University of São Paulo
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Compatibility of insecticides used to control *Bemisia tabaci* Biotype B (Hemiptera: Aleyrodidae) with the predatory mirid *Macrolophus basicornis* (Hemiptera: Miridae) in tomato

Thaís Fagundes Matioli

Thesis presented to obtain the degree of Doctor in Science. Area: Entomology

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Dedication

I dedicate this thesis to
Our Lord Jesus Christ, my Savior.

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"Do what you ought and concentrate on what you are doing".

Saint Josemaría Escrivá

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RESUMO

Compatibilidade de inseticidas utilizados no controle de *Bemisia tabaci* Biótipo B (Hemiptera: Aleyrodidae) com o mirídeo predador *Macrolophus basicornis* (Hemiptera: Miridae) em tomateiro

Mirídeos predadores foram identificados no Brasil para, possivelmente, serem utilizados no controle biológico aumentativo das principais pragas do tomateiro, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) e *Bemisia tabaci* (Gennadius) Biótipo B (Hemiptera: Aleyrodidae). Estudos ecotoxicológicos com a espécie *Macrolophus basicornis* (Stal) (Hemiptera: Miridae) têm sido realizados com produtos químicos usados para controle de *T. absoluta*. Entretanto, existe a necessidade de conhecer a toxicidade de inseticidas usados para o controle de *B. tabaci* sobre esta espécie predadora. Objetivou-se com o presente estudo, avaliar a compatibilidade dos principais inseticidas registrados para a mosca-branca na cultura do tomateiro com a espécie *M. basicornis*. Os inseticidas testados foram acetamiprido, bifentrina, buprofezina, ciantraniliprole, etofenproxi + acetamiprido, piriproxifeno + acetamiprido e espiromesifeno. Os testes foram feitos em condições de laboratório e semi-campo seguindo as metodologias da Organização Internacional para o Controle Biológico e Integrado de Animais e Plantas Nocivas (IOBC) e de outros métodos ecotoxicológicos para insetos benéficos. Com os resultados das primeiras análises toxicológicas realizadas em laboratório para avaliação da toxicidade aguda, os inseticidas foram separados em grupos de risco-reduzido e amplo-espectro. Buprofezina, ciantraniliprole e espiromesifeno são de risco-reduzido e foram testados quanto aos efeitos subletais em laboratório. Buprofezina e espiromesifeno causaram redução do tamanho de tíbias de descendentes da geração que entrou em contato com os resíduos inseticidas. Acetamiprido, bifentrina, etofenproxi + acetamiprido e piriproxifeno + acetamiprido são inseticidas de amplo-espectro. Em condições de semi-campo, foram testados quanto às suas persistências, efeitos no comportamento e também foi feita quantificação de resíduos por meio de cromatografia líquida (HPLC-UV). Quanto à persistência, bifentrina é vida curta (< 5 dias), etofenproxi + acetamiprido e piriproxifeno + acetamiprido são levemente persistentes (5 – 15 dias) e acetamiprido é um inseticida persistente (> 31 dias). Além disso, os resíduos de acetamiprido foram quantificados em 0, 5, 15 e 31 dias após a pulverização (DAP) por HPLC-UV. Os resultados obtidos foram de 30.80 mg i.a. L^{-1} (0 DAP), 29.97 mg i.a. L^{-1} (5 DAP), 21.56 mg i.a. L^{-1} (15 DAP) and 15.45 mg i.a. L^{-1} (31 DAP). Os estudos indicam que os insetos são afetados pelos inseticidas, exceto por ciantraniliprole. As informações do presente trabalho contribuírão para auxiliar que esta espécie de mirídeo seja utilizada para o controle de *B. tabaci* na cultura do tomateiro com as premissas do MIP.

Palavras-chave: Controle biológico, Ecotoxicologia, Inimigo natural, Inseticidas, Manejo integrado de pragas

ABSTRACT

Compatibility of insecticides used to control *Bemisia tabaci* Biotype B (Hemiptera: Aleyrodidae) with the predatory mirid *Macrolophus basicornis* (Hemiptera: Miridae) in tomato

