on resistance levels to key pests in commercial cultivars available on the marketplace. This contributes to delaying transfer of knowledge to coffee growers, who end up not cultivating the modern cultivars. Therefore, more research under field and laboratory conditions is needed to assess the resistance levels in commercial cultivars, characterize the resistance types, and identify chemical and morphological mechanisms involved.

Coffee cultivars have been developed using classical and molecular breeding techniques, mainly by public research institutions (Guerreiro Filho, 2006). Fundação Procafé launched the cv. Siriema AS1, a hybrid from a cross between *Coffea arabica* (cv. Mundo Novo) x *C. racemosa*. This cultivar possess resistance to coffee leaf miners and coffee leaf rust, and is the only cultivar recognized as resistant to the insect pest (Carvalho et al., 2013). For coffee berry borer, little is known about resistant cultivars, with resistant germplasm only being identified in non-commercial *Coffea* species (Sera et al., 2010). In recent years, studies have evaluated the categories and levels of resistance to coffee leaf miner and coffee berry borer, and the main results will be presented herein.

In Lavras, southern Minas Gerais, *L. coffeella* infestation was evaluated monthly over three consecutive crop seasons. The study investigated the resistance in 30 coffee genotypes, of which 28 are commercial cultivars. Temperature and rainfall monthly averages were also recorded to correlate these abiotic factors to coffee leaf miner population fluctuations. When comparing the resistant cultivar Siriema with the susceptible Catuaí Vermelho IAC 144, for example, coffee leaf miner infestation was much more reduced by the resistant genotype in almost the entire evaluation period, with accentuated differences in months of higher population peaks (from August to November). Therefore, cv. Siriema has moderate resistance to *L. coffeella* in the field and can serve as an important control tactic in IPM. Rainfall was also an important factor in regulating coffee leaf miner populations, which can help in terms of optimizing the period of pest sampling control decision-making (Figure 1).

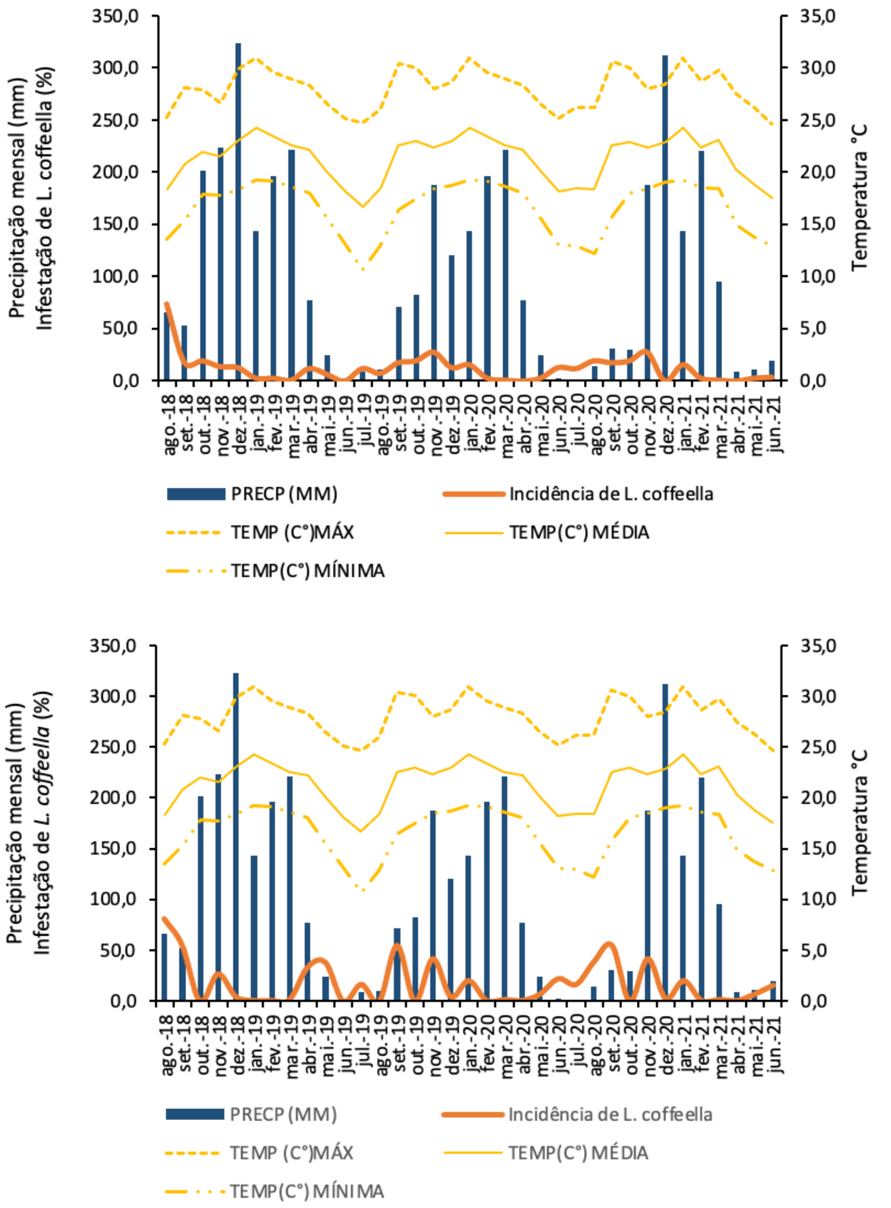

Figure 1. Mean percentages of leaves mined by coffee leaf miner in resistant (Siriema - A) and susceptible (Catuaí Vermelho IAC 144 - B) cultivars and mean monthly temperature and rainfall over three years of evaluation in Lavras, MG (Brazil).

Oviposition preference assay with *L. coffeella* was also carried out in the laboratory using a free-choice approach, comparing cv. Siriema progenies and the commercial cultivar Arara, and characterizing the types and levels of resistance. Most dual-choice comparisons showed a higher number of eggs in the cv. Siriema progenies than in cv. Arara, ruling out the presence of antixenosis-resistance (Table 1).

Genotype	Eggs/cm^2 (Siriema)		Eggs/cm^2 (Arara)		*P*-value
T70	0.21 ± 0.05	a	0.19 ± 0.04	a	0.758
T71	0.22 ± 0.04	a	0.08 ± 0.01	b	0.004
T72	0.19 ± 0.04	a	0.07 ± 0.02	b	0.024
T73	0.27 ± 0.05	a	0.13 ± 0.03	b	0.040
T66	0.13 ± 0.01	a	0.13 ± 0.03	a	0.987
T67	0.16 ± 0.02	a	0.13 ± 0.02	a	0.436
T65	0.12 ± 0.02	a	0.03 ± 0.02	b	0.005
T69	0.30 ± 0.05	a	0.10 ± 0.03	b	0.005
T68	0.37 ±0.09	a	0.14 ± 0.03	b	0.026

Table 1. Number of Leucoptera coffeella eggs in coffee genotypes.

Means followed by the same letter within rows do not differ by t-test (*p*>0.05).

In a no-choice bioassay with *L. coffeella* using genotypes selected from the previous oviposition preference assay, the insect parameters most affected were larval survival and leaf injury intensity. While the cultivar Arara provided high larval survival (99%), the genotypes T69 and T70 caused only 35-38% survival. These results confirm the presence of moderate level of antibiosis-resistance in the genotypes of cv. Siriema (Table 2).

Genotype	Larval survival (%)		Pupal survival (%)		Leaf injury scores (1-4)	
Arara	99.0 ± 0.9	a	96.4 ± 2.1	a	2.9 ± 0.04	a
T68	97.0 ± 3.0	a	98.6 ± 1.3	a	3.1 ± 0.08	a
T66	44.5 ± 8.3	b	98.0 ± 1.9	a	1.8 ± 0.1	b
T70	38.6 ± 5.2	b	100.0 ± 0.0	a	1.5 ± 0.1	b
T69	35.9 ± 7.7	b	100.0 ± 0.0	a	1.6 ± 0.2	b
T71	58.4 ± 9.7	b	82.8 ± 9.9	a	2.0 ± 0.2	b
p-value	<0.0001		0.078		<0.0001	

Table 2. Larval and pupal survival of *Leucoptera coffeella* in coffee genotypes.

Means followed by the same letter within columns do not differ by Tukey's test (*p*>0.05).

Experiments with coffee leaf miner are ongoing in the field and laboratory using kaolin-based products (aluminum silicate) as an IPM strategy. These products have been applied in coffee plantations to prevent scald burn injury in plants. Kaolin sprays have shown positive effects on pest reduction in other crops. The partial results obtained for coffee leaf miner showed that the highest kaolin doses decreased the number of *L. coffeella* eggs under a free-choice assay in the laboratory. Such an effect might be related to light reflection alteration on the leaf surface due to the white color of the kaolin powder. This, therefore, may have modified adult leaf miner behavior; yet, abrasive effects on insect cuticles or other unidentified factors may also be related.

Population fluctuation and resistance to coffee leaf miner in four *C. canephora* genotypes were evaluated in experimental plots in the field in Lavras (Santos, 2022). During the months of higher population peaks, mainly in October of each year, the genotypes Conilon 213 and Conilon LB1 were less infested, especially Conilon 213, which showed a moderate resistance level to coffee leaf miner.

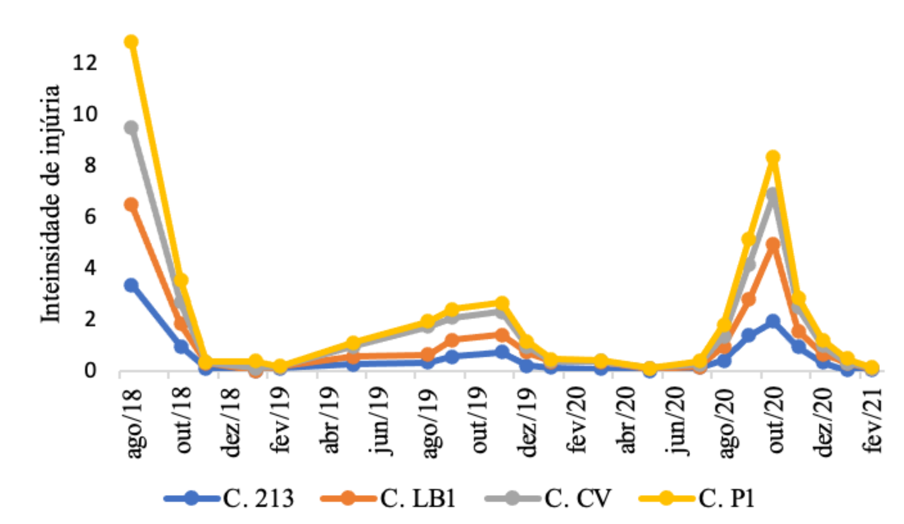

Figure 2. Infestations of *Leucoptera coffeella* in *Coffea canephora* genotypes in the field.

The same arabica coffee genotypes tested in the field (Lavras, MG) were tested for the resistance to coffee berry borer (Alves, 2021). In this study, the number of bored fruits and the presence of biological forms within the fruits were recorded. The berries were also classified during evaluations according to their maturation stages. This was made to exclude occurrence of resistance from phenological asynchrony, in which the phase of greater susceptibility of host plants coincides with lower pest population peaks and may lead to erroneous interpretation on the presence of resistance.

Among the main results (Alves, 2021), the lowest *H. hampei* infestation in the upper third of coffee plants was found in the cultivar Arañas RH. This cultivar, in turn, did not differ from Acauã, IPR 100, IPR 102, Arara, IPR103, Acauã Novo, Clone 312, Clone 224, Aranãs RV, Pau Brasil, and Asa Branca. In the middle third of plants, the cultivars IPR 103 and Arara had the lowest infestations, not differing from Acauã, IPR 100, Acauã Novo, Arañas RH, IPR 102, and Clone 224. The cultivar Siriema showed greater coffee berry borer infestation than the other cultivars in both thirds of plants. In the upper third, the cultivar Catiguá MG-3 had the second highest infestation, not differing from Catiguá MG2, Guará, Saira II, Catiguá MG-1, Araponga, IAPAR 59, Oeiras, and Catucaí Amarelo. In the middle third, the cultivar Catucaí Amarelo was the second most infested, not differing from Catiguá MG-3, Oeiras, Araponga, Catiguá MG-1, and Pau Brasil.

According to the results, cv. Siriema was highly susceptible and was not affected by fruit precocity regarding higher *H. hampei* infestation. These effects may have been overlapped on Catuaí Amarelo, IAPAR 59, and Araponga, which were moderately and earlier infested. The cultivars Aranãs RH, Acauã, Arara, IPR 103, IPR 100, and IPR 102 may have characteristics that affected *H. hampei* colonization, regardless of fruit maturation. Moreover, the cultivar Pau Brasil showed a higher percentage of fruits bored only on the edges. Such a result may indicate the presence of morphological characteristics or volatile or non-volatile compounds that caused insects to leave fruits after perforation. Those cultivars that stood out in terms of lower coffee berry borer infestations deserve further detailed research regarding plant resistance.

In coffee production, a uniform fruit maturation during harvest is stimulated by application of growth regulators, also called bioregulators. Potassium acetate is a precursor of aminoethoxyvinylglycine, which in turn, inhibits the enzyme 1-carboxylic acid-1-aminocyclopropane synthase (ACC synthase). This enzyme produces ethylene from 1-carboxylic acid-1-aminocyclopropane (ACC) in ripening fruits (Even-Chen et al., 1982). Increasing ethylene concentrations in fruit rises climacteric respiration, initiating ripening by increasing respiration rates and synthesis of enzymes related to flavor, aroma, color, and water loss (Taiz et al., 2017).

Field and laboratory studies (Dias, 2019; Martins, 2022) were performed to evaluate the effect of applying ethylene-synthesis inhibitor (Mathury™) on *H. hampei* colonization and development in fruits treated with different doses and application periods. Due to the mode of action of the bioregulator, its application could impair coffee berry borer colonization and affect oviposition and larval development, reducing pest infestation due to the influence on fruit and seed water contents, as humidity is a limiting condition for the pest development.

Since ethylene is one of the main phytohormones in signaling pathways and resistance to insects, along with jasmonic acid and salicylic acid (Souza; Boiça Júnior, 2014; Taiz et al., 2017), changes in its concentrations may have additional effects on *H. hampei* behavior and development.

