

TOPICS IN

AGRICULTURAL ENTOMOLOGY

XIII

JOACIR DO NASCIMENTO | CLAUDIANE MARTINS DA ROCHA
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.

Outros organizadores
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Érica Ayumi Taguti
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Ricardo Antonio Polanczyk

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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Atena
Editora
Ano 2022

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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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 <https://doi.org/10.22533/at.ed.4432201092>

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

Amanda Cristiane Queiroz Motta

Nayma Pinto Dias

Ricardo Antonio Polanczyk

1 | 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
	Cry1Aa, Cry1Ab, Cry1Ac and Cry1B	> 2000 ng cm ²	No strict correlation between binding and toxicity, non-toxic δ-endoproteins	Aranda et al., 1996
<i>Spodoptera frugiperda</i> Noctuidae	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
<i>Spodoptera littoralis</i> Noctuidae	Cry1C	0.17, 2.40, 3.74, 5.39 and 5.46 µg g ⁻¹	Protein was hydrolyzed faster in the resistant than in the susceptible strain	Moussa et al., 2020
<i>Spodoptera eridania</i> 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
<i>Spodoptera cosmioides</i> Noctuidae	Cry1Ac, Cry1Fa, and Cry2Aa	>10000, 853.4 and 1132.1 ng cm ² (LC ₅₀)	Growth inhibition	Rabelo et al., 2020b
<i>Helicoverpa armigera</i> Noctuidae	Cry1Ac	0.071 µg ml ⁻¹ (LC ₂₅) and 0.119 µg ml ⁻¹ (LC ₅₀) 2.5µg and 4µg g ⁻¹	Decreased fertility, increased malformed adults, fecundity, and fecundity period The growth rate of Knock out of HaREase gene was repressed significantly	Kannan and Uthamasamy, 2006 Guan et al., 2019
<i>Sesamia nonagrioides</i> 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
<i>Sesamia nonagrioides</i> 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
<i>Anticarsia gemmatilis</i> 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
<i>Ostrinia furnacalis</i> 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
<i>Chlosyne lacinia</i> 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 ($LC_{50} = 0.46 \text{ mg mL}^{-1}$) 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-aposomatic 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 ($1.47 \pm 4.42 \text{ ng Cry1F.10 eggs}^{-1}$) in a toxin concentration of about 28 ± 83 times lower than that detected in Cry1F Bt maize leaves.

3 | 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. xylostella* 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 | 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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ÉRICA AYUMI TAGUTI - Graduated in Biological Sciences (Licentiate and Bachelor's Degree) from the State University of Northern Paraná - Campus Luiz Meneghel (UENP/CLM), Bandeirantes, PR. Master's in Biological Sciences (Entomology) from the Federal University of Paraná (UFPR), in partnership with Embrapa Soja (Londrina PR). She is currently a PhD student in Agronomy (Agricultural Entomology) at the Universidade Estadual Paulista, Campus de Jaboticabal, (UNESP / FCAV), working on identification of sugarcane parasitoids. Url Lattes:

<http://lattes.cnpq.br/5817867834045082>

FABIANA SANTANA MACHADO - Bachelor degree in Agronomy from the State University of Maringá (UEM). Scientific Initiation Scholarship PIBIC-AF-IS (2016-2020), carrying out research with induced resistance of plants against pest attacks. Currently, she is a Master student from the Graduate Program in Agricultural Entomology at São Paulo State University Júlio de Mesquita Filho (UNESP/FCAV), working in the research field related to Microbial Pest Control. Laboratory of Microbial Control of Arthropod Pests (LCMAP) member. Url Lattes: <http://lattes.cnpq.br/4680129365297530>