Predatory mirids were identified in Brazil to possibly be used in the augmentative biological control of the main tomato pests, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Bemisia tabaci* (Gennadius) Biotype B (Hemiptera: Aleyrodidae). Ecotoxicological studies with the species *Macrolophus basicornis* (Stal) (Hemiptera: Miridae) have been carried out with chemical products used to control *T. absoluta*. However, there is a need to know the toxicity of insecticides used to control *B. tabaci* on this species. The objective of the present study was to evaluate the compatibility of the main insecticides registered for the whitefly in the tomato crop with the species *M. basicornis*. The insecticides tested were acetamiprid, bifenthrin, buprofezin, cyantraniliprole, etofenprox + acetamiprid, pyriproxyfen + acetamiprid and spiromesifen. Tests were carried out under laboratory and semi-field conditions following the methodologies of the International Organization for the Integrated Biological Control of Noxious Animals and Plants (IOBC) and other ecotoxicological methods for beneficial insects. With the results of the first toxicological analyzes carried out in the laboratory to assess acute toxicity, the insecticides were separated into broad-spectrum and reduced-risk groups. Buprofezin, cyantraniliprole and spiromesifen are low-risk and have been tested for sublethal effects in the laboratory. Buprofezin and spiromesifen caused a reduction in the size of tibias of descendants of the generation that came into contact with insecticide residues. Acetamiprid, bifenthrin, etofenprox + acetamiprid and pyriproxyfen + acetamiprid are broad-spectrum insecticides. Under semi-field conditions, they were tested for their persistence, effects on behavior and residues were also quantified by liquid chromatography (HPLC-UV). As for persistence, bifenthrin is short-lived (< 5 days), etofenprox + acetamiprid and pyriproxyfen + acetamiprid are slightly persistent (5 – 15 days) and acetamiprid is a persistent insecticide (> 31 days). Furthermore, acetamiprid residues were quantified at 0, 5, 15 and 31 days after spraying (DAS) by HPLC-UV. The results obtained were 30.80 mg a.i. L^{-1} (0 DAS), 29.97 mg a.i. L^{-1} (5 DAS), 21.56 mg a.i. L^{-1} (15 DAS) and 15.45 mg a.i. L^{-1} (31 DAS). Studies indicate that insects are affected by insecticides, except for cyantraniliprole. The information from the present work will contribute to help the implementation of this mirid species for the control of *B. tabaci* in the tomato crops with the MIP premises.

Keywords: Biological control, Ecotoxicology, Insecticides, Integrated pest management, Natural enemy

1. GENERAL INTRODUCTION

Predatory mirids are very effective controlling pests in agricultural crops (Calvo et al., 2012; Wheeler and Krimmel, 2015). In Europe, the species *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) are commercially used as augmentative biological control agents to reduce tomato pests (Van Lenteren, 2012). *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Bemisia tabaci* (Gennadius) Biotype B (Hemiptera: Aleyrodidae) are the main tomato crop pests causing phytosanitary problems all over the world (De Barro et al., 2011; Biondi et al., 2018). The success of the use with the Miridae family, aroused the interest of researchers in South America to search predators to control these pests.

The species *Macrolophus basicornis* (Stal), *Engytatus varians* (Distant) and *Campyloneuropsis infumatus* (Carvalho) (Hemiptera: Miridae) were found occurring naturally in the state of Minas Gerais, Brazil (Bueno et al., 2013). Since that, studies have been conducted to implement these predators as biological control agents in Integrated Pest Management (IPM) programs to reduce *T. absoluta* and *B. tabaci* in tomato crops (Silva et al., 2016; Van Lenteren et al., 2016; Van Lenteren et al., 2017; Passos et al., 2017, Passos et al., 2018; Silva et al., 2018; Soares et al., 2019; Van Lenteren et al., 2019). Their predatory capacity is similar to those species commercialized in Europe (Bueno et al., 2013), and they are attracted to the volatiles emitted by tomato plants when attacked by whitefly and tomato leafminer (Silva et al., 2018). In addition, the mirids are zoophytophagous, which make them able to maintain themselves in the culture by feeding on the contents of plants (Calvo et al., 2012; Lins et al., 2014). However, even with excellent biological characteristics, beneficial organisms can be affected by insecticides used for chemical pest control.

The risk assessment of insecticides used to control *T. absoluta* have been evaluated for *M. basicornis* (Wanumen et al., 2016; Passos et al., 2017; Passos et al., 2018; Soares et al., 2019). The registered products are from different chemical groups and their mode of actions varies between the active ingredients (MAPA, 2022). Under laboratory conditions, researchers assessed the acute toxicity and sublethal effects on the predator. The insecticides acting on the central nervous system of insects caused high mortality on the predator. Abamectin, chlorfenapyr, indoxacarb and imidacloprid caused acute toxicity to nymphs and adults and were considered harmfull to the species. Tests were also done under semi-field conditions for the harmful insecticides to access their persistence, which showed that indoxacarb was short life, abamectin and chlorfenapyr were slightly persistent, and imidacloprid was persistent. (Wanumen, et al., 2016; Passos et al., 2017). On the other hand, chemicals that did not cause death, as the growth regulators, induced sublethal effects by reducing body size, offspring number, and also caused behavior changes (Passos et al., 2018;

Soares et al., 2019). These studies help in decision making for the use of this natural enemy in the field. However, further tests should be carried out with products used to control *B. tabaci*, which has not been done so far for this species.