In field experiments (Lavras, MG), ethylene-synthesis inhibitor (Mathury™) applications at 80 days after flowering (DAF) with 2 and 15 L ha^{-1}, and at 110 DAF with 15 L ha^{-1} caused, respectively, 84 and 93% reductions in the number of *H. hampei* pupae inside fruits collected from the middle third of arabica coffee plants. For *H. hampei* adults, reductions were by 50, 76, and 55% for treatments applied at 80 DAF with 2 and 15 L ha^{-1} and at 110 DAF with 2 L ha^{-1}, respectively. Moreover, *H. hampei* females showed a lower preference for fruits treated in the field at 80 DAF with 15 L ha^{-1} or at 110 DAF with 2 L ha^{-1} when compared to untreated fruits in a free-choice assay (Martins, 2022).

A complementary study on *H. hampei* development in fruits subjected to the same field treatments was evaluated in a no-choice assay in the laboratory (Dias, 2019). The results showed significantly lower adult survival (6.7 to 17.8%) in fruits treated with ethylene-synthesis inhibitor (Mathury™) at 80 DAF with 15 L ha^{-1} and 110 DAF with 2 and 15 L ha^{-1} compared to untreated coffee fruits (46.6%). The number of *H. hampei* larvae in fruits under the same treatments was also reduced (0.07 to 0.16 larvae) as compared to the control (1.46 larvae).

Other studies were carried out under field and laboratory conditions (Padilla, 2022) to evaluate IPM strategies for *H. hampei*. The treatments tested rotation or mixture of chemical insecticides (chlorpyrifos, acetamiprid+bifenthrin, and metaflumizone), entomopathogenic fungus *B. bassiana*, and an adjuvant (Openeem Plus™) based on neem extract (*Azadirachta indica* A. Juss.). The neem extract-based product may also have biostimulant effects on plants due to its constituent compounds, which are extracted from virtually all neem plant parts but the fruits and kernels.

In the field experiment, the strategy constituted by Chlorpyrifos+Openeem/ Openeem/Clorpyrifos+Openeem in the first, second-, and third-monthly applications, respectively, stood out with the highest number of fruits without *H. hampei* adults in the second evaluation, reducing infestation by 55%. In the third evaluation, the applications of Sperto/Boveril/Verismo showed the highest number of bored berries without adults inside, and the adult infestation was reduced by 41% (Figure 3).

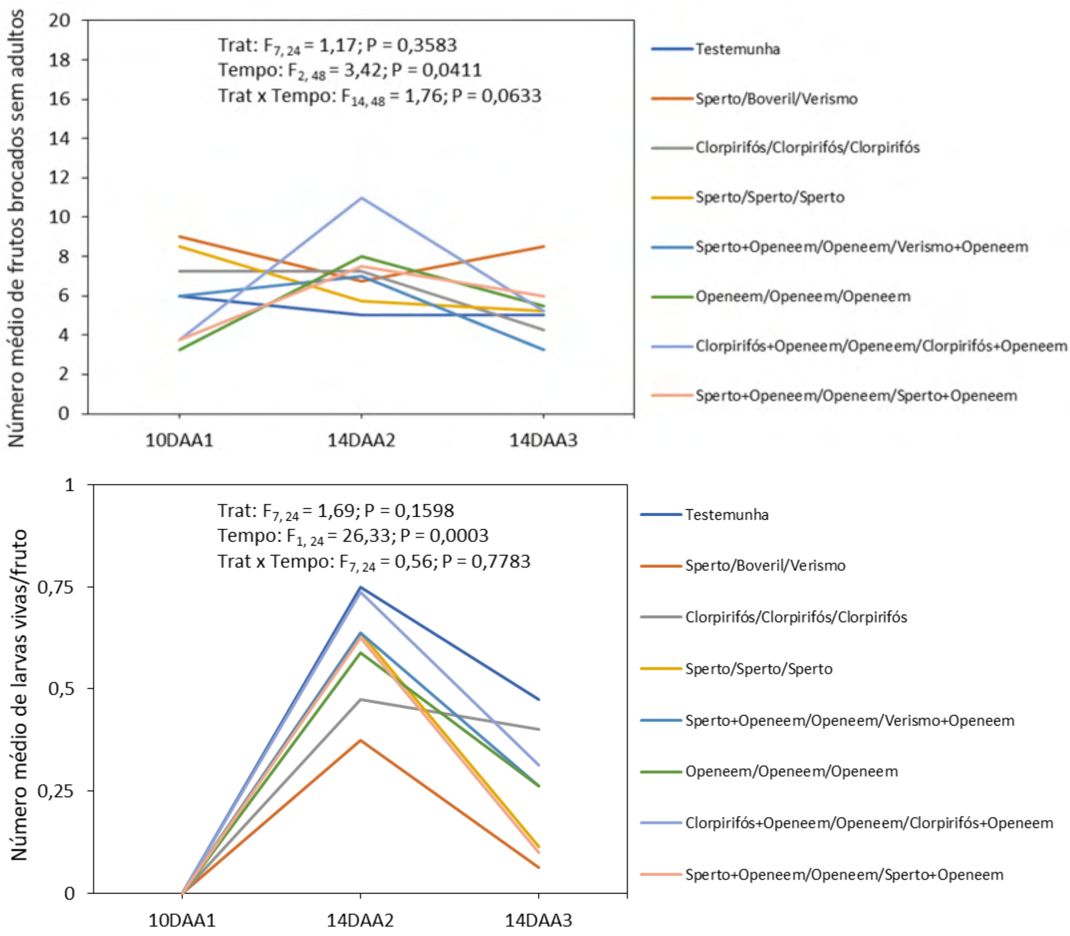

Figure 3. Numbers of bored berries without *H. hampei* adults (A) and larvae per fruit (B) in three evaluations after applications of insecticides, bioinsecticide, and neem extract-based adjuvant in rotation or mixture.

As for the number of larvae in fruits (Padilla, 2022), the treatment consisting of Sperto/Boveril/Verismo in three monthly applications, respectively, had the lowest mean number of live *H. hampei* larvae per fruit (50% control efficiency). In the third evaluation, the treatments Sperto/Boveril/Verismo, Sperto+Openeem/Openeem/Sperto+Openeem, and Sperto/Sperto/Sperto showed the lowest infestations of live larvae per fruit, reducing larval infestations by 87, 79, and 76%, respectively (Figure 3).

A field bioassay was conducted in *voile*-fabric cages attached to branches with cherry fruits in arabica coffee plants artificially infested with *H. hampei* adults after 40 days of neem extract (Openeem Plus™) application. As the main results of this field bioassay, the number of *H. hampei* eggs was significantly reduced with the application of the neem-based product

(4.92 eggs) relative to the control (7.92 eggs) without application (Padilla, 2022).

The effect of neem extract (Openeem Plus^{TM}) application over artificial diet on *H. hampei* biological development was evaluated in Petri dishes under laboratory conditions (PADILLA, 2022). The percentage of dishes with eggs was four-fold higher in the control treatment. The results were similar for *H. hampei* larvae and pupae. The number of adults was numerically higher in the control, while in dishes with artificial diet treated with neem extract, adults did not emerge until 40 days of evaluation (Figure 4). The number of eggs also showed a significant difference, being lower in artificial diet treated with neem extract. Based on the field and bioassay results (Padilla, 2022), neem extract impaired coffee berry borer performance, reducing oviposition of females fed fruits treated with the botanical product.

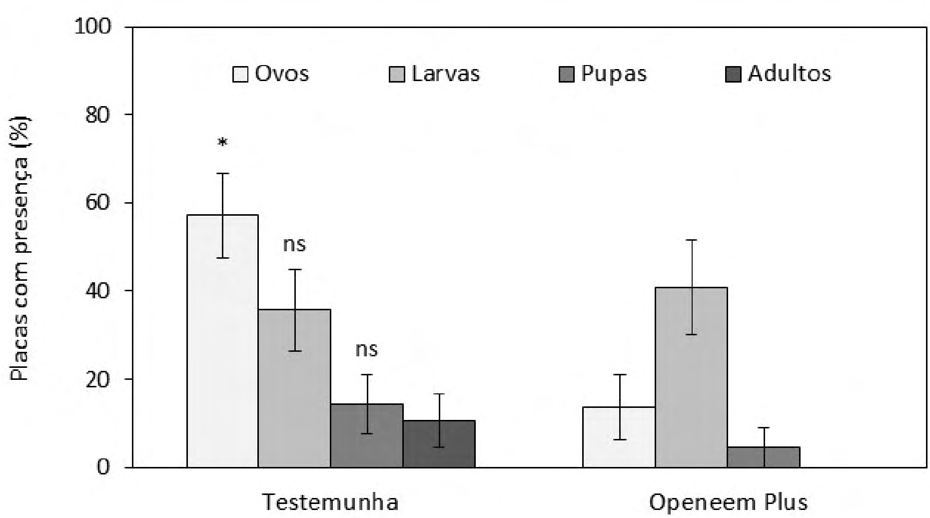

Figure 4. Percentage of *H. hampei* eggs, larvae, pupae, and adults in Petri dishes with artificial diet treated or not with neem extract adjuvant (Openeem Plus^{TM}).

The physiological effect of neem extract-based product (Openeem Plus^{TM}) reducing *H. hampei* oviposition can be explained by adult malnutrition in treated fruits, affecting egg production and maturation. This is because *H. hampei* is synovigenic, that is, insect feeding during the adult stage influences egg production.

The information generated from that research (Padilla, 2022) is relevant for coffee berry borer IPM. The proposed pest management strategies may contribute to reducing chemical insecticide applications and show neem extract compatibility with *B. bassiana*-

based bioinsecticide and with some insecticides. Still, further studies should be carried out to assess the ability of neem extract to trigger induced defense responses in coffee fruits against coffee berry borer.

6 I FINAL CONSIDERATIONS

Coffee leaf miner and coffee berry borer are the major biotic threats in terms of coffee productivity and quality for the main coffee-growing regions in Brazil. Such problems impact economic revenues from the production of this important agricultural commodity. These key pests of coffee crop are mostly controlled by application of chemical insecticides, which are often highly toxic. However, they do not provide the expected effects in terms of pest population reductions mainly due to cases of pest resistance. Therefore, novel IPM strategies must be developed and deployed in coffee plantations to improve pest control efficiency and sustainability.

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CAPÍTULO 11

CHALLENGES OF DIGITAL AGRICULTURE IN PEST MANAGEMENT

David Luciano Rosalen

1 I INTRODUCTION

This chapter aims to provide a basic reference for consultation encompassing Digital Agriculture within the scope of Agricultural Entomology. In item 2, the Concept of Digital Agriculture is shown since the historical evolution of agriculture and the emergence of the so-called Industry 4.0, as well as its respective disruptive technologies.

In item 3, basic concepts of GNSS (Global Navigation Satellite System) Positioning, Remote Sensing, and Geographic Information Science, together with their applications in the context of Agricultural Entomology, are shown. Finally, item 4 discusses some challenges relating the three mentioned geotechnologies with Agricultural Entomology.

2 I DIGITAL AGRICULTURE CONCEPT

Agricultural labor productivity can be measured as cereal equivalent per worker per year. This factor increased highly over the evolution of agricultural technology, increasing by 10 times at the time of agricultural mechanization in the between-war period, and 100 times in the late twentieth century after the Green Revolution (Mazoyer; Roudart, 2008).

This technological evolution, according to Feldens (2018), went through the following stages:

1. Modern agriculture, before 1900 (use of hand tools and animal traction);
2. Mechanized agriculture, after 1920 (mechanization);
3. Green Revolution, after 1960 (intensive use of mechanization, inputs, and breeding);
4. Digital revolution, after 1990 (beginning of automation and digitization of agriculture).

Massruhá et al. (2020) also divided technological evolution into four stages, but differently:

1. Agriculture 1.0, 1900-1950 (Animal Traction), characterized using animal traction, family and subsistence farming, with the sale of surpluses;
2. Agriculture 2.0, 1950-1990 (Green Revolution), characterized by monocropping, monodisciplinary activities, synthetic inputs use, and adaptive research;
3. Agriculture 3.0, 1990-2015 (Integrated Systems), characterized

by integrated crops, multidisciplinary activities, higher efficiency, and systemic research;

4. Agriculture 4.0, 2015 - present days (biologically-based Agriculture), characterized by complex systems, transdisciplinary activities, biological inputs, and complex research.

Stages 3.0 and 4.0 break with the Agriculture-2.0 paradigm (Green Revolution), which is characterized by monocropping, monodisciplinary vision, intensive use of synthetic inputs, and adaptive agricultural research. Notably, the questioning of the Green Revolution principles is not new, with its starting point being the publication of the book “Silent Spring” in 1962, by Rachel Carson. In this work, the serious environmental effects of the large-scale use of agrochemicals are presented. For more details, consult Rachel Carson’s original work (Carson, 2010) and the work of Bonzi (2013), which analyzes the impact of that book’s publication.

It is also important to analyze the origin of the term 4.0. According to Pereira and Simonetto (2018), this terminology originated in Germany in 2011, from a government project, Industry 4.0, which would be the Fourth Industrial Revolution and is characterized by:

1. Internet of Things (IoT);
2. Cloud computing (CC);
3. Cognitive Computing (CoC);
4. Cyber-physical System (CPS).

IoT is the ability to connect all sorts of devices (machines, gadgets, cell phones, cars, among others) to the internet and make them smart, considering autonomy and privacy issues (Ali; Ali; Badawy, 2015).

On the other hand, CC is not only data storage but also data processing in large providers with high hardware capacity as described in Madhavaiah and Bashir (2012). The CC service is monopolized by the so-called “Big Techs”: Microsoft (Microsoft Azure), Google (Google Cloud Platform), and Amazon (Amazon Web Services - AWS). Besides these, there are also IBM Cloud, Oracle Cloud, CloudStigma, GoDaddy, VMware Center Server, DigitalOcean, and Hyve.