GABRIELA LARA LEITE ALCALDE - Graduated in Agronomic Engineering from the University of Marília (2016), with a postgraduate degree in Georeferencing of Rural Properties from the Faculty of Technology of Piracicaba (FATEP) and currently studying Mechanization in Precision Agriculture at Fatec Pompeia "Shunji Nishimura. by the company CYGNI Agrociência and the Division of Agronomic Operations, both of the ORION Group. Member of the Improvement Committee of Orion Tecnologia e Sistemas Agrícolas, in the Research and Development area. Has experience in the area of Precision Agriculture, biological products and nematodes. Url Lattes: <http://lattes.cnpq.br/6988329838825984>

GABRIELA PELEGRINI - Agronomist from the Universidade Estadual Paulista "Júlio de Mesquita Filho" in Jaboticabal. Member of the research group of the Center for Studies in Genetics and Maize Improvement FCAV/UNESP (2015-2017) and of the research group on Silviculture FCAV/UNESP (2013 and 2014). Curricular internship working in the production of corn matrix seed and conducting the applications of Progiib 400 for the control of tillers in corn (2017). Agricultural exchange in the United States acting in the production process of vegetables in the greenhouse and field (2018). Url Lattes: <http://lattes.cnpq.br/1751759899207529>

GABRIEL GONÇALVES MONTEIRO - Graduated in Biological Sciences, Bachelor and Licentiate from the State University of Northern Paraná - UENP Campus Luiz Meneghel, Bandeirantes - Paraná, with an exchange period, one and a half years, at the University of Adelaide, Adelaide, Australia. Environmental Technician from ETEC Centro Paula Souza, Pedro D'Arcádia Neto, Assis - São Paulo. Master's Degree in Agronomy (Agricultural Entomology) from Universidade Estadual Paulista - UNESP, Campus of Jaboticabal, Jaboticabal - São Paulo. He is currently a Doctoral Student in Agronomy (Agricultural Entomology), UNESP, and Professor at the Adventist University Center of São Paulo - UNASP, São Paulo - São Paulo. He has experience in the areas of English speaking, environmental preservation and management in water resources and invertebrate zoology; with an emphasis on entomology. Url Lattes: <http://lattes.cnpq.br/6784040792180280>

GUILHERME DUARTE ROSSI - Graduated in Agronomy from the Federal University of Lavras (2005), Master in Agrochemistry from the Federal University of Lavras (2007) and Doctor in

Entomology from the Higher School of Agriculture "Luiz de Queiroz" (ESALQ/USP) (2012). He developed a postdoctoral project at the Department of Entomology and Acarology at ESALQ / USP and is currently Assistant Professor at the Department of Agricultural Production Sciences (Phytosanity Sector) at FCAV / UNESP-Jaboticabal. He works mainly on the following topics: lepidopteran digestion and host-parasitoid interaction. Url Lattes: <http://lattes.cnpq.br/2485806465712882>

IVANA LEMOS SOUZA - Graduated in Agronomy (UESC - Santa Cruz State University (BA), Master's and Doctorate in Entomology (UFLA - Federal University of Lavras). OR). Has experience in the area of biological control, nutrient analysis of the intestinal content of insects, identification of parasitoids, insect breeding, insect biology, olfactometry and volatile analysis, among others. State of São Paulo "Júlio de Mesquita Filho" (UNESP) at the Faculty of Agrarian and Veterinary Sciences and works with entomophagous agents, *Bacillus thuringiensis*, in pest control. Url Lattes: <http://lattes.cnpq.br/1344147899355434>

IWLIANNY LUIZA PEREIRA DOS SANTOS - Master's student in Agronomy (Agricultural Entomology) at The State University of São Paulo (Júlio de Mesquita Filho) - FCAV/Unesp, Jaboticabal, SP. Agronomist at The Associated Colleges of Uberaba (FAZU), Uberaba, MG (2020). He has experience in Agronomy, with emphasis on Agricultural Entomology, working mainly in insect breeding, insect biology and biological control. Url Lattes: <http://lattes.cnpq.br/4192209794428511>