The phytophagy of *T. absoluta* is different from that of *B. tabaci*, due to this, there are variances between the insecticides registered for their controls (MAPA, 2022). Some insecticides used to control the whitefly are systemic, because it is sucking-sap insect (Biondi et al., 2018; Perring et al., 2018). This fact further contributes for testing the insecticides toxicity on the predator considering its zoophytophagy, which causes the insects to have greater forms and exposure to the products. Furthermore, *B. tabaci* causes direct and indirect damage to plants in addition to transmitting viruses (Jones, 2003), which increases the chances of using insecticides and the need to use other control methods, as biological control, in a sustainable way. Therefore, it is necessary to study the compatibility of the main products used to control the whitefly with the mirid *M. basicornis*.

The hypotheses of this study are that insecticides used to control *B. tabaci* cause toxicity to the predator and, when they do not cause death, they cause sublethal effects that impair predation capacity, by reducing its body size, offspring number and changing its behavior. We investigated the compatibility of seven insecticides with *M. basicornis* nymphs and adults under laboratory and semi field conditions seeking to contribute to the IPM in tomato. Similar to the works in the literature, we followed the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) methodologies (Hassan et al., 1998) and also used other methodologies used in ecotoxicological studies (Preetha et al., 2010; Passos et al., 2017; Morales et al., 2019).

The main objectives of this work were: (I) investigate the acute toxicity, median lethal concentration (LC_{50}), risk quotient (RQ) and survival time of the main insecticides used to control the whitefly in tomato fields in Brazil; (II) evaluate the sublethal effects of the harmless insecticides to the *M. basicornis* nymphs and adults; and (III) assess the persistence and toxicological effects of harmful insecticides on adults, their behavior effects and the residues quantification over time.

References

Biondi, A., Guedes, R.N.C., Wan, F.H., Desneux, N., 2018. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: past, present, and future. Ann. Review Entomol., 63, 239-258. https://doi.org/10.1146/annurev-ento-031616-034933

Bueno, V.H.P., Van Lenteren, J.C., Lins, J.C., Calixto, A.M., Montes, F.C., Silva, D.B., Santiago, L.D, Pérez, L.M., 2013. New records of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) predation by Brazilian Hemipteran predatory bugs. J. Appl. Entomology, 137, 29-34. https://doi.org/10.1111/jen.12017

Calvo, F.J., Lorente, M.J., Stansly, P.A., Belda, J.E., 2012. Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. Entom. Experim. Applic., 143, 111-119. https://doi.org/10.1111/j.1570-7458.2012.01238.x

De Barro, P.J., Liu, S.S., Boykin, L.M., Dinsdale, A.B., 2011. *Bemisia tabaci*: a statement of species status. Ann. Review Entomol., 56, 1-19. https://doi.org/10.1146/annurev-ento-112408-085504

Hassan, S.A., Hafes, B., Degrande, P.E., Herai, K., 1998. The side-effects of pesticides on the egg parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae), acute dose-response and persistence tests. J. Appl. Entomology, 122, 569-573. https://doi.org/10.1111/j.1439-0418.1998.tb01547.x

Jones, D. R., 2003. Plant viruses transmitted by whiteflies. European journal of plant pathology, 109, 195-219. https://doi.org/10.1023/A:1022846630513

Lins, J.C., van Loon, J.J., Bueno, V.H., Lucas-Barbosa, D., Dicke, M., van Lenteren, J.C., 2014. Response of the zoophytophagous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* to volatiles of uninfested plants and to plants infested by prey or conspecifics. BioControl, 59, 707-718. https://doi.org/10.1007/s10526-014-9602-y

MAPA, 2022. Ministério da Agricultura, Pecuária e Abastecimento, Brazil. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons, accessed on 10 March 2022.