Within the remote sensing scope, image processing can be cited as a CC example. This tool has undergone a major revolution, with any user being allowed to process several images using state-of-the-art algorithms. Formerly, such high-level processing was restricted to companies and/or research institutions that owned advanced and expensive hardware and software resources. If on one side cloud processing has led to greater democratization and ease, on the other side, the dependence on a good internet connection and the fact that few companies have an information monopoly on the Internet, as shown by Öhman and Aggarwal (2020). In this context, the wireless field (Wi-Fi) connection in Brazilian rural areas is often poor, leading to the search for local solutions to circumvent such a problem, e.g., LoRaWAN (Low Power WAN; Protocol for IoT), as described by Silva et al. (2017).

CoC is a multidisciplinary field and its main goal is to develop computational models for decision-making based on neurobiological brain processes, Cognitive Sciences, and Psychology (Gutierrez-Garcia; Lopez-Neri, 2015). Big Data Analytics and Artificial Intelligence (Machine Learning and Deep Learning) techniques are part of the CoC (Pereira; Simonetto, 2018).

CPS consist of a coordinated combination of computational and physical resources, such as collision avoidance systems in autonomous navigation (NSF, 2008). In this way, physical entities (robots or other devices) are controlled by computational elements.

Huang et al. (2020) developed an agricultural CPS consisting of an intelligent robotic vehicle controlled by neural networks, with crop growth and pest and disease detection models in its decision-making mechanism.

Within the above context, Agriculture 4.0 should cover technologies that also characterize Industry 4.0. However, according to Fonseca et al. (2020), the concept of Agriculture 4.0 is broader as it encompasses agricultural production systems, inputs used, and applied research modality. Thus, it goes beyond the simple use of technologies; in this approach, Agriculture 4.0 would be a new paradigm in agricultural production.

Another important point to be discussed is the terminology “Precision Agriculture”, “Smart Agriculture” and “Digital Agriculture”. The definition of Precision Agriculture by the International Society of Precision Agriculture (ISPA) is given below:

> Precision Agriculture is a management strategy that gathers, processes and analyzes temporal, spatial and individual data and combines it with other information to support management decisions according to estimated variability for improved resource use efficiency, productivity, quality, profitability and sustainability of agricultural production. (ISPA, 2022, np).

The “Smart” concept emerged in the last decade (2010-2020), within the scientific and industrial communities, which fostered the so-called “Smart Vision”. In this case, “smart” means integrated, intelligent, fast, as well as technologically, economically, politically, and culturally sustainable (doing more with less, improving the quality of life for all).

This new vision integrated disciplines in the problem-solving process (Castrignanò et al., 2020). Therefore, Smart Agriculture (Smart Agriculture or Smart Farm) proposes, from disruptive technologies such as the IoT, intelligent solutions to various agricultural problems. Intelligent Agriculture can therefore be understood as a step beyond Precision Agriculture, as this would initially be restricted to local actions using geotechnologies, such as GNSS Positioning, Remote Sensing, and Geographic Information Science. Therefore, Intelligent Agriculture would be a milestone for a new stage of Precision Agriculture.

In this context, Precision Agriculture can be divided into three steps. The first (1990-2000) consists of using GNSS technology to map soil physicochemical properties and automation processes for Variable Rate Application (VRA). The second (2000-2010) comprises the progress of autonomous navigation of agricultural machinery and the increase in VRA use, besides the beginning of the use of low-altitude aerophotogrammetry (drones). The third (from 2010 onwards) encompasses the adoption of Smart vision and Industry 4.0 technologies. Therefore, “Digital Agriculture” may be referred to as the junction of Precision Agriculture and Intelligent Agriculture.

The Food and Agriculture Organization of the United Nations (FAO) states that the process of digitizing agriculture would be the “Digital Agricultural Revolution,” and this would not be limited to the concept of Precision Agriculture, even in its third stage. The concept of Digital Agriculture by FAO indicates that the digitalization process is not limited only to the agricultural production process itself (as in Precision Agriculture), but also to the entire agri-food value chain: Pre-production, Production, and Post-production, including agricultural research in pre-production. Such a concept is also adopted by Fonseca et al. (2020).

Precision Agriculture is also called “Site-Specific Management - SSM”. It must adjust the use of inputs as a function of the spatial and temporal variability of production factors within a given area, hence locally customized. Accordingly, Precision Agriculture is partly analogous to Industry 4.0 since this, as indicated by Lasi et al. (2014), allows industrial products to be customized according to customer preferences, without losing mass production advantages.

3 I GEOTECHNOLOGIES AND AGRICULTURAL ENTOMOLOGY

As seen in the previous item, Digital Agriculture involves the use of GNSS positioning, Remote Sensing, and Geographic Information Science, as well as Industry 4.0-related technologies. This item presents some concepts and applications that make use of these technologies in the scope of Agricultural Entomology.

3.1 GNSS Positioning

GNSS positioning has revolutionized Geodesy and Surveying, as it allows georeferenced data collection on the earth's surface, in any geographic position and 24 hours a day. It also allows three-dimensional mapping, both statically and kinematics, the latter being unprecedented in terms of topographic surveys in general.

According to Monico (2004), the GNSS system encompasses three segments: Space (satellite constellation), Control (satellite tracking and control stations), and User (receivers used in field surveys). The GNSS system also includes global and local systems. Global systems allow navigation and mapping in any terrestrial location, while locals serve as a complement to global systems in specific geographic regions.

Examples of global systems are NAVSTAR-GPS (United States), GLONASS (Russia), BEIDOU (China), and Galileo (European Union). As complementary local systems and their respective regions of operation, there are Wide Area Augmentation – WAAS (North America), European Geostationary Navigation Overlay System - EGNOS (Europe), System for Differential Corrections and Monitoring – SDCM (Russia), Multifunctional Transport Satellite-based Augmentation System - MSAS (Japan), Quasi-Zenith Satellite System - QZSS (Japan), and GPS Aided Geo Augmented Navigation System - GAGAN (India).

Regarding application in Agricultural Entomology, GNSS positioning can be used in georeferencing of pest sampling in the field for Integrated Pest Management - IPM. This georeferencing can be done through smartphones, using general mapping applications, such as GeoMapp (Figure 1), developed by UNESP (Rosalen; Monteiro, 2021), or other specific to Agricultural Entomology.

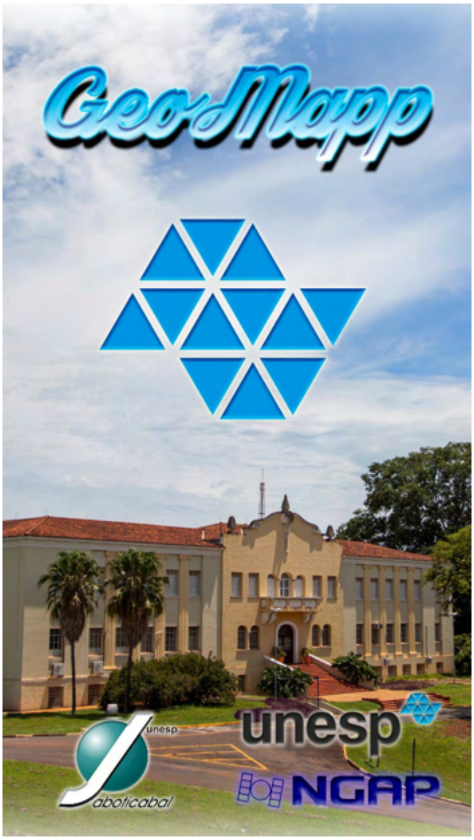

Figure 1 – GeoMapp splash screen (Source: Monteiro [2021]).

FAW Monitoring and Early Warning System (FAMEWS), for example, is aimed at sampling *Spodoptera frugiperda* (Lepidoptera: Noctuidae). This application was developed by FAO for Android system v.5 or higher and allows data collection on *S. frugiperda* at a local, national, and global levels, identifying priority areas and promoting early warning mechanisms for all interested parties. Another interesting app is OsBeeHives, which is aimed at inspecting beehives in beekeeping activities. This application, using the IoT concept, allows monitoring of hives remotely. Moreover, Cubero et al. (2020) used GNSS positioning as a field robot navigation feature intended for pest detection in horticulture.

3.2 Remote sensing

The American Society for Photogrammetry and Remote Sensing (ASPRS) defines Remote Sensing as “techniques used to collect and process object information without direct physical contact” (ASPRS, 2022, np).

Remote Sensing collects data at different levels on which platforms and their respective sensors are located. There are orbital levels used by satellites, aerial used by manned or unmanned aircraft, and terrestrial used by agricultural machinery and/or static platforms. The aerial level can be divided into aerial and low-altitude aerial, the latter being used by Remotely Piloted Aircraft (RPA), the so-called drones. The terrestrial level, also called Proximal, can be divided into terrestrial (field) and laboratory (bench).

Apart from data collection levels, there are sensors with different characteristics. Optical sensors, for example, are passive sensors and may have one or more spectral resolutions, which can record different spectral bands. Sensors with few non-continuous and relatively wide spectral bands (e.g., 4 to 10) are called multispectral. On the other hand, sensors with several bands (e.g., more than 40), narrow and continuous, are called hyperspectral sensors.

Active sensors, unlike optical sensors, have their energy; therefore, they do not require sunlight for data collection. Some active sensors record bands in the visible spectrum (Light Detection and Ranging - LIDAR) or the microwave spectrum (Radio Detecting and Ranging - RADAR). More details about the different sensor types used in Remote Sensing can be found in Barros et al. (2021).

Studies on the relationship of Low Altitude Aerophotogrammetry and Proximal Remote Sensing with Agricultural Entomology have increased significantly in recent years. For example, Pinto et al. (2020) studied Hyperspectral Proximal Remote Sensing and gas exchange parameters to characterize the responses of peanut (*Arachis hypogaea*) plant leaves to herbivory by *Stegasta bosqueella* (Lepidoptera: Gelechiidae) and *Spodoptera cosmioides* (Lepidoptera: Noctuidae), two main pests in this crop. The authors observed that the spectral range from 777.42 to 1,000 nm can be used to classify *S. bosqueella* and *S. cosmioides* lesions and that these pests should be considered individually in sampling programs for IPM purposes.

Concerning Remote Sensing with optical sensors, Feng et al. (2022) evaluated *S. frugiperda* infestation in corn (*Zea mays* L.) through image processing using Convolutional Neural Network (CNN), in particular: ResNeSt50, ResNet50, EfficientNet, and RegNet. Images were captured by an RGB sensor embedded in RPA. Results indicated the following hit rates: 98.77%, 97.59%, 97.89%, and 98.07% for evaluated networks, respectively.

An important point to be highlighted in optical sensor data processing is the use of different vegetation indices, with the Normalized Difference Vegetation Index (NDVI) being one of the best known and used. There are structural and biochemical indices; in Barros et al. (2021), a brief list of some of these indices can be found, as well as in Bagheri (2020).

In terms of active sensors, Song et al. (2020) used a continuous wave LIDAR system

(Continuous Wave - CW) to monitor insects and verified that insect abundance varies with time of day and weather conditions. They could also identify insect species, analyze wing beat frequencies, and ultraviolet light attraction. The authors used LIDAR to evaluate flying insect populations, as its sensors can be used on vegetation canopy rather than directly on insects to evaluate their behavior.

Most phytosanitary problems trigger plant defense mechanisms, changing and/or reducing vegetative growth, and hence decreasing biomass, leaf area index, or other canopy parameters. Accordingly, detection of canopy changes by active sensors can reveal the occurrence of plant-health problems such as pest infestation (Barros et al., 2021).

Undoubtedly, both passive and active sensors can be embedded in orbital, air, and ground platforms. In this sense, Iost Filho et al. (2020) reviewed the literature extensively on Low Altitude Aerial Photogrammetry applied to MIP.

3.3 Geographic Information Science

Geographic Information System (GIS) and Geographic Information Science (GISc) concepts must be distinguished. For ASPRS, GIS is "an information system capable of encoding, storing, transforming, analyzing, and displaying geospatial information" (ASPRS, 2022, np). On the other hand, GISc, as conceptualized by Prof. Michel F. Goodchild (Goodchild, 1992), is a set of fundamental research questions, which GIS can solve. Such issues can be enumerated as data capture, measurement, and storage; spatial analysis; spatial data modeling and theories; data structures; algorithms and processes; data display; analytical tools; in addition to ethical, institutional, and managerial issues. Despite being more related to Engineering issues than Science itself, Artificial Intelligence and Expert Systems could be added (Goodchild, 2010). Therefore, GIS, as a computational tool, answers the questions "what?" and "where?" are phenomena that occur in geographic space, whereas GISc will add the questions "how?" or "why?" they occur.

GISc has been widely applied in spatial distribution studies on pest infestations. For instance, Arends et al. (2022) characterized spatiotemporally injuries caused by *Helicoverpa zea* (Lepidoptera: Noctuidae) in corn (*Zea mays* L.), which is fundamental for Bt technology crops (transgenic plants).

4 I FINAL CONSIDERATIONS

Industry 4.0, Precision Agriculture, Smart Agriculture, and Digital Agriculture concepts shall be clearly defined. For Precision Agriculture, the concept established by ISPA is recommended, while for Digital Agriculture the FAO concept, both discussed in item

2 of this chapter.

Regarding item 3, in terms of GNSS positioning, the challenge of Digital Agriculture, in the context of Agricultural Entomology, is developing applications to map pest infestation, to be integrated into IPM programs. Another point is designing lightweight and versatile insect tracking systems integrated with GNSS technology. Lastly, integrating GNSS positioning with applications for pest monitoring through smart traps.

There is still a wide range of research to be conducted on Remote Sensing, from developing specific sensors for monitoring and early detection of pest infestations to specific research that could support such development. Furthermore, image processing techniques involving both evaluation and development of vegetation indices and Convolutional Neural Networks should be developed to detect injuries and infestations.

A challenge to be met in Geographic Information Science is its use in pest spatial distribution studies and respective mathematical modeling of pest behavioral dynamics under field situations. Finally, Machine and Deep Learning algorithms must be developed in R and Python. Furthermore, QGIS open-source plugins applied to Agricultural Entomology are a wide and fascinating field to be explored.

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CAPÍTULO 12

USE OF REMOTE SENSING TO IDENTIFY AND MANAGE NEMATODES IN SOYBEAN CROPS

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1 I INTRODUCTION

Brazil is an agricultural power worldwide, especially for its production of grains, biofuel, and livestock, among others. Of these, soybeans are the main crop in Brazilian agribusiness. Studies showed that, in the last 30 years, soybean production in Brazil has increased significantly due to the development of techniques and improvement of processes incorporated into the crops. This increase in soybean production has brought concern regarding the environment, soil, and water conservation, besides the need to increase productivity without expanding the cultivated areas. Therefore, farmers must take more precise and assertive measures.