JOÃO RAFAEL SILVA SOARES - Agronomist from the Federal University of Viçosa. Master's degree from the postgraduate program in Phytotechnics at the Federal University of Viçosa, with emphasis on Integrated Pest Management. He is currently a doctoral student at the graduate program in Agricultural Entomology at the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Jaboticabal campus. Activities developed in the areas of Integrated Pest Management, natural biological control, toxicology, insecticide selectivity, insect bioecology, insect-plant interaction and ecological niche modeling. He received a scientific initiation scholarship in the area of research on coffee pests through the partnership between FUNAPE / Embrapa Café and the UFV Integrated Pest Management laboratory. He participated in the Science Without Borders Program as a CAPES Scholar at Michigan State University from 2014 to 2015. Url Lattes: <http://lattes.cnpq.br/1783969206754489>

JOICE MENDONÇA DE SOUZA - Agronomist graduated from Faculdades Associadas de Uberaba - FAZU, in 2018. During two years of graduation (2016 and 2017) she was a scientific initiation scholarship, developing the course conclusion project in the line of research in Conservative Biological Control. She has a master's degree from the Postgraduate Program in Agronomy (Agricultural Entomology) at FCAV / Unesp, Jaboticabal / SP. She is currently a PhD student in the Postgraduate Program in Agronomy (Agricultural Entomology) at FCAV-Unesp, Jaboticabal / SP. Url Lattes: <http://lattes.cnpq.br/5723386032221415>

LANA LETICIA BARBOSA DE CARVALHO - Currently she is a doctoral student at Graduate Program in Agronomy - Agricultural Entomology at São Paulo State University Júlio de Mesquita Filho (UNESP/FCAV), Jaboticabal -SP Campus. Bachelor degree in Agronomy from the Federal Rural University of the Amazon - Parauapebas –PA Campus (2017). During the undergraduation she worked with Amazonian cultures entomofauna and she was a student mentor of Agricultural and Forestry Entomology disciplines. Master degree in Agronomy (Agricultural Entomology) from the Graduate Program in Agronomy (PGAgro) at Federal Rural University of Amazon Belém -PA Campus (2019). She was Master degree level scholarship holder in EMBRAPII (Brazilian Company for Research and Innovation) at the Laboratory of Pathology and Microbial Control at ESALQ/USP working with association of entomopathogenic fungi (*Beauveria* sp.) and soil fertilizers, *Metarhizium rileyi* cultivation, insects and mites creation, insects and mites bioassays and entomopathogenic fungi production in liquid and solid media. Url Lattes: <http://lattes.cnpq.br/3918313011391120>

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 (CONBRAEF) 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/5742970506065459>

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 Nematode 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 - Licenciature 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

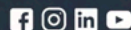
working with pollinating beetles, environmental preservation, use of natural resources in the production of bio-jewels. Master's student in Agricultural Entomology at the Universidade Estadual Paulista Júlio de Mesquita Filho (Unesp -FCAV). Url Lattes: <http://lattes.cnpq.br/6354990263477827>

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Os eventos de soja transgênica contidos nas variedades de sojas Enlist E3® e Conkesta E3® são desenvolvidos e pertencem conjuntamente à Corteva Agriscience e à M.S. Technologies L.L.C. Enlist® Colex-D[®] deve ser usado em dessecação da soja, em pré-plantio (aplique/plante) e em pós-emergência das sojas Enlist E3® e Conkesta E3®.

ATENÇÃO PRODUTO PERIGOSO À SAÚDE HUMANA, ANIMAL E AO MEIO AMBIENTE; USO AGRÍCOLA; VENDA SOB RECEITUÁRIO AGRONÔMICO; CONSULTE SEMPRE UM AGRÔNOMO; INFORME-SE E REALIZE O MANEJO INTEGRADO DE PRAGAS; DESCARTE CORRETAMENTE AS EMBALAGENS E OS RESTOS DOS PRODUTOS; LEIA ATENTAMENTE E SIGA AS INSTRUÇÕES CONTIDAS NO RÓTULO, NA BULA E NA RECEITA; E UTILIZE OS EQUIPAMENTOS DE PROTEÇÃO INDIVIDUAL.

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