Morales, S.I., Martínez, A.M., Figueroa, J.I., Campos-García, J., Gómez-Tagle, A., Lobit, P., Smagghe, G., Pineda, S., 2019. Foliar persistence and residual activity of four insecticides of different mode of action on the predator *Engytatus varians* (Hemiptera: Miridae). Chemosphere, 235, 76-83. https://doi.org/10.1016/j.chemosphere.2019.06.163

Passos, L.C., Soares, M.A., Collares, L.J., Malagoli, I., Desneux, N., Carvalho, G.A., 2018. Lethal, sublethal and transgenerational effects of insecticides on *Macrolophus basicornis*, predator of *Tuta absoluta*. Entomol. Gen. 38, 127–143. https://doi.org/10.1127/entomologia/2018/0744

Passos, L.C., Soares, M.A., Costa, M.A., Michaud, J.P., Freire, B.C., Carvalho, G.A., 2017. Physiological susceptibility of the predator *Macrolophus basicornis* (Hemiptera: Miridae) to pesticides used to control of *Tuta absoluta* (Lepidoptera: Gelechiidae). Biocontrol Sci. Technol. 27, 1082–1095. https://doi.org/10.1080/09583157.2017.1381879

Perring, T.M., Stansly, P.A., Liu, T.X., Smith, H.A., Andreason, S.A., 2018. Whiteflies: Biology, ecology, and management. In Sustainable management of arthropod pests of tomato, 73-110. Academic Press. https://doi.org/10.1016/B978-0-12-802441-6.00004-8

Preetha, G., Stanley, J., Suresh, S., Samiyappan, R., 2010. Risk assessment of insecticides used in rice on miridbug, *Cyrtorhinus lividipennis* Reuter, the important predator of brown planthopper, *Nilaparvata lugens* (Stal.). Chemosphere, 80, 498-503. https://doi.org/10.1016/j.chemosphere.2010.04.070

Silva, D.B., Bueno, V.H., Montes, F. C., Van Lenteren, J.C., 2016. Population growth of three mirid predatory bugs feeding on eggs and larvae of *Tuta absoluta* on tomato. BioControl, 61, 545-553. https://doi.org/10.1007/s10526-016-9736-1

Silva, D.B., Bueno, V.H., Van Loon, J.J., Peñaflor, M.F.G., Bento, J.M.S., Van Lenteren, J.C., 2018. Attraction of three mirid predators to tomato infested by both the tomato leaf mining moth *Tuta absoluta* and the whitefly *Bemisia tabaci*. Journal of chemical ecology, 44, 29-39. https://doi.org/10.1007/s10886-017-0909-x

Soares, M.A., Passos, L.C., Campos, M.R., Collares, L.J., Desneux, N., Carvalho, G.A., 2019. Side effects of insecticides commonly used against *Tuta absoluta* on the predator *Macrolophus basicornis*. J. Pest Sci. 92, 1447–1456. https://doi.org/10.1007/s10340-019-01099-4

Van Lenteren, J.C., 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl, 57, 1-20. https://doi.org/10.1007/s10526-011-9395-1

Van Lenteren, J.C., Bueno, V.H., Smit, J., Soares, M.A., Calixto, A.M., Montes, F.C., De Jong, P., 2017. Predation of *Tuta absoluta* eggs during the nymphal stages of three Neotropical mirid predators on tomato. Bulletin of Insectology, 70, 69-74.

Van Lenteren, J.C., Bueno, V.H.P., Burgio, G., Lanzoni, A., Montes, F.C., Silva, D.B., Jong, P.W., Hemerik, L., 2019. Pest kill rate as aggregate evaluation criterion to rank biological control agents: a case study with Neotropical predators of *Tuta absoluta* on tomato. Bull. Entomol. Research, 109, 812-820. https://doi.org/10.1017/S0007485319000130

Van Lenteren, J.C., Hemerik, L., Lins, J.C., Bueno, V.H., 2016. Functional responses of three Neotropical mirid predators to eggs of *Tuta absoluta* on tomato. Insects, 7, 34. https://doi.org/10.3390/insects7030034

Wanumen, A.C., Carvalho, G.A., Medina, P., Vinuela, E., Adán, Á., 2016. Residual acute toxicity of some modern insecticides toward two mirid predators of tomato pests. J. Econ. Entomology, 109, 1079-1085. https://doi.org/10.1093/jee/tow059

Wheeler, A.G., Krimmel, B.A., 2015. Mirid (Hemiptera: Heteroptera) specialists of sticky plants: adaptations, interactions, and ecological implications. Ann. Review Entomol. 60, 393-414. https://doi.org/10.1146/annurev-ento-010814-020932

2. RISK ASSESSMENT OF INSECTICIDES USED IN TOMATO TO CONTROL WHITEFLY ON THE PREDATOR *Macrolophus basicornis* (HEMIPTERA: MIRIDAE)