In this scenario, precision agriculture plays a fundamental role in agricultural processes. This technology is related to a set of tools, including automation of most procedures, which reduces manual work in data collection, increases equipment operational efficiency, and promotes remote sensing use. The latter has helped monitor production areas through satellites and drones.

Satellite images are tools used in remote sensing to favor and facilitate the identification of crop anomalies, whether due to biotic or abiotic factors. Among these irregularities are soil compaction and fertility, plant phytosanitary conditions, and water stress occurring concurrently within the same area. Regarding phytosanitary problems, this resource is widely used to identify the presence of nematodes.

Nematodes have low mobility in the soil, and their economic damages may take a few crops to appear, showing late epidemiological evidence. Furthermore, these phytoparasites occur in hot spots, which characterizes one of their main symptoms. In addition, they commonly remain within the same areas throughout crop harvests, confirming their recurrence in crops (Otoboni, 2018).

The use of remote sensing through satellite images to identify anomalies has become more viable and accurate. And, because these images have geographic information, points of attention can be marked for a directed field inspection. Thus, soil and roots can be collected at specific points and, after examining samples, it is confirmed whether the main limiting factor in these areas is nematodes.

Plants have spectral properties that vary with their composition, morphology, and internal structure. These characteristics can be observed through remote sensing. Spectroradiometry allows observing these changes through sensor wavelengths, generally in the visible (RGB) and near-infrared region of the electromagnetic spectrum (Batista; Rudorff, 1990; Rudorff et al., 1997).

Reflected wavelengths are used to calculate vegetation indexes, through which vegetation covers can be evaluated and characterized as a function of plant developmental characteristics. The best index to estimate crop productivity and vegetation health is the Normalized Difference Vegetation Index - NDVI (Taylor et al., 1997). This index helps monitor crops and identify anomalies such as fertility problems, water deficit, pest attacks, and mainly the presence of nematodes. This index also allows dimensioning and georeferencing the problem with the area. Therefore, phytonematode management and control can be done locally, which means that inputs can be applied in the right place and the right amount, thus bringing environmental and economic benefits to farmers.

For that purpose, currently developed methods and techniques can provide such site-specific management and/or control, with emphasis on those for the application of chemical or biological products directly into soybean planting furrows (Otoboni, 2018). That said, many studies on Precision Agriculture have shown significant results, especially those on the use of vegetation indexes for diagnosis and management and/or control of nematodes in soybean crops. Throughout this chapter, methods and results will be presented, and how this technology contributes to diagnosis and guidance for the use of fundamental management practices to control nematodes.

2 I ORBITAL SENSORS

Remote sensing (RS) is defined as a set of techniques that aim to obtain information and data from objects without physical contact (Lillesand; Kieffer, 1994). The remote sensing data types consist of the information to be obtained, at the size and dynamics of the object/phenomenon of interest. These data are obtained through sensors that differ from each other according to functionality and capacity (spatial, spectral, and radiometric resolution).

Sensors collect information from different data acquisition levels such as orbital (through satellites), aerial (at high and low altitudes), and terrestrial (through ground measurements in the field and laboratory). The sensors can also be classified according to their energy sources, spectral regions, and energy transformation types.

Regarding energy sources, sensors are classified as active, when they produce their radiation (Ex: RADAR); and passive, when they depend on an external source, commonly solar radiation (Ex: Landsat - TM). As for the spectral region, they are divided into optical (when using mirrors, lenses, and prisms) and microwaves. Regarding energy transformation, they are divided into non-imagers (generate accurate and timely information in digit or graphic form) and imagers (represent the spatial variation of spectral response, i.e., generate images).

For orbital sensors to explore features of interest in an orbit, this must: be circular to ensure resolution of all images regardless of Earth region; allow cyclic surface imaging, ensuring revisits in the same places; be synchronous with the sun, that is, passing over any point on the earth's surface at the same time so that lighting is constant; be on satellite pass time.

Given the above, the satellites used in remote sensing should have these characteristics. These devices orbit from one pole to the other at a distance of about 800 km from the Earth's surface. They also can cover the entire planet after a certain period, due to the synchronicity of their speed with the Earth's rotation, which characterizes their temporal resolution. Furthermore, each sensor can discriminate objects according to their size at the level of detail, which is called spatial resolution (Soares et al., 2016).

3 I CHARACTERIZATION OF SYMPTOMS VIA REMOTE SENSING (RS)

The growing food production demand has aroused interest from technology-related sectors to develop tools to assist in the detection of pests, diseases, and nematodes in the most diverse production systems. This is because the diagnosis traditionally performed in extensive infested areas becomes expensive since a large number of samples and other complementary laboratory analyses are required. Therefore, using remote sensing (RS) to detect plant symptoms has been an efficient alternative. This method allows obtaining plant data in an indirect and non-destructive way (Lillesand & Kiefer, 1994; Shiratsuchi et al., 2014; Xue & Su, 2017; Liu & Wang, 2021), as the sensor will record the electromagnetic radiation emitted.

This tool allows identifying specific points at which crop yield is restricted. This way, potential pests/diseases/nematodes can be identified and managed. If compared to plants with disease symptoms, asymptomatic plants will show differences when placed in contact with electromagnetic radiation. Thus, the parameter used to obtain data is the electromagnetic wave intensity when reaching the sensor (Aggarwal & Dun, 2005; Gao et al., 2020).

Process stages must be well known for remote sensing to be successful. The process can occur in five stages: 1) electromagnetic radiation; 2) transmission of energy from generating source and later scattering; 3) reflection and emission (also known as interaction), in which part of the absorbed energy is transformed into heat with two main points, reflectance (ability to emit radiant energy) and absorbance (absorption of radiant energy); 4) transmission of information to remote sensor (transmittance, expressed in percentage or numbers between 0 and 1); and 5) sensor data output processing (Aggarwal & Dun 2005; Soares et al., 2016).

After RS operating stages, the so-called “spectral signature” or spectroradiometric data must be analyzed (Khaled et al., 2018). In addition to this, “Vegetation Indices (IV)” should also be mentioned, and these are obtained from values associated with reflectance. Thus, after measuring reflectance, one can observe that for the same analyzed object and/or condition, reflectance is altered according to the type of radiation.

In the case of nematodes for example, changes can be observed as leaf chlorophyll content reduces. Thus, when the plant is infected, reflectance changes are due to reduced levels of chlorophyll and its structure.

Several phytopathogens, including nematodes, are commonly observed in soybean crops. Among the nematode species found, *Heterodera glycines* and *Pratylenchus brachyurus* are the most frequently recorded. These species can be detected by a specific spectral band. In this sense, Arantes et al. (2021) detected the occurrence of *H. glycines* and *P. brachyurus* at the beginning of soybean flowering (R1) by spectral changes, in the city of Rio Verde during the 2017/2018 harvests. For the analysis, the authors performed flights with the Phantom 4 Advanced, Sequoia, and Sentera drones over five patches (Figure 1).

The red wavelength best explained variability in *H. glycines* data in the soil and roots, as well as in second instar juvenile data in the soil. Thus, at the beginning of flowering (R1), the spectral reflectance of soybeans allowed association with the number of *H. glycines* and *P. brachyurus* individuals in the soil and roots (Arantes et al., 2021).

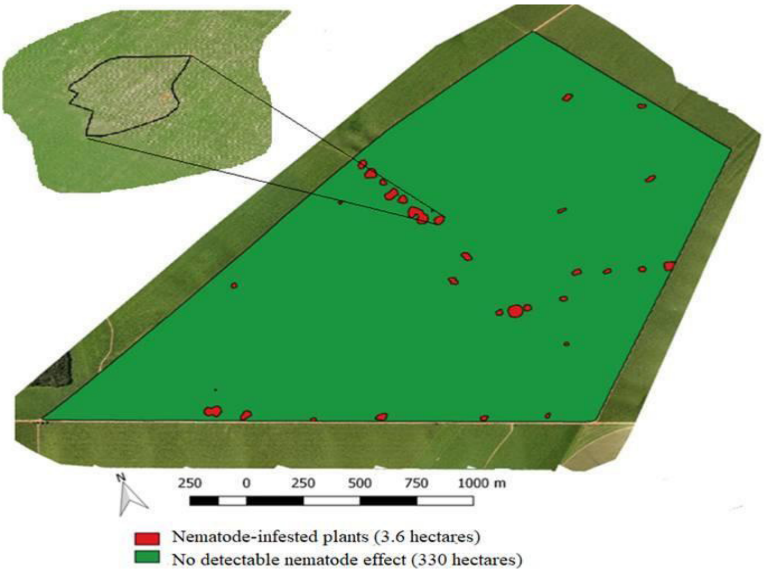

Figure 1 – Mapping of *Heterodera glycines* using a low-cost sensor (Phantom 4 Advanced).
Source: Arantes et al. (2021).

4 I FIELD INSPECTION

Once signs of stress are detected in crops through remote sensing techniques, or a drop in productivity is verified through harvest maps, a field inspection is necessary to validate potential causes of symptoms. Geostatistical maps and images can be used to define the most infested areas in the field and, consequently, generate maps for site-specific treatments for nematodes and/or applications at variable rates of nematicides (Soares et al., 2016).

Precision farming techniques allow directing field inspections and nematological sampling in regions suspected of nematode infestation. This is because stress zones are easily detected by remote sensing and delimited through georeferencing, thus allowing for optimizing sampling (Otoboni, 2014).

Soybean nematodes show distinct clinical signs, with distribution in hot spots or patches. Moreover, the nematode genus causing damage can be easily distinguished by simple visual observation of the crop root system. The visual verification of the presence or absence of nematodes in the field should be complemented by sampling and nematological analysis of soil and roots. With this, not only the genus is determined, but also the species, strain, and population levels allowing the elaboration of the most appropriate management strategy for each case (Soares et al., 2016; Dinardo-Miranda & Miranda, 2017).

In Brazil, the rainy season varies with its region since the country has continental dimensions. This time of the year, specifically during soybean flowering, is the most appropriate to perform sampling and inspection in the field. This is because nematode populations are in full development during that period, as there is a great availability of food (mainly radicles) and suitable environmental conditions (humidity and temperature), making them reach peak populations (Goulart, 2010; Galbieri et al., 2016).

Crops attacked by root-knot nematodes (*Meloidogyne* spp.) have clumps of poorly developed plants, with yellowish leaves, high pod abortion, and premature ripening (Grigolli & Asmus, 2014). Moreover, the root system shows deformations, with the appearance of a tumor/gall, which can vary in size and number with nematode population level, cultivar susceptibility, and soil fertility (Asmus, 2001; Soares & Nascimento, 2022).

Soybean cyst nematode females (*H. glycines*) can be seen on the external part of roots from the thirtieth day after sowing. They are initially white in color but become dark brown when dead and turned into a cyst. The distribution of this species is also in hot spots, wherein poorly developed plants with intense leaf chlorosis are observed. This is due to the difficulty of plants in absorbing water and nutrients, dying prematurely in severe cases (Tylka, 2012).

Likewise, poorly developed plants are also observed in hot spots caused by root-lesion nematodes (*Pratylenchus* spp.), but with no leaf chlorosis or yellowing. For this species, dark spots can be observed in isolated spots or the entire root system. Such a pattern is due to the migratory behavior of this endoparasite, penetrating and moving intensely in the root system. As a result, many cells are ruptured, expelling enzymatic toxins, which cause the death of cells that are later invaded by soil fungi and bacteria (Goulart, 2009; Grigolli & Asmus, 2014).

The reniform nematode (*Rotylenchulus reniformis*) is frequently found in cotton crops. This parasite has also caused damage to soybeans, mainly due to intense plant unevenness within stands, showing nutritional deficiency symptoms and/or soil compaction. Not only plant shoot, but also roots are underdeveloped, with small layers of soil adhered to egg masses on its surface (Dias et al, 2010; Grigolli & Asmus, 2014).

Hoes or straight shovels are recommended for nematode sampling, as the entire root system is sampled without major physical damage. Soybean roots and rhizosphere soil should be collected within the arable layer (0 – 20 cm), as it is the most fertile horizon with high rootlet concentrations (Norton & Niblack, 1991). Moreover, hot spots must be sampled individually, collecting as many plants as needed to obtain nearly 100 g of roots and 1 L of soil (Goulart, 2010; Silva & Machado, 2019).

All material collected must be deposited in a bucket and homogenized to form a composite sample for each hot spot. Then, this composite sample will be deposited in a plastic bag properly identified, with soil enveloping roots to prevent their exposure. Immediately after collection, the samples must be stored in a Styrofoam box until arriving at the laboratory to minimize sunlight and temperature degradation, and thus not negatively impact the analyses (Dinardo-Miranda & Miranda, 2017).

Sampling should be hot spot directed, not sampling the central part where the most weakened plants are found. These plants have limited root systems, which may directly impact nematode population analysis, not representing the reality (Silva &Machado, 2019). Therefore, symptomatic plants should be collected between the outer boundary of hot spots and halfway from their central point (Dinardo-Miranda; Miranda, 2017; Batista, 2018).

5 I PRODUCTIVITY AND NEMATODE MAPS

Composite soil analyses have been traditionally used on farms. In these, several points are sampled randomly and subsequently homogenized to obtain a single sample. This sample theoretically represents the entire area and its properties (Molin, 1997). Despite the vast use of composite analysis, soils have different properties, particularities, and infestations, varying according to the evaluated location (Schueller, 1992; Wieda & Borgelt, 1993). In this sense, productivity maps can help to improve the accuracy of results.

A productivity map is a precision agriculture tool and may represent different spatial and temporal variability sources. They are built using several points, i.e., a wide-ranging sampling. Thus, it can represent the crop area more faithfully, indicating potential problems therein (Riselo et al., 2020). Although it determines productivity spatial variability, it is unable to define its causes. Therefore, it must be combined with other resources, such as soil sampling. Together, they may indicate regions where a crop is under greater stress (Doege, 1999; Schemberger, et al., 2017). The most suitable precision agriculture technique is that where site-specific applications can be guided assertively, thus minimizing process costs (Molin, 1997).

