Topics in Agricultural Entomology XIII

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Daniel Dalvan do Nascimento | Edimar Peterlini
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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.

Outros organizadores
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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
Telefone: +55 (42) 3323-5493
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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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CAPÍTULO 1

QUALITY CONTROL IN MASS REARING OF INSECTS

Matheus Moreira Dantas Pinto

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1 | 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 I 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.

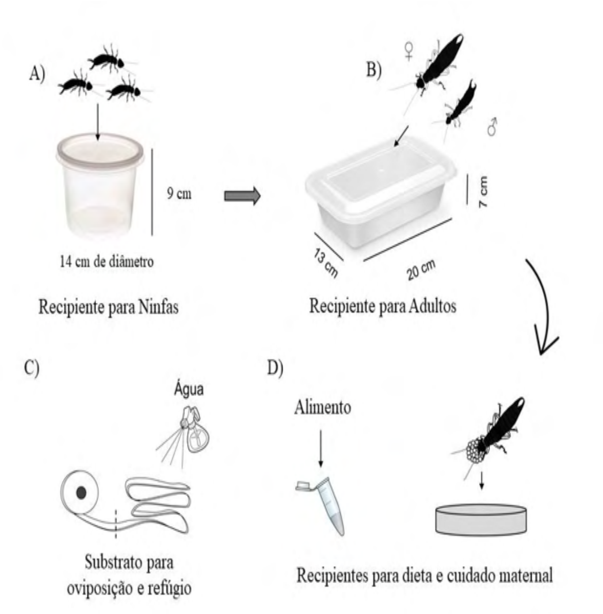

Figure 1 - Scheme of the rearing method for *Euborellia annulipes* under laboratory conditions. Containers used for rearing A) nymphs and B) adults; C) substrate for insect refuge and oviposition; and D) containers for providing food and maternal care (Source: Nunes, 2020).

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.

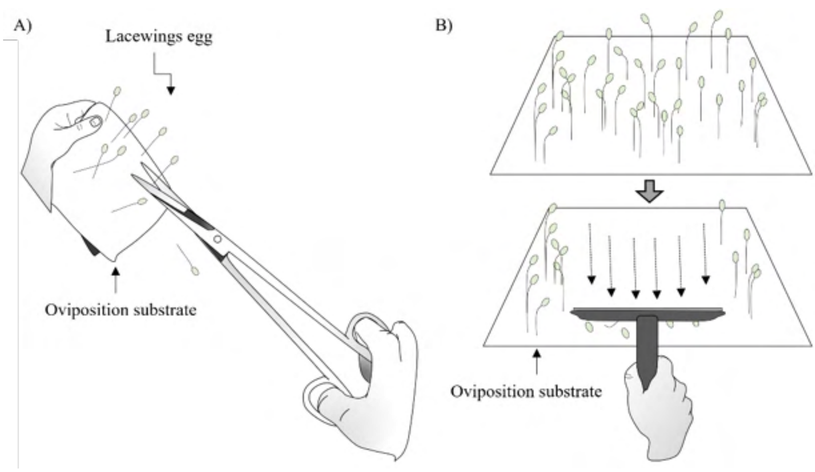

Figure 2 - A) Collection of eggs in voile fabric with the aid of scissors; B) Collection of eggs on bond paper with the aid of a knife blade.

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 I 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 I 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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