Abstract

The generalist mirid predator *Macrolophus basicornis* may contribute to Integrated Pest Management (IPM) of *Bemisia tabaci* in tomato crops. It is important to know the compatibility of the chemicals used to control this pest with this promising biological control agent. Seven insecticides were tested to investigate their toxicity to the predator. For four of the products, the LC_{50} for adults were determined. Buprofezin, cyantraniliprole and spiromesifen did not cause lethality and were classified as harmless. Acetamiprid, bifenthrin, etofenprox + acetamiprid and pyriproxyfen + acetamiprid caused acute toxicity and were classified as harmful. LT_{50} for all harmful insecticides were relatively low, ranging from 1.8 to 3.2 days. Moreover, these four insecticides have low LC_{50}, with acetamiprid (0.26 mg a.i. L^{-1}) as the lowest, followed by bifenthrin (0.38 mg a.i. L^{-1}), etofenprox + acetamiprid (4.80 mg a.i. L^{-1}) and pyriproxyfen + acetamiprid (8.71 mg a.i. L^{-1}). However, the calculated risk quotient (RQ) values demonstrated that these insecticides were mostly ecologically safe for this predator, except for acetamiprid, classified as slightly to moderately toxic. The present study can contribute to the use of *M. basicornis* as a biological control agent on tomato crops and to compatible use with the insecticides tested, according to IPM strategies.

Keywords: biological control; ecotoxicology; integrated pest management; natural enemy.

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2.1. Introduction

The family Miridae contains a significant number of predator species used in augmentative biological control in tomato crops [1, 2]. The genus *Macrolophus* has been used in Europe to control *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Bemisia tabaci* (Gennadius) Biotype B (Hemiptera: Aleyrodidae), the main tomato pests [2]. In Brazil, *Macrolophus basicornis* (Stal) (Hemiptera: Miridae) has a considerable potential to be reared in biofactories and released in the field to control the whitefly *B. tabaci* [3–9]. Some studies have shown that this natural enemy can easily establish in the field because of its zoophytophagy, a trait that aids it to remain where it is released without the presence of prey, since it can consume the sap from the crop [7, 10–12]. Despite the benefits of using this natural enemy, *B. tabaci* is controlled by different insecticides, due to its direct and in-direct damage on tomato [13,14], which may harm this possible new biological control agent.

It is important to understand the acute toxicity of insecticides used in pest control and the ecological risks to natural enemies prior to introducing a new biological control agent in any crop [15, 16]. Hence, the development of new strategies in integrated pest management

(IPM) programs involves compatibility studies of tactics, especially chemical and biological control [17]. Recent studies have demonstrated the effects of chemical products used to control *T. absoluta* on the predator *M. basicornis* [18–21]. Studies are needed to assess the toxic effects on *M. basicornis* using chemical products against *B. tabaci*.

Both the whitefly and the mirid predator have sucking mouthparts that introduce the stylets into the tissues [4, 14]. Many insecticides used to control *B. tabaci* are systemic and can affect the mirid, which feeds on plant tissue as a source of water and nutrients. The insecticides function by contact exposure to reach the pest nymphs and adults that remain on the leaves and residues may harm *M. basicornis* individuals on treated surfaces. Chemical groups have different modes of action on pests. Many are broad-spectrum and can kill a wide range of many natural enemies [22, 23], including *M. basicornis*. Other, reduced-risk insecticides are more selective to the predators and cause no or low lethal effect [18, 20, 24].

The first step of risk assessment is to determine the acute toxicity of commonly used insecticides to natural enemies in a laboratory bioassay, using the recommended field rates. One way to classify the chemicals is according to the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC), evaluating their lethal effect on the target species to determine the physiological selectivity [25]. Another important classification is the risk quotient (RQ), which classifies the chemicals that will be ecologically selective, related to the ways that the insects are exposed in the field [26, 27]. This evaluation can help to determine the possible risks of pesticides to natural enemies in the field, quantifying the concentrations for parasitoids and predators, as estimated for *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) [28], three *Trichogramma* species (Hymenoptera: Trichogrammatidae) [27,29] and the mirid *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae) [26, 30].

The present study was conducted to assess the acute toxicity and RQ of seven insecticides commonly used to control *B. tabaci* in tomato crops on the mirid predator *M. basicornis*. We investigated the acute toxicity and median lethal concentration (LC_{50}) of the insecticides that are currently most often used (acetamiprid, bifenthrin, buprofezin, cyantraniliprole, etofenprox + acetamiprid, pyriproxyfen + acetamiprid and spiromesifen). We hypothesized that it is possible to find compatible insecticides with the predator to enable it to be used as a biological control agent, adding to the IPM tactics.