Antonio et al. (2012) studied *P. brachyurus* population (individuals/ g roots), and soybean productivity and losses (sacks per hectare) in the city of Vera, Mato Grosso State - Brazil (12º08'25.67" and 55°11'42.71") during the 2011/2012 crop seasons (Figure 2). These authors observed that productivity is correlated with the root nematode population. In areas where the population was higher, productivity was lower, with an estimated loss of 21%.

Studies have highlighted the importance of productivity maps to identify and locate nematodes. As the presence of nematodes is harmful to crops, identifying their specific location can help accurate control, reducing costs.

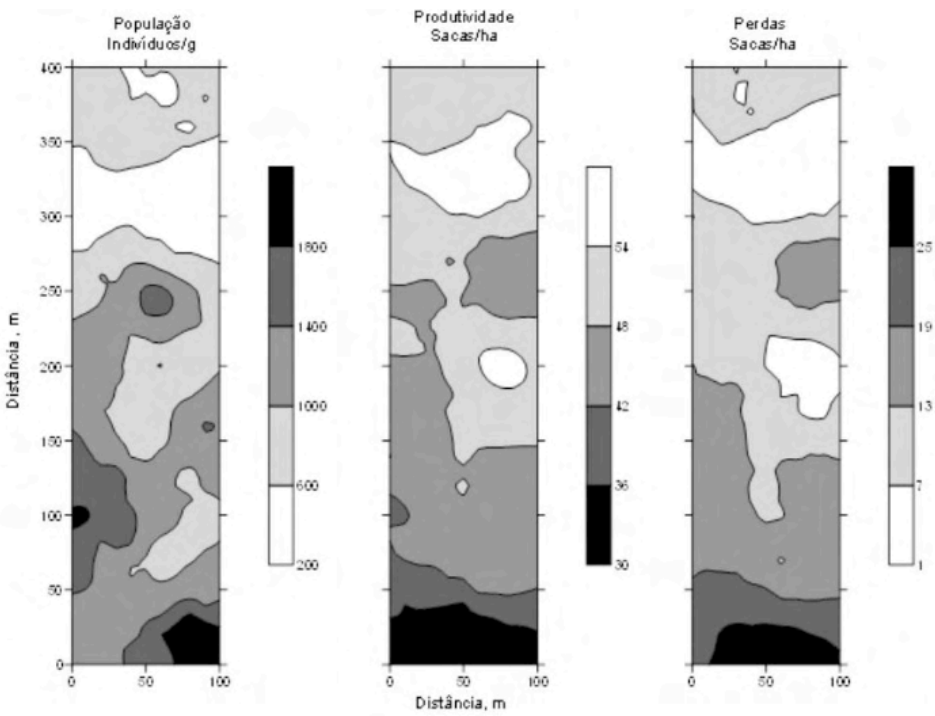

Figure 2 – Maps of root nematode population, estimated soybean yield and losses in the study area (Antonio et al., 2012).

6 I SITE-SPECIFIC MANAGEMENT AND APPLICATION OF CHEMICAL AND/ OR BIOLOGICAL PRODUCTS

The main indication of the presence of nematodes in the field is the occurrence of hot spots. Such a pattern results in an aggregated distribution in areas with greater water accumulation due to the unevenness of the stand. Due to its low mobility in the field, economic damages have late-type epidemiological evidence. This means that nematodes are found in hot spots located at the same sites for several years, at which their occurrence prevails in the field (Soares et al., 2016).

Nematode hot spots in crops can be detected and mapped by sets of remote sensing techniques. These processes are achieved by analyzing differences between reflectance from nematode-infested and healthy plants, due to physiological changes caused by nematodes. Therefore, site-specific operations can be conducted, such as application at a variable rate only in the areas of occurrence. The data obtained with the aid of a spectroradiometer express numerical values. These numbers are grouped into wavelength ranges to calculate vegetation indexes, relate variables, and explore spectral occupations of an area (Almeida et al. 2016).

The rational use of these technologies to monitor, analyze, and control spatial and temporal variations of factors limiting production can guide decision-making for site-specific applications and management of inputs, increasing economic and environmental gains (Jacinto; Miranda & Laskoski, 2020). Chemical control of nematodes over the total area significantly increases soybean production costs, given the increase in the amount of input used. On the other hand, site-specific control uses the necessary amount of nematicide, thereby reducing quantity, cost, and environmental impacts (Golhani et al., 2018). According to Soares (2016), input savings in site-specific applications can represent up to 90% when compared to applications in total area.

The description and mapping of hot spots within stands is the first step in spatial variability studies. Through these, the best method for the site-specific application of pesticides can be determined. Nematode hot spots can remain in the same place for several years due to the biological characteristics of each species. Thus, farmers must identify the species and determine its population level at each point. To do so, nematological analysis of soil and root samples is needed. Although applications in the total area have the same efficiency as those performed in specific points of heterogeneous areas, they have higher costs.

Nematode hot spots in crops can be delimited with the aid of the Global Navigation Satellite System (GNSS). Then, samples are collected on the contours of the hot spots due to potential expansions of nematodes within the areas, thus site-specific control and management maps can be designed (Otoboni, 2014). After mapping, site-specific applications of nematicides are carried out at the points set on the map. Equipment containing electronic controllers, onboard computer, and GPS receiver allows the spray to be applied automatically in a furrow opened by the seeder-planter. Through this technology, chemical and biological product, or even a mixture of both when compatible, can be applied without the need for conventional operations in machinery. With the help of a map programmed in an onboard computer, the deposit is only made in a demarcated area. After the operation, the onboard computer generates a new map representing the areas that have been treated.

Given the current site-specific management technologies and available methods of operation, nematode management is becoming increasingly efficient. Operations in large areas are still a challenge, but with the new technologies introduced in the market and studies focused on the theme, site-specific control of nematodes in large areas will soon reach efficiency.

7 I FINAL CONSIDERATIONS

The process traditionally used for diagnosing areas with the presence of nematodes and determining the severity levels in production areas is expensive. Furthermore, in some situations, such as large areas, it becomes unfeasible to perform it since a large number of samples is required.

In this context, precision agriculture can offer high efficiency and precision to diagnose the occurrence of nematodes, as well as identify areas requiring management practices for control by site-specific, partial, or total area applications. In recent years, there have been advances in equipment and sensors, with great potential for use in pesticide application, using classification models and detection of the occurrence of phytosanitary problems in production fields.

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CAPÍTULO 13

ENDOPHYTIC ENTOMOPATHOGENIC MICROORGANISMS IN PEST MANAGEMENT

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1 | INTRODUCTION

Endophytic microorganisms were first mentioned in the early 19th century, but only at the end of the 20th century they became more relevant in scientific research. Microorganisms were believed to be neutral, that is, cause no benefits or harm to plants. However, over the years the benefits from plant-microorganism interaction, have been discovered and, in many cases, playing an important role in plant protection and development (Azevedo et al., 2000; Santos & Varavallo, 2011).

Currently, endophytic microorganisms are widespread in academia and have been used to improve agriculture, such as: phosphate solubilization, plant nutrition, plant growth promotion, among other advantages that are being discovered every day. Among the advantages of plant-microorganism interaction, pest control with endophytic entomopathogenic microorganisms is a technology in increasing development.

Two entomopathogenic microorganisms most used endophytically in research are fungi and bacteria. Both can penetrate plant tissues and establish a symbiotic relationship. The activation of plant mechanisms when in contact with these microorganisms can increase tolerance to different biotic and abiotic stresses.

Bacteria and plants have beneficial relationships. Through endophytic interaction, bacteria help plant growth and development, in addition to acting as pest and disease control agents (Kloepper et al. 1992; Barka et al. 2002; Mcgee, 2002). Biological control by endophytic bacteria has been widely studied and appears as an alternative to excessive use of chemicals in cropping areas (Pozzebon & Santos, 2016).

Fungi are the most studied entomopathogenic microorganisms used endophytically. Their implications and uses, ranging from growth promotion to plant protection against pathogens, stem from their promising results in insect control studies. Entomopathogenic species such as *Beauveria bassiana*, *Metarhizium* spp., and *Isaria fumosorosea* have been reported to have high pathogenic potential in insects when inoculated into plants (Mantzoukas & Lagogiannis, 2019).

Most studies on entomopathogenic endophytic fungi have focused on chewing pests. However, research on sucking insect pests has gained notoriety, mainly with fungus of the species

B. bassiana. Tests with endophytic entomopathogenic fungi have already been carried out for aphids, mealybugs, leafhoppers, whiteflies, and bed bugs of the Pentatomidae family (Sword; Tessnow & Ek-Ramos, 2017; Jaber; Araj & Qasem, 2018; Rondot & Reineke, 2018; Gonzalez-Mas et al., 2019).

In this chapter, some topics related to endophytic entomopathogenic microorganisms used for pest control will be addressed. We will begin by discussing the two most widespread microorganisms in agriculture, entomopathogenic bacteria and fungi, and their endophytic applications. We will also argue about sucking insect control by endophytic entomopathogenic fungi. Finally, we will provide a brief overview of perspectives on the use of plant colonization by entomopathogenic fungi for insect pest control.

2 | ENDOPHYTIC ENTOMOPATHOGENIC BACTERIA

Bacteria are inhabitants of both internal and external tissues of most plants (Xie et al., 2020). To be endophytic, when colonizing internal plant tissues, they cannot have any negative effect on plants (Joo et al., 2021). An endophytic colonization provides a protective environment for microorganisms against ultraviolet radiation, scarcity of nutrients, excess rainfall, and extreme temperature fluctuations (Silva et al., 2006; Francis et al., 2010).

Likewise, several natural plant processes are improved by interactions with endophytic bacteria, inducing production of secondary metabolites, especially defense-related phytohormones such as jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) (Lazebnik et al., 2014), which are responsible for activation of acquired (ASR) and induced (ISR) systemic resistance pathways (Filho et al., 2010).

Bacteria of the *Enterobacter*, *Bacillus*, *Methylobacterium*, *Agrobacterium*, *Serratia*, *Acinetobacter*, *Arthrobacter*, and *Pseudomonas* genera are the most commonly microorganisms endophytically associated with plants. Baseline studies demonstrated *Bacillus thuringiensis* isolates colonizing cotton plant tissues (Monnerat et al., 2003). Praça et al. (2012) proved that *B. thuringiensis* isolates can act as endophytic organisms in cabbage plants, with a toxic effect against *Plutella xylostella* (Lepidoptera: Plutellidae). Macedo et al. (2012) selected and characterized native isolates of *B. thuringiensis* and observed that three of them caused mortality of above 75% in *Diatraea saccharalis* (Lepidoptera: Pyralidae).

In this sense, endophytic bacteria may act as biological control agents, protecting the host through synthesis of antimicrobial molecules and induction of ISR (Compant et al., 2010). These microorganisms can also produce toxins that play a role in insect control, becoming a great alternative for producers while reducing the use of agrochemicals, and hence environmental contamination (Pozzebon & Santos, 2016).

3 | ENDOPHYTIC ENTOMOPATHOGENIC FUNGI

Entomopathogenic fungi have been widely used as bioinsecticides, and a new line of research on endophytic species has been intensifying. These fungi can naturally colonize different host-plant tissues, including roots, stems, leaves, reproductive systems, and fruits (Baron & Rigobelo, 2022). Still, successful attempts to artificially introduce entomopathogenic fungi into plants have already been reported in several studies (Vega et al., 2008; Vega, 2018).

Application of entomopathogenic fungi via aerial conidia is advantageous compared to other methods. Among the benefits are the infection via insect integument, low risks of environmental imbalance and toxicity to humans and invertebrates, besides high capacity/reach of colonization in plants. Such advantages can be intensified when the fungi are endophytic. Examples of that include pest control by inducing systemic resistance and production of secondary metabolites, as well as promotion of plant growth by improving nutrition, producing siderophores and phytohormones, increasing photosynthetic efficiency, and relieving abiotic stresses (e.g., salinity).

4 | ENDOPHYTIC ENTOMOPATHOGENIC FUNGI FOR BIOLOGICAL CONTROL OF INSECT PESTS

Endophytic colonization of entomopathogenic fungi (Ascomycota, Hypocreales) can be beneficial to plants as it reduces pest infestation in economically important crops (Klieber & Reineke, 2016). Plant defense mechanism against insect and mite pests is not fully known yet. However, a few studies have reported systemic induction of resistance and production of secondary metabolites as causes of pest control by endophytic fungi.

Studies have shown that entomopathogenic fungi secrete chemical molecules into host plants soon after colonization. An initial plant response is to produce secondary metabolites, including alkaloids, flavonoids, and phenolic compounds to combat potential pathogenic threats (Zaynab et al., 2018).

After fungal infection, plant-secreted secondary metabolites are at first a barrier to colonization by endophytic entomopathogenic fungi. To overcome this obstacle, these fungi produce detoxification and degradation enzymes such as -1,3-glucanases, chitinases, laccases, cellulases, and amylases. Endophytes produce a variety of species-specific secondary metabolites of bioactive structure (e.g., phenolic acids, benzopyrones, quinones, and steroids) (Tan & Zou, 2001), which are widely used as agrochemicals, antibiotics, antiparasitic, and antioxidants.

Fungal secondary metabolites are also used for signaling, defense, or interacting with the host plant. They may also influence the host plant secondary metabolite profile and directly influence pathogen attack (Baron & Rigobelo, 2022). In this way, these fungi can improve the self-defense system of plants by activating systemic resistance pathways that protect them against pathogens and pests (Chadha et al., 2015). Endophytic colonization may have a priming effect, preparing plants for new infections by entomopathogenic microorganisms (Latz et al., 2018).

5 I ROLE OF ENDOPHYTIC ENTOMOPATHOGENIC FUNGI IN PLANT GROWTH PROMOTION

Endophytic entomopathogenic fungi have been used to improve plant growth (Akutse et al., 2013). According to the literature, plant growth is promoted due to the activation of mechanisms by endophytic entomopathogenic fungi, such as: improvement in plant nutrition, production of phytohormones, increase in photosynthetic efficiency, etc.

Endophytic entomopathogenic fungi can improve macronutrient or micronutrient uptakes from organic matter and increase their supply to host plants (Rana et al., 2020). For instance, Behie & Bidochka (2014) found that five *Metarhizium* species and *B. bassiana* can kill insect larvae in endophytically colonized plants, and transfer nitrogen from insects to plants. Numerous reports in the literature have highlighted an improvement in phosphorus uptake by fungal inoculation due to endophytic interactions (Ortega-García et al., 2015; Baron et al., 2018).

Although little explored, endophytic entomopathogenic fungi can also promote plant growth by stimulating phytohormone production. Rivas-Franco et al. (2020) verified corn colonised endophytically by *M. anisopliae* show increased levels of certain phytohormones. Among the most produced phytohormones are auxins (Aux), gibberellins (GAs), and cytokinins (CKs).

The main auxin produced by endophytic fungi is indole-3-acetic acid (IAA). It is a plant growth regulator and has several positive effects on shoot and root developments (Jaroszuk-Ściseł; Kurek & Trytek, 2014). Gibberellins are other hormones secreted by endophytic fungi that are essential for various plant processes, including seed germination, stem elongation, sexual expression, flowering, fruit formation, and senescence (Bömke & Tudzynski, 2009).

Endophytic entomopathogenic fungi have also been reported to influence plant photosynthetic rate. Moustaka, Meyling & Hauser (2021) noted that *Metarhizium brunneum* and *Beauveria bassiana*-inoculated plants tend to have higher photosynthetic rates than those non-inoculated, mainly after an insect pest attack. Photosynthetic rate is known to directly affect crop yields and determine plant growth and development.

6 I CONTROL OF SUCKING INSECTS BY ENDOPHYTIC ENTOMOPATHOGENIC FUNGI

Studies in the literature on endophytic entomopathogenic fungi, such as *Beauveria bassiana* and different *Metarhizium* species, are regularly used to control lepidopteran or coleopteran pests. Yet, new researches have been using them to control sucking insects, mainly due to a lack of biological products registered against these pests.

Aphids (Hemiptera, Aphididae) are the most studied sucking pests using endophytic entomopathogenic fungi. These insects directly affect plants by sap suction, damaging the final product. Indirect damages such as injection of toxins or acting as disease vectors are also observed; therefore, more punctual control methods are required, such as the use of pathogenic plants.

Aphis gossypii is an efficient vector of cucumber mosaic and cucurbit aphid-borne yellow viruses. Endophytic colonization of melon plants with *B. bassiana* promoted decreases in *A. gossypii* feeding and virus inoculation rates *(Gonzalez-Mas et al., 2019)*. Thus, in addition to controlling *A. gossypii* (Lopes et al., 2014) *B. bassiana* may reduce virus transmission by aphids and be used in integrated pest management (IPM) programs.

Although *B. bassiana* is the most tested entomopathogenic fungus for endophytic use, other species have been widely described in the literature. Mantzoukas & Lagogiannis (2019) reported mortality rates of green peach aphid (*Myzus persicae*) above 80% in sweet pepper (*Capsicum annum*) inoculated with *B. bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea*.

Rondot & Reineke (2018) evaluated inoculation of grapevines with *B. bassiana* and had promising results against two piercing-sucking insects; they observed infestation reductions for vine mealybug (*Planococcus ficus*) in semi-field tests and grape spittlebug (*Empoasca vitis*) in a field vineyard.

The use of endophytic entomopathogenic fungi has also shown to be efficient in controlling whitefly (*Bemisia tabaci*), especially when combined with other strategies. The combined application of these microorganisms and plant extracts, such as *Calotropis procera*, has an additive effect on mortality at almost all developmental stages of that insect (Jaber; Araj & Qasem, 2018).

Despite the agriculture damages caused by bed bugs of the Pentatomidae family, few are the reports in the literature concerning the use of endophytic entomopathogenic fungi to control such pests. However, this endophytic technology might be extremely efficient, as already observed by Sword, Tessnow & Ek-Ramos (2017). These authors noted the feeding preference of bed bugs for cotton plants not inoculated with *B. bassiana*. Behavioral responses indicated that insects were repelled before contacting with plant tissues of colonized-plants, highlighting the crucial role of volatile compounds in mediating negative responses (Sword; Tessnow & Ek-Ramos, 2017).

7 I PERSPECTIVES ON THE USE OF PLANTS COLONIZED BY ENDOPHYTIC ENTOMOPATHOGENIC MICROORGANISMS IN PEST CONTROL

Studies on the application of endophytic entomopathogenic agents in pest control have intensified in recent years. This increase in interest is mainly due to the positive results of research on the plant-host relationship for pathogen control. Besides the interest in the pathogenic potential of these endophytic microorganisms, several potential implications, such as plant growth promotion, have also raised lots of interest by researchers.

As discoveries advance, endophytic fungi and bacteria have become new biological resources. These microorganisms have great research value and broad development prospects, opening doors not only for pest control, but also for plant development and crop productivity improvement (Xie et al., 2020).

Throughout the text, some cases of use of this technique for pest control were reported; however, it is not yet used commercially on a large scale directly in agriculture. Despite the numerous findings about mechanisms of this interaction and reaction of plants to pathogen attacks, there is still much to be studied and revealed about this symbiotic relationship.

Modern procedures, such as genetic, physiological, and semiochemical techniques, among others, have allowed to better understand how such microorganisms can benefit plants, especially when attacked by pathogens. Accordingly, research on the use of these microorganisms in pest control tends to intensify increasingly. Furthermore, the search for answers on how the technique works and can be applied will create possibilities for the use of endophytic microorganisms on a large scale in agriculture, mainly due to current demands for more sustainability and reduction of agricultural inputs.

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CAPÍTULO 14

Bacillus thuringiensis CRY PESTICIDAL PROTEINS SUBLETHAL EFFECTS ON TARGET LEPIDOPTERA AND THEIR IMPACT ON THE AGROECOSYSTEM

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1 I INTRODUCTION

Farmers worldwide have preferred broad-spectrum pesticides for pest control due to their knock-out and long-term effects (Bel et al., 2017; Dudhbale et al., 2017). However, when misused, they may cause problems such as human diseases and/or intoxication and environmental pollution, besides affecting ecosystem population dynamics and services, and increasing selection for resistance in pest populations (Nicolopoulou-Stamati et al., 2016; Sanchez-Bayo & Wyckhuys, 2019; Sparks et al., 2020).

Microbial control agents, such as the entomopathogenic bacterium *Bacillus thuringiensis* (Bt) which are based on Cry pesticidal proteins, have been widely adopted against major defoliating pests in corn and cotton (Lacey et al., 2015; do Nascimento et al., 2022). Given their effectiveness, Bt biopesticides represent around 75% of the biopesticides market share, and they could grow even more in the coming years (Rao & Jurat-Fuentes, 2020).

Pathogen and host-insect interactions are evaluated in terms of pathogenicity and virulence. Pathogenicity is a qualitative concept in which a microorganism kills or not a host, whereas virulence varies with abiotic factors and characterizes its ability to cause disease. The former is applied to groups or species, and the latter is intended for within-group or species comparisons, i.e., quantitative evaluations (Shapiro-Ilan et al., 2005).

According to the above conceptions, a Bt strain can be pathogenic despite its low mortality (<80%) in screening assays, mainly against low susceptibility pests, such as *Spodoptera frugiperda* (Lepidoptera: Noctuidae). In such a situation, the strain is ruled out from selection to produce promising pesticides. Otherwise, most larval survival may provide evidence of a fitness cost, i.e., a "price has been paid" to allow larval survival, such as growth and pupation inhibition, and interference with adult emergence or even oviposition. These effects are named "sublethal effects" and are frequently reported in the literature (Asano et al., 1993; Babu et al., 2002; Li and Bouwer, 2012; Chauhan et al., 2017)

Sublethal effects are defined as biological, physiological, demographic, or behavioral effects on individuals or populations that survive exposure to a toxicant at a lethal, sublethal dose or concentration, or low doses (Desneux et al., 2007; Mohan et al., 2008; De França et al., 2017). A few resistance and susceptibility approaches have been used to understand those effects. In a remarkable paper, Aranda et al. (1996) stated that Cry-1 pesticidal protein binding to *S. frugiperda* epithelial membrane receptors may be reversible or irreversible. A reversible binding is when the toxin is dissociated from the receptor site, while an irreversible interaction shows no dissociation and results in insect death (Schnepf et al., 1998). Therefore, toxicity is a complex process in which binding is an essential but insufficient step to cause insect mortality, among which irreversible bindings can lead to Bt sublethal effects.

Furthermore, Castagnola & Jurat-Fuentes (2016) described that damage to insect digestive system by entomopathogens and their toxins activate a defensive response mediated by repeat and arylphorin genes. This response usually consists of epithelial regeneration, replacing diseased cells with newly differentiated midgut cells. This mechanism depends on the proliferation and differentiation of midgut stem cells and appears to allow insects to survive exposure to entomopathogens.

This chapter was proposed to review the sublethal effects of Bt Cry pesticidal proteins on different pest lepidopteran species, in addition to their effects on agroecosystems (target and non-target organisms). Laboratory tests under lethal concentration, low doses, or sublethal evidence, including life parameters and binding competition, were selected for cross-resistance considerations. The impacts of sublethal effects on agroecosystems based on ecological interactions and pest behavior were also discussed.

2 I SUBLETHAL CONCENTRATIONS AND EFFECTS ON LEPIDOPTERA SPECIES

Due to abiotic factors (UV, humidity, and temperature), Bt formulation applications may result in ingestion of sub-lethal doses of the biopesticide by a fraction of the pest population, promoting toxin tolerance and resistance in the long term (Chauhan et al., 2017). Several studies have been carried out on the sublethal effects of Cry proteins on lepidopteran pests of commodities (Table 1), with most of them assessing their interference with insect development. However, few studies have evaluated protein-binding mechanisms and resistance evolution-related aspects.

The species studied included the complex *Spodoptera* (*Spodoptera littoralis, S. litura, S. cosmioides*, *S. frugiperda*, and *S. eridania*), which has been reported as critical in soybean-, cotton-, and corn-producing regions in North America, South America, Asia, and Africa (Hosny et al., 1986; Cruz et al., 1999; Santos et al., 2010; Aguirre et al., 2016; Dudhbale et al., 2017).

Lepidopteran species	Cry protein	Concentration	Reported effects	Reference
Spodoptera frugiperda Noctuidae	Cry1Aa, Cry1Ab, Cry1Ac and Cry1B	> 2000 ng cm^{-2}	No strict correlation between binding and toxicity, non-toxic δ-endoproteins	Aranda et al., 1996
	Cry1Ca	10, 8, 6, 4, 2 and 1 mg cm^{-2}	Changes in defense and oxidative stress-related genes were transcriptionally enhanced, and metabolic-related genes were repressed	Rodríguez-Cabrera et al., 2008
Spodoptera littoralis Noctuidae	Cry1C	0.17, 2.40, 3.74, 5.39 and 5.46 $\mu g\ g^{-1}$	Protein was hydrolyzed faster in the resistant than in the susceptible strain	Moussa et al., 2020
Spodoptera eridania Noctuidae	Cry1Ac, Cry1Fa, and Cry2Aa	>10000, >3000 and 11 ng cm^{-2} (LC_{50})	Did not cause any mortality or growth inhibition, caused only growth inhibition and growth inhibition plus mortality	Rabelo et al., 2020a
Spodoptera cosmioides Noctuidae	Cry1Ac, Cry1Fa, and Cry2Aa	>10000, 853.4 and 1132.1 ng cm^{-2} (LC_{50})	Growth inhibition	Rabelo et al., 2020b
Helicoverpa armígera Noctuidae	Cry1Ac	0.071 µg ml^{-1} (LC_{25}) and 0.119 µg ml^{-1} (LC_{50})	Decreased fertility, increased malformed adults, fecundity, and fecundity period	Kannan and Uthamasamy, 2006
		2.5μg and 4$\mu g\ g^{-1}$	The growth rate of Knock out of HaREase gene was repressed significantly	Guan et al., 2019
Sesamia nonagrioides Noctuidae	Cry1Aa, Cry1Ab, Cry1Ac and Cry2	0.35 and 0.035mg kg^{-1}	Higher mortality, longer developmental time, extra molts, and higher sensitivity to critical daylength for diapause induction	Eizaguirre et al., 2005
Sesamia nonagrioides Noctuidae	Cry1Ab	0.35, 0.9, and 2 mg kg^{-1}	Higher levels of juvenile hormone, low level of ecdysteroids, consequently longer larval development, more larval molts, and pupation difficulty	Pérez-Hedo et al., 2011
Anticarsia gemmatalis Erebidae	Cry1Aa, Cry1Ab, Cry1Ac and Cry2	0.46 mg mL^{-1} (LC_{50})	Structural damage and death of the midgut epithelial cells of this insect	Castro et al., 2019
Ostrinia furnacalis Crambidae	Cry1Ac	0.05, 0.2, 0.8, 3.2, 12.8 µg g^{-1}	Larval growth and development delayed, pupation, pupal weight, and adult emergency also decreased	Ma et al., 2008
Chlosyne lacinia Crambidae	Cry1Ac	100 and 2.0 ng ml^{-1} (LC_{10})	F1 larvae had higher mortality and longer development time	Paula et al., 2014

Table 1. Sublethal effects on lepidopteran pests exposed to sublethal concentrations.

Transcriptional studies have been performed to identify midgut cell responses in Lepidoptera pests exposed to Bt proteins. Rodríguez-Cabrera et al. (2008) suggested that transcriptional profiles of midgut cells in Cry toxin poisoning should be early determined to better understand the biochemical and molecular aspects of insect detoxification. Another study by those authors provided the transcriptional responses of *S. frugiperda* third-instar larvae exposed to Cry1Ca sublethal concentrations (10, 8, 6, 4, 2,1, and 0 mg cm^{-2} diet), with sixteen genes being associated with a known biological process of *S. frugiperda*. The authors also found that defense (*serpin*-like) and oxidative stress-related (*catalase*-like) genes were transcriptionally up-regulated, while metabolic-related (*lipase 1*-like and *glycosyl hydrolase*-like) genes were down-regulated, in toxin-fed insects after 15 minutes of treatment. Serpins regulate insect innate immunity by inhibiting serine proteinase cascades, starting immune responses such as melanization and production of antimicrobial peptides (Meekins et al., 2018). Catalase is a robust antioxidant enzyme that breaks down toxic reactive oxygen species (ROS), which are also actively released to respond to bacterial attacks in insects (Molina-Cruz et al., 2008; Diaz-Albiter et al., 2011). In turn, glycosyl hydrolase is a carbohydrate-active enzyme (Cantarel et al., 2008), while lipase has a key role in insect lipid acquisition, storage, and mobilization (Santana et al., 2017).