2.2. Materials and Methods

2.2.1. Insects

Individuals of *M. basicornis* were obtained from the established rearing colony, more than eight generations old, at the Laboratory of Insect Biology in the Entomology and Acarology Department, "Luiz de Queiroz" College of Agriculture (ESALQ/USP), Piracicaba, Brazil. The insects were originally collected in the state of Minas Gerais, Brazil (21°08.596′S and 045°03.466′W, 808 m altitude) in tobacco (*Nicotiana tabacum* L.) fields. The method used was proposed by Bueno et al. [4], in which adults and nymphs were kept on tobacco plants in acrylic cages (60 × 30 × 30 cm) and fed with eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) offered ad libitum. The cages were kept in a climate-controlled room at 25 ± 2 °C, 70 ± 10% RH and 12:12 h (L:D). Before the experiments, adults were kept in cages with tobacco plants for oviposition. After 48 h, the plants were moved to an insect-free cage. This made it possible to obtain predators with the same age, either third-instar nymphs or adults (<3 days old) from the plants [20].

2.2.2. Insecticides

The commercial insecticides are registered for the control of *B. tabaci* in Brazilian tomato crops and were tested on *M. basicornis* at the highest recommended field doses (Table 1).

Table 1. Active ingredient, trade name, chemical group, exposure route, mode of action and field application rate of the principal insecticides used to control *Bemisia tabaci* in tomato crops in Brazil.

Active ingredient	Trade Name	Chemical Group	Exposure Route	Mode of Action	Field Rate (g or mL 100 L^{-1})		Field Rate (g a.i. ha^{-1})
					a.i.	c.p.	
Acetamiprid	Mospilan WG	Neonicotinoid	Systemic	Competitive modulator of nicotinic acetylcholine receptors	21.8	30	87
Bifenthrin	Seizer® 10 EC	Pyrethroid	Contact and ingestion	Sodium channel modulator	1.5	15	15
Buprofezin	Applaud® 25 WP	Thiadiazinone	Contact	Chitin synthesis inhibitors	50	200	500
Cyantraniliprole	Benevia® 10 OD	Diamide	Systemic and contact	Ryanodine receptor modulator	12.5	125	50
Etofenprox + acetamiprid	Eleitto® 30 + 16,7 OD	Pyrethroid + Neonicotinoid	Systemic and contact	Sodium channel modulator + competitive modulator of nicotinic acetylcholine receptors	12 + 6.8	40	120 + 66.8
Pyriproxyfen + acetamiprid	Privilege® 10 + 20 OD	Pyridyloxypropyl ether + Neonicotinoid	Contact, ingestion, translaminar and systemic	Juvenile hormone mimics + Nicotinic acetylcholine receptor (NACHR) competitive modulators	3 + 6	30	30 + 60
Spiromesifen	Oberon® 24 SC	Cetoenol	Contact and ingestion	Inhibitors of acetyl CoA carboxylase	14.4	60	144

EC (Emulsifiable Concentrate); OD (Oil Dispersion); SC (Suspension Concentrate); WG (Water-dispersible Granules); WP (Wettable Powder); c.p. (commercial product); a.i. (active ingredient).

2.2.3. Insecticide exposure for testing acute toxicity

Five-week-old tomato plants (cv. Santa Clara) grown in greenhouse conditions were sprayed with each of the insecticides listed in Table 1, using a hand-held sprayer (Light Sprayer—Breeze, 500 mL capacity; Guarany) until the run-off point (~50 mL per plant). Distilled water was used as the control treatment [31].

After drying for 2 h, the leaves were collected from treated plants and transferred to the laboratory. Each leaf had its petiole inserted into a flask (20 mL) filled with water to maintain turgidity during the bioassay and provided a lid with an opening for the petiole (Supplementary Material Figure S1). Each tube was transferred to a cage (12 cm high × 5 cm diameter) (PET crystal, 500 mL; Copozan), with each unit representing one repetition. In each cage, 15 adults of *M. basicornis* (<3 days old) or 15 third-instar nymphs were released and the cage was covered with voile fabric to prevent accumulation of toxic gases and retain the insects. *M. basicornis* individuals were fasted for 24 h before the beginning of the experiments to ensure that they started to feed as soon as they came into contact with the insecticide residues. As an alternative food source for *M. basicornis*, *E. kuehniella* eggs (0.4 g) were offered per cage. The design was randomized with 6 replicates per treatment.

M. basicornis were left on the treated leaves for 72 h under controlled room conditions (25 ± 2 °C, 70 ± 10% RH and 12:12 h L: D). After this period, untreated leaves were provided to assess the survival rate and median lethal time (LT_{50}). The insects' survival was assessed every 24 h. The insects were considered dead when they were unable to walk at least the distance of their own body length after being touched with a fine brush.