Laboratory approaches using low Bt doses to assess sublethal effects have been increasingly comprehensive once the efficacy of pesticidal Cry protein is threatened by the possibility of pest resistance. Moussa et al. (2020) conducted a laboratory investigation to evaluate the resistance development in *S. littoralis* against Cry1C. Fourth-instar larvae were exposed to the protein for the subsequent twelve generations. The resistance ratio increased from one generation to another until it reached 32.12 folds in F12. The authors compared a resistant with a susceptible strain and reported that Cry1C protein was hydrolyzed faster in the resistant population; therefore, *S. littoralis* could develop resistance to Bt proteins while exposed to a diet mixed with the protein for subsequent generations. Such rapidity may have been due to the associated particles in the spore/crystal mixture, which may delay resistance development in cotton leafworm strains compared to the purified proteins used by Moussa et al. (2020).

Spodoptera eridania, known as southern armyworm, is a pest under expansion in cotton and soybean fields, recently found in the African continent (Goergen, 2018). This pest has lower susceptibility to Cry1Ac and Cry1F than to Cry2Aa. The highest Cry1Ac concentration (10000 ng cm^{-2}) did not cause mortality or growth inhibition, while the highest Cry1F concentration (3000 ng cm^{-2}) caused only growth inhibition. According to the authors, the higher growth inhibition and mortality rates in southern armyworm larvae exposed to Cry2Aa when compared to Cry1Ac and Cry1F support the hypothesis that the former does not share the same binding sites with the latter two proteins, which is critical for toxicity to armyworms (Rabelo et al., 2020a). These Bt proteins were evaluated on *S. cosmioides* (Rabelo et al., 2020b), which showed greater growth inhibition when exposed to Cry1Fa than to Cry1Ac and Cry2Aa. While Cry1Fa and Cry2Aa had similar toxicity, Cry1Ac was at least 11.7 times less toxic than Cry1Fa. Therefore, the effect of Cry protein on the insect organism depends on the species.

Helicoverpa armigera is a highly polyphagous pest (Riaz et al., 2021) and shows high susceptibility to the pesticidal Cry1Ac protein (Da Silva et al., 2018). For this bollworm species, Kannan and Uthamasamy (2006) related the sublethal effects of Cry1Ac protein to decreased fertility, as well as increased number of malformed adults, egg viability, and fecundity period when the species was exposed to low (LC_{25}) and medium (LC_{50}) lethal concentrations. Guan et al. (2019) reported that the same protein can increase HaREase gene knockout rates, which are significantly repressed in *H. armigera* second-instar larvae fed an artificial diet with Cry1Ac (2.5 or 4 mg g^{-1}). These authors also found that *HaREase* participates in the lepidopteran immune stress processes and affects cotton bollworm resistance to Bt. Such findings may provide a novel strategy to enhance the sensitivity of insects to Bt proteins by inhibiting immune-related genes.

Ostrinia furnacalis, an important corn pest in China, was evaluated by Ma et al. (2008) for growth, development, and mortality of its neonates and third-instar larvae after being fed a Cry1Ac diet. The protein reduced the pest growth and development, with its increased concentrations reducing larval development; still, third-instar larvae were eight times more tolerant to Cry1Ac than neonates. After being fed the diet for ten days, the larvae weight decreased significantly but their development time extended. Moreover, pupation rates and pupal weights decreased significantly.

Sesamia nonagrioides is the major corn pest in the Mediterranean Basin. In a study, newly hatched larvae (< 24 h old) were used to evaluate the effect of sublethal Bt concentrations (0.35 and 0.035 mg kg^{-1}) on larval development. All larval instars treated with the commercial Dipel DF formulation (Cry1Aa, Cry1Ab, Cry1Ac, and Cry2Aa) showed an increase in the number of days to pupate, regardless of the concentration, besides additional ecdysis (Eizaguirre et al., 2005). Another study (Pérez-Hedo et al., 2011) evaluated Cry1Ab for the same pest but evaluated the hormonal balance after larvae were fed from molting to pupation or death with a semiartificial diet with sublethal concentrations (0, 0.35, 0.9, and 2 mg/kg diet) of active Cry1Ab with trypsin. Surviving larvae showed higher levels of juvenile hormone (JH), but ecdysteroid levels did not increase enough for pupation, prolonging larval development and the number of molts. This response may be considered a defense mechanism that allows some larvae to survive protein ingestion (Pérez-Hedo et al., 2011).

Anticarsia gemmatalis, one important defoliating pest of soybeans in Brazil, has been recently evaluated for toxicity to *B. thuringiensis kurstaki* strain HD-1 (Castro et al., 2019). Median lethal concentrations (LC_{50} = 0.46 mg mL^{-1}) showed toxicity to *A. gemmatalis* fourth-instar larvae after 108 hours. Cytopathological changes mediated by Cry pesticidal proteins in the larval midgut cause cellular disorganization, microvillus degeneration, cell fragmentation and protrusion, peritrophic membrane rupture, and cell vacuolization. Cells also show a progressive increase in nuclei with condensed chromatin, and numerous lysosomes are found in the intestine of toxin-exposed insects. Moreover, apoptosis (a morphological pattern of programmed cell death) occurs in the midgut cells of larvae exposed to Bt (Castro et al., 2019).

Paula et al. (2014) reported that *C. lacinia* exposed to sublethal or low concentrations of Cry1Ac undergoes adverse effects during the first offspring generation (F1) such as higher mortality and longer development time when compared to the F1 larvae of parents that did not ingest Cry1Ac. In addition, this species can absorb the protein and transfer it to its eggs.

Souza et al. (2018) evaluated the resistance to Cry1F in non-aposematic larvae of *S. frugiperda* and the possibility of the species transferring the protein from a genetically engineered maize variety to its offspring. The authors reported that Cry1F was transferred to the offspring (1.47 ± 4.42 ng Cry1F.10 $eggs^{-1}$) in a toxin concentration of about 28 ± 83 times lower than that detected in Cry1F Bt maize leaves.

3 I BT SUBLETHAL EFFECTS ON AGROECOSYSTEMS

Although evaluating the sublethal effect of pesticidal Cry proteins under field conditions remains a challenge given the strong influence of abiotic and biotic factors, some laboratory approaches have allowed highlighting some aspects of sublethal effects in agroecosystems.

Some interesting insights have been found in sublethal assays with *A. gemmatalis*, *S. frugiperda*, *S. eridania*, *C. includens*, and *H. armigera* assessed for larval weight every two days, from 9 to 19 days after treatment. As a remarkable result, surviving larvae of both *Spodoptera* species (*S. frugiperda* and *S. eridania*) treated with Bt had weight gains of 300 and 500%, respectively, concerning control larvae. Such an improvement is due to insect defense mechanisms in which larvae increase their food ingestion to repair midgut damage as a strategy to survive Bt infection (Castagnola & Jurat-Fuentes, 2016).

Leob et al. (2001) observed that more mature cultured midgut cells are destroyed as Bt toxin titers increase. The authors also described a significant increase in the number of immune-positive cells in *Chloride virescens* (Lepidoptera: Noctuidae) larvae treated with two Bt strains. This fact indicates an upregulation of the synthesis of an MDF1 (Lepidopteran midgut differentiation factor), directing increased stem cell differentiation.

Under field conditions, the recovery of insects from Bt infection seems like a "nightmare" since surviving larvae would consume more leaves or stems than would healthy ones. This increase in feeding can increase yield losses and impair biological control adoption by farmers. To avoid such a situation, Bt spray conditions must be optimized such as volume, temperature, humidity, and larval age at spraying. Mass et al. (2021) emphasized that interactions among farmers, extension and private services, and scientists should be enhanced to establish an initiative-taking response to agriculture challenges.

The interactions between *B. thuringiensis* infecting larvae and important ecosystem services, such as predators, are another interesting issue. In this context, Santos et al. (2020) reported that Bt-infected larvae of *Corcyra cephalonica* and *Plutella xylostella* had no negative effect on the predator *Xylocoris sordidus*. Magalhães et al. (2020), in turn, highlighted that *Podisus nigrispinus* consumption of Bt-infected *P. xylostella* larvae increased as prey quality decreased, i.e., its predatory behavior was more aggressive to allow sufficient food intake for its development. On the other hand, Dibelle et al. (2013) said that *P. nigrispinus* had its phytophagy, reproductive capacity, and biological cycle affected by Bt treatment, but its predatory capacity against *P. xllostella* was not altered. Overall, predatory responses to infected prey seem to change according to prey density, Bt strain and concentration, and bioassay method.

In agroecosystems, insect parasitoids have been important natural enemies, with host quality being a determinant of their field performance. In this regard, Eerb et al. (2001) examined parasitoid-pathogen interactions to quantify the effects of Bt sublethal doses force-fed gypsy moths (*Lymantria dispar*) and determine whether Bt sublethal doses affect host acceptance and suitability of gypsy moths for the parasitoid *Compsilura concinnata*. The study showed that gypsy moths were minimally affected by sublethal Bt doses, with its fourth-instar larvae development time and male pupal mass being reduced. The authors also observed that non-infected hosts were preferentially attacked and super-parasitized by *C. concinnata*. In short, gypsy moth exposure to both sublethal Bt doses and parasitoid attack reduced parasitism rates and host larval survival. Parasitoids in super-parasitized and Bt-treated gypsy moths had shorter larval development times and reduced pupal masses than those in untreated larvae. In turn, parasitoids in larvae parasitized alone had larger pupal masses than those developing in super-parasitized ones. Timing of Bt infection relative to parasitism is a major factor in gypsy moth mortality but not in parasitoid potential fecundity. Indeed, the authors emphasized that combining parasitism and Bt treatment provided a synergistic effect on gypsy moth mortality and a shorter period than the use of parasitoids alone.

Guedes et al. (2017) pointed out that sublethal exposure is an important condition for shaping community stress through inadvertent selection, hormesis, hormetic priming, an induced shift in dominance, impairment of species interactions, and eventual pest outbreaks. The author emphasized that most research on insecticide-induced community stress conducted with terrestrial arthropods has focused on natural enemies of arthropod pest species, frequently even neglecting their associated host complex.

4 I FINAL CONSIDERATIONS

Evaluating the sublethal effects of Bt insecticidal proteins provides important insights to understand their relationships with host and non-target organisms. Thus, integrated pest management must take them into account in determining their efficiency and selectivity. Notably, such a research approach requires significant laboratory work effort due to the various evaluations performed over a lengthy period, which may include more than one generation of the target pest studied. Another important aspect to highlight is the complexity inherent to the fauna of agroecosystems, making these studies impossible under field conditions. These limitations, however, can be mitigated by mathematical model-based studies.

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Letícia Barbosa de Lacerda - PhD student in Agricultural Entomology at the Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, Jaboticabal. Master in Agronomy (2022) and Agricultural Engineer from the Federal University of Paraíba - Center for Agrarian Sciences, Campus II, Areia - PB (2020). She has experience in Agronomy, with emphasis on Agricultural Entomology, working mainly in Biological Pest Control. Url Lattes: http://lattes.cnpq.br/8129296567964231

LORENA TOZI BOMBONATO - Graduated in Animal Science from Universidade Estadual Paulista FCAV/UNESP (2017), finishing Agronomic Engineering also from Universidade Estadual Paulista FCAV/UNESP, with experience in the area of nematology. Currently developing a mandatory internship at the company Suzano – FuturaGene with experience in the area of Regulatory Affairs of the Eucalyptus crop. Url Lattes: http://lattes.cnpq.br/9967431700437398

MARCELLE BEZERRA SILVA - He holds a bachelor's degree in Biological Sciences from FCAV-Unesp, Jaboticabal-SP (2019). He carried out training to complement educational training from August 2015 to November 2015, under the guidance of Prof. Nilza Maria Martinelli, at FCAV-Unesp, Jaboticabal/SP. He was a fellow of the Unesp Student Support Program of the Dean of University Extension-Scholarship Academic Support and Extension I, from March 2016 to February 2017, under the guidance of Prof. Laura Satiko Okada Nakaghi. He performed an internship at the Laboratory of Applied Ecology (APECOLAB), Department of Agricultural Production Sciences at FCAV-Unesp, Jaboticabal / SP, from July 2017 to June 2018, under the guidance of Prof. Dr. Odair Aparecido Fernandes. He performed an internship at the then Department of Plant Health at FCAV-Unesp, Jaboticabal / SP, from September 2019 to December 2019, under the guidance of Prof. Dr. Sérgio Antonio De Bortoli, where he carried out the Completion of Course Work. She is currently a Master's student at the Postgraduate Course in Agronomy (Agricultural Entomology) at FCAV-Unesp, Jaboticabal / SP, under the guidance of Prof. Dr. Sergio Antonio De Bortoli. She works on the following topics: Entomology and Pest Control. Url Lattes: http://lattes.cnpq.br/1780807921252824

MARCELO DA COSTA FERREIRA - Teacher Visitor Senior at Univ. Lisbon - Instit. Superior of Agronomy (ISA-UL, 2021 - CAPES/PRINT, Proc. - 88887.571103/2020-00). Teacher Incumbent (2018) and Free Teacher (2010) by UNESP jaboticabal/SP. Postdoctoral internship at the Silsoe Spray Application Unit in the UK (2007-08; 2010-11). Agronomist Eng. (1996), Master (2000) and Doctor (2003) by UNESP of Jaboticabal. Coordinated the Agronomy Course at UNESP jaboticabal (2013-15). Member of the University Council of UNESP - Rectory (2014-15). Coordinator of the Center for Studies and Development of Application Technology - NEDTA - UNESP. Responsible for the Application Technology Area, in undergraduate and graduate disciplines. He was Head of the State and Plant Health of UNESP jaboticabal (2009-11); He was General Secretary of the Brazilian Association of Agricultural Engineering - SBEA (2009-11); is a member of the Association of Applied Biologists of the United Kingdom (since 2008); Speaker at several national and international scientific events; Coordinated the Work Program for completion of agronomy course at UNESP jaboticabal (2009-11); guides undergraduate and graduate students; Creator of events such as Brazilian Congress of Plant Health (CONBRAF) and Workshop on the use of Adjuvants in Phytosanitary Syrups. Advisor to FAPESP and scientific journals. Works in Plant Health Products Application Technology, from history and legislation to the technical aspects of research, development and use itself. Url Lattes: http://lattes.cnpq.br/3661533094675596