2.2.4. Determination of LC_{50} of harmful insecticides

The median lethal concentration (LC_{50}) was estimated for those insecticides that were harmful to adults of *M. basicornis* in the acute toxicity test (Section 2.2.3). The procedures for the LC_{50} bioassays were similar to the methods in Section 2.2.3. The design was completely randomized, with 6 replicates per treatment and 15 *M. basicornis* adults (<3 days old) in each cage. The bioassays were performed with different concentrations per insecticide, below the recommended field concentration (Table 1), according to procedures described by Finney [32]. The following insecticide concentrations (in mg a.i. L^{-1}) were used: five concentrations of acetamiprid (0.03, 0.3, 1.0, 15 and 30); eight concentrations of bifenthrin (0.015, 0.075, 0.15, 0.75, 1.5, 7.5, 15 and 22.5); six concentrations of etofenprox + acetamiprid (0.18, 0.93, 9.34, 18.68 and 93.4); and five concentrations of pyriproxyfen + acetamiprid (0.09, 0.45, 0.9, 4.5, 9.0 and 45.0). Mortality was assessed 72 h after insecticides exposure to calculate the LC_{50} and the live insects were checked every 24 h. The insects were considered dead when they were unable to move at least the distance of their own body length after being touched with a fine brush.

2.2.5. Statistical analysis

The data for the total number of live insects per replicate after 24, 48 and 72 h were checked for normality and homoscedasticity using the Shapiro–Wilk and Bartlett tests. If the assumptions of ANOVA were met, one-way ANOVA with Scott–Knott post-hoc ($p < 0.05$) was used to ascertain differences among treatment means. If the data did not satisfy the normality and variance homogeneity, Kruskal–Wallis non-parametric one-way ANOVA with Dunn with Bonferroni correction post-hoc ($p < 0.05$) was used through the “ExpDes”, “easyanova” and “dunn.test” packages in the R software [33]. The mortality percentage values after 72 h were corrected according to the Schneider–Orelli formula [34]: M_a (%) = [(Mt - Mc)/ (100 - Mc)] × 100, where M_a is the corrected mortality, M_t the mortality observed in the treatment and M_c the control mortality.

The data for survival of mirids exposed to insecticides over time were analyzed using Kaplan–Meier estimators (Log-Rank method). The survival curves and the median lethal time (LT_{50}) were compared using the Holm–Sidak test, in SigmaPlot version 12.3 (Systat Software, San José, CA, USA).

The data obtained from the tests to estimate the LC_{50} were submitted to a binomial model with the log-logistic regression (drfit) function for dose-response analysis in the statistical program R [33, 35].

2.2.6. Toxicity classification

Insecticides were classified in the toxicological categories of residual effects for tests in extended laboratory analysis, with the corrected mortality (M_a) according to the IOBC, in which: class 1 = harmless (M_a < 25%); class 2 = slightly harmful (25 ≤ M_a ≤ 50%); class 3 = moderately harmful (51 ≤ M_a ≤ 75%); and class 4 = harmful (M_a > 75%) [25].

To assess the ecological risk of the harmful insecticides, the risk quotient (RQ) was calculated from the LC_{50} values at 72 h after exposure, based on the formula: RQ = recommended field rate (g a.i. ha–1)/LC50 of beneficial insects (mg a.i. L−1). To understand the ecological selectivity of the harmful insecticides, the calculated RQ values estimate the possible effect that can occur in the field. According to the results, the insecticides were classified as safe (RQ < 50), slightly to moderately toxic (50 < RQ ≤ 2500) or dangerously toxic (RQ > 2500) [26].

2.3. Results

The toxicity for nymphs and adults of the insecticides tested varied widely. The number of live insects in the groups exposed to buprofezin, cyantraniliprole and spiromesifen were similar to the control treatment, while the number of live insects exposed to acetamiprid, bifenthrin, etofenprox + acetamiprid and pyriproxyfen + acetamiprid significantly differed from the other treatments for both third-instar nymphs (Table 2) and adults (Table 3). The data obtained for the most lethal insecticides showed an increasing toxicity over time for nymphs (24 h: 2 = 37.3, *df* = 7, *p* < 0.001; 48 h: 2 = 36.7, *df* = 7, *p* < 0.001; 72 h: 2 = 39.0, *df* = 7, *p* < 0.001) (Table 2) and for adults (24 h: F = 34.9, *df* = 7, *p* < 0.001; 48 h: F = 34.9, *df* = 7, *p* < 0.001; 72 h: F = 34.9, *df* = 7, *p* < 0.001) (Table 3).