MÁRCIO APARECIDO DE MELO - Agronomist graduated from the Educational Institution Vale da Jurumirim Faculdade Eduvale de Avaré - SP. Emphasis on the subjects: Zoology, Agricultural Entomology, Pests of Cultivated Plants and Phytopathology. It is based on Taxonomy with ease of identifying some species of insects. It has affinity with Biological Control and natural enemies of the Order: Neuroptera, family: Chrysopidae and Hemerobiidae. He acted as Entomology Monitor in the identification of some species of the orders: Coleoptera, Diptera, Hymenoptera, Neuroptera and Hemiptera. Domain of AutoCAD 2D and 3D in the realization of floor plans and three-dimensional also performing other technical drawings. He participated in all Scientific Initiation Congresses (CONINCE) by Faculdade Eduvale presenting scientific works and was awarded with Scientific Merit at XIII CONINCE as the best oral presentation of a Simple Abstract. He also had work presented at the IX BRAZILIAN LATIN AMERICAN CONGRESS OF ENTOMOLOGY. Currently, Master's student at FCAV - Unesp, Campus de Jaboticabal at PPG in Agronomy (Agricultural Entomology). Url Lattes: http://lattes.cnpq.br/9540611382349340

MARIA THALIA LACERDA SIQUEIRA - Graduated in Agronomic Engineering from the Federal Rural University of the Amazon (UFRA). Internship by the Scientific Initiation Program PIVIC-UFRA (2018-2019), Scientific Initiation Scholarship PIBIC-EMBRAPA (2019-2021) by the Citrus Genetic Improvement Program (PMG citrus). Currently studying for a Master's degree in Vegetal

Production at the Universidade Estadual Paulista Júlio de Mesquita Filho. It develops research activities related to pesticide application technology, plant health, with emphasis on the following topics: citrus psyllid management and insects of agricultural importance. Url Lattes: http://lattes.cnpq.br/0287385437369577

MARINA GUIMARÃES BRUM DE CASTRO - Graduated in Agronomy from the Federal University of Viçosa (2019). During graduation she worked as an intern in the areas of molecular biology and entomology. She was a PIBIC/Fapemig fellow for one year (2017-2018). She worked with student representation at the Academic Center of Agronomy (2018-2019) in the position of people manager and at the Collegiate of Phytopathology (2018) in the position of student representative. She helped in the establishment and operation of the AGRO UFV Internship Center (2019), a student initiative supported by the course coordination that brought the job market closer to UFV agricultural science students. Url Lattes: http://lattes.cnpq.br/5742970506065459

MATHEUS CARDOSO DE CASTRO - Biologist from the Faculty of Engineering of the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP/FEIS), Ilha Solteira Campus. Master in Agronomy (Agricultural Entomology) from the Faculty of Agrarian and Veterinary Sciences of the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP/FCAV), Campus of Jaboticabal. Currently, he is a doctoral student at the Faculty of Agrarian and Veterinary Sciences of the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP/FCAV), Campus of Jaboticabal. He works in the field of Entomology, with an emphasis on Agricultural Entomology, working mainly on the following topics: Agricultural Acarology, Mite Management, Taxonomy, Integrated Pest Management and selectivity of phytosanitary products. Url Lattes: http://lattes.cnpq.br/5012039466504685

MATHEUS MOREIRA DANTAS PINTO - Agronomist graduated from the Federal Institute of Education, Science and Technology of Pará, where he worked as an intern at the Laboratory of Agricultural Zoology, gaining experience with Agricultural Entomology in research on the bioecology of fruit flies and their parasitoids. Master's degree from the Agronomy program (Agricultural Entomology) of the Universidade Estadual Paulista "Júlio de Mesquita Filho", FCAV/UNESP and Doctoral candidate from the same program and working at the Laboratory of Biology and Insect Breeding - LBCI, in which he develops laboratory and field work involving biology and breeding of insect pests and natural enemies, predators and parasitoids, with emphasis on the predator group of the Chrysopidae family. Url Lattes: http://lattes.cnpq.br/8341019790296616

MORGANA BAPTISTA GIMENES - Student of the Agronomic Engineering course at the Federal University of Triângulo Mineiro - UFTM. Currently a member of the Center for Study, Research and Extension in Plant Health - NEPEF, holding the position on the board as secretary of finance. She has experience in the area of nematology, entomology and phytopathology, working mainly with the following crops: sugarcane and cowpea. Url Lattes: http://lattes.cnpq.

br/6609577249562899

NICOLE DE PAULA SOUZA - Agronomic Engineer and Master's Student at the Postgraduate Program in Agronomy (Agricultural Entomology) at the São Paulo State University "Júlio de Mesquita Filho" Campus of Jaboticabal. Belonging to the Laboratory of Insect Biochemistry, coordinated by Prof. Dr. Guilherme Duarte Rossi, develops research in the area of digestive trypsin inhibitors. Url Lattes: http://lattes.cnpq.br/7596364660994217

NILZA MARIA MARTINELLI - Graduated in Agronomy (1975) - UNESP - Jaboticabal Campus, Master's (1979) and Doctorate (1985) in Entomology, from ESALQ/USP. He completed post-doctoral internships in 1987 and in 1994/1995 at the Muséum National d'Histoire Naturelle-Paris-France. She is Assistant Professor at the Department of Plant Health at FCAV / UNESP, responsible for the Basic Entomology course in the Undergraduate Course in Agronomy and Insect Morphology at the Graduate Program. She is the coordinator of the Laboratory of Hemiptera Biosystematics (LABHEM) at the Department of Plant Health at FCAV / UNESP. She works mainly in identification of cicada species (Hemiptera-Cicadoidea), bioecology of soil pests and bioecology and systematics of Hemiptera and Coleoptera. Url Lattes: http://lattes.cnpq.br/5338275205137898

ODAIR APARECIDO FERNANDES - Graduated in Agronomic Engineering from FCAV/UNESP, Jaboticabal, SP (1983), Master's in Biological Sciences - Entomology from FFCL/USP, Ribeirão Preto, SP (1987) and PhD in Entomology - University of Nebraska ? Lincoln, NE, USA (1995). He is currently a full professor at FCAV / UNESP, Jaboticabal, SP, where he is a professor in the undergraduate courses in Agronomic Engineering and Biological Sciences; professor-advisor of the postgraduate programs in Agronomy (Agricultural Entomology), FCAV/UNESP and Entomology, USP, Ribeirão Preto, SP; visiting professor, Department of Entomology, University of Nebraska -Lincoln, NE, USA. He has experience in the field of Agronomy, with an emphasis on Agricultural Entomology. The main areas of research involve Applied Insect Ecology with emphasis on understanding the natural factors of insect population regulation and insect-plant interactions with a view to improving biological control programs and integrated pest management in agroecosystems. Url Lattes: http://lattes.cnpq.br/1458288287757880

PEDRO GOMES PEIXOTO - Doctoral student of the Postgraduate Program in Agronomy: Agricultural Entomology at Unesp in Jaboticabal. Master in Environmental Sciences. Specialist in Agroecology. They have experience in the areas of Community Ecology and Entomology of agricultural, natural and urban areas. Url Lattes: http://lattes.cnpq.br/1543067014008672

PEDRO HENRIQUE URACH FERREIRA - Graduated in Agronomic Engineering from USP - ESALQ (2015), with two semesters at the University of Queensland, Australia. He holds a Master's in Plant & Soil Science with an emphasis in Application Technology from Mississippi State University, USA (2018). He is currently a doctoral student at the Graduate Program in

Agricultural Entomology, Department of Agricultural Production Sciences, UNESP, Jaboticabal and president of the Center for Study and Development in Application Technology, NEDTA. He works mainly on the topics of application technology and phytosanitary treatment. Url Lattes: http://lattes.cnpq.br/3267902817746109

PEDRO HENRIQUE VASQUES BOCALINI - He is currently working as a technical assistant in the Nematoide Command project, at FMC Química do Brasil. Graduated in Agronomic Engineering from FCAV/UNESP, with experience in nematology, soil and crop management, and precision agriculture. Url Lattes: http://lattes.cnpq.br/0974363755907809

PEDRO LUIZ MARTINS SOARES - Teacher, Doctor and nematologist at Unesp in Jaboticabal. Currently is Teacher Nematology Assistant (compulsory subject of the Agronomic Engineering course) and Agricultural Nematology (Postgraduate course in Agronomy, concentration area in Agricultural Entomology), at the aforementioned institution. He has experience and has worked with nematodes, in different cultures and for over 20 years. It works and works with the following topics: identification of nematode species of economic importance, genetic resistance, crop rotation, cover crops, biological control, chemical control, physical control and integrated management of nematodes. In addition to all this expertise, he has extensive experience in the field, visiting agricultural areas across the country. Url Lattes: http://lattes.cnpq.br/4772641951244235

SERGIO ANTONIO DE BORTOLI - Graduated in Agronomy from Universidade Estadual Paulista (UNESP), Jaboticabal, SP (1975). Degree in Law from the Faculty of Education São Luís (FESL), Jaboticabal, SP (2005). Master's degree in Entomology from the Luiz de Queiroz Higher School of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP (1979). PhD in Entomology from the Luiz de Queiroz Higher School of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP (1980). He is a Lecturer in Agricultural Pests at FCAV-Unesp, Jaboticabal/SP (1986). He participated in Post-Doctoral programs at "University of Illinois at Urbana-Champaign", Urbana-Champaign, IL, USA (1991-92), at "Oregon State University", Corvallis, OR, USA (1992 and 1996-98) , and at "The University of Tennessee, Knoxville, TN, USA (2020). He is currently a Collaborating Professor at the Faculty of Philosophy Sciences and Letters of Ribeirão Preto (FFCLRP), University of São Paulo (USP), Ribeirão Preto, SP, together to the postgraduate program in Entomology, and Professor (Since 1990) at the Faculty of Agrarian and Veterinary Sciences (FCAV), Universidade Estadual Paulista (UNESP), Jaboticabal, SP. Has experience in the field of Agricultural Entomology, with emphasis on pests agriculture, working mainly on the following topics: insect biology, insect breeding, insect nutrition and biological control aiming at integrated pest management. Url Lattes: http://lattes.cnpq.br/9277721969335158

SABRINA JUVENAL DE OLIVEIRA - Graduated in Agronomy from the Federal University of Ceará (2019). Working at PET Agronomia UFC, at the Laboratory of Applied Entomology and at

Embrapa Agroindústria Tropical. Has experience in Agronomy, with emphasis on Phytotechnics. Master in Agronomy (Agricultural Entomology) at UNESP in Jaboticabal and PhD student at the same Institution working in Insect Ecology. Url Lattes: http://lattes.cnpq.br/2363531526486028

SANDY SOUSA FONSÊCA - Graduated in Agronomic Engineering from the Federal University of Recôncavo da Bahia. She worked in the area of Agricultural Entomology, working at Embrapa Cassava and Fruticultura, with the main insect pest of the banana tree, Cosmopolites sordidus, where research was developed with damage analysis through image treatment and population fluctuation. He completed his master's degree at Universidade Estadual Paulista, working in the area of plant resistance to insects, working with insect resistance Spodoptera frugiperda to different bean genotypes. Currently studying for a doctorate at the same University, in the area of Insect Physiology. Url Lattes: http://lattes.cnpq.br/9626539447113103

THAYNARA ARANTES SOARES JUNQUEIRA - Graduated in Agronomic Engineering from the State University of Minas Gerais (UEMG). He was a UEMG-PAPq fellow (2018). He is currently a Master's student in Agronomy (Agricultural Entomology) from the State University of São Paulo Júlio de Mesquita Filho (Unesp -FCAV). He has experience in research through an undergraduate internship with Mineração Morro Verde, where he participated in environmental education activities, environmental compensation, production of native seedlings in a forest nursery since seed collection, definitive planting and maintenance. Volunteer of the Silver Stream Revitalization project in Pratápolis-MG. Url Lattes: http://lattes.cnpq.br/2157495741404865

THIAGO NASCIMENTO DE BARROS - Bachelor in Biological Sciences from the Catholic University Dom Bosco - UCDB (2017-2020). CNPq PIBIC fellow in the area of Biotechnology (UCDB - 2018/2019) and intern at Embrapa Gado de Corte in the area of Veterinary Entomology (2020). He is currently a Master's student in Agronomy (Agricultural Entomology), FCAV-Unesp, Jaboticabal/SP. Url Lattes: http://lattes.cnpq.br/2445622539932596

VINÍCIUS FERRAZ NASCIMENTO - Doctoral student in Agronomy (Agricultural Entomology) at FCAV-Unesp, Jaboticabal/SP. Master in Biodiversity and Nature Conservation from the Federal University of Juiz de Fora - UFJF (2021). Bachelor in Agronomic Engineering from the Federal Institute of Education, Science and Technology of the South of Minas - IFSULDEMINAS (2018). He has experience in the field of Agronomy, with emphasis on entomology, pest control, manufacture of compounds of botanical origin, organic agriculture, agroecology, coffee production and information technology. Url Lattes: http://lattes.cnpq.br/9140434849610951

YASMIM ESTEVES IZIDRO - Graduated in Biological Sciences - Licentiate modality, at the State University of Minas Gerais - UEMG, Passos-MG Campus (2021), has experience in research as a CNPq grantee (2018) as the author of a scientific initiation project in conjunction with INCT Hympar Southeast. She was a PAEX fellow (2019) with an extension project involving environment and art. Development of extension projects in the area of Botany and Ecology working with pollinating beetles, environmental preservation, use of natural resources in the production of bio-jewels. Master's student in Agricultural Entomology at the Universidade Estadual Paulista Júlio de Mesquita Filho (Unesp -FCAV). Url Lattes: http://lattes.cnpq.br/6354990263477827


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