Table 2. Number of live third-instar nymphs (mean ± SE) of *Macrolophus basicornis* 24, 48 and 72 h after contact with insecticidal residues in tomato leaves, correct mortality (M_a) after 72 h, and IOBC classification of insecticides.

Treatment	Number of live nymphs (n = 15)			M_a (%)*	Class[1]
	24 h	48 h	72 h		
Control	14.5 ± 0.3 a	14.0 ± 0.3 a	13.8 ± 0.3 a	-	-
Acetamiprid	3.3 ± 1.7 b	1.0 ± 0.7 b	0.2 ± 0.2 b	99.9	4
Bifenthrin	4.2 ± 1.3 b	0.7 ± 0.5 b	0.0 ± 0.0 b	100.0	4
Buprofezin	14.5 ± 0.2 a	14.0 ± 0.2 a	13.8 ± 0.3 a	0.0	1
Cyantraniliprole	14.5 ± 0.2 a	14.0 ± 0.5 a	13.6 ± 0.5 a	0.8	1
Etofenprox + acetamiprid	4.0 ± 0.9 b	0.7 ± 0.3 b	0.2 ± 0.2 b	99.9	4
Pyriproxyfen + acetamiprid	3.0 ± 1.1 b	1.7 ± 0.9 b	1.2 ± 0.9 b	87.4	4
Spiromesifen	14.8 ± 0.2 a	14.6 ± 0.2 a	14.0 ± 0.4 a	0.0	1
$\chi 2$	37.3	37.6	39.0	-	-
df	7	7	7	-	-
p	< 0.001	< 0.001	< 0.001	-	-

Means followed by the same letter in the columns do not differ by the Bonferroni test.
*Corrected mortality (Ma) by the Schneider-Orelli formula [31].
[1] Toxicological class according to IOBC ("International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section") in which: class 1 = harmless (Ma < 25%); class 2 = slightly harmful (25 ≤ Ma ≤ 50%); class 3 = moderately harmful (51 ≤ Ma ≤ 75%); class 4 = harmful (Ma > 75%) [25].

Table 3. Number of alive adults (mean ± SE) of *Macrolophus basicornis* 24, 48 and 72 h after contact with insecticidal residues in tomato leaves, correct mortality (M_a) after 72 h, and IOBC classification of insecticides.

Treatment	**Number of live adults (n = 15)**			**M_a (%)***	**Class[1]**
	24 h	**48 h**	**72 h**		
Control	14.5 ± 0.2 a	14.5 ± 0.2 a	14.3 ± 0.3 a	-	-
Acetamiprid	4.1 ± 1.1 c	2.2 ± 0.9 c	0.2 ± 0.2 c	98.6	4
Bifenthrin	6.8 ± 0.8 b	5.2 ± 0.8 b	2.8 ± 0.3 b	80.3	4
Buprofezin	14.0 ± 0.4 a	14.0 ± 0.4 a	14.0 ± 0.4 a	2.1	1
Cyantraniliprole	14.3 ± 0.3 a	14.0 ± 0.4 a	13.8 ± 0.5 a	3.5	1
Etofenprox + acetamiprid	6.2 ± 0.9 b	2.6 ± 1.0 c	0.5 ± 0.3 c	96.5	4
Pyriproxyfen + acetamiprid	7.8 ± 0.9 b	5.2 ± 0.9 b	1.8 ± 0.5 b	87.4	4
Spiromesifen	14.5 ± 0.2 a	14.3 ± 0.2 a	14.3 ± 0.2 a	0.0	1
CV (%)	17.9	19.8	12.9	-	-
F	34.9	60.9	288.2	-	-
df	7	7	7	-	-
p	< 0.001	< 0.001	< 0.001	-	-

Means followed by the same letter in the columns do not differ by the Scott-knott test.
*Corrected mortality (Ma) by the Schneider-Orelli formula [31].
1 Toxicological class according to IOBC ("International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section") in which: class 1 = harmless (Ma < 25%); class 2 = slightly harmful (25 ≤ Ma ≤ 50%); class 3 = moderately harmful (51 ≤ Ma ≤ 75%); class 4 = harmful (Ma > 75%) [25].

After 72 h of exposure of third-instar nymphs to insecticide residues on tomato leaves, buprofezin, cyantraniliprole and spiromesifen caused less than 1% mortality (Table 2). For acetamiprid, bifenthrin, etofenprox + acetamiprid and pyriproxyfen + acetamiprid, mortality ranged from 91.3% to 100% (Table 2). For adults, spiromesifen, buprofezin and cyantraniliprole caused 0, 2.1 and 3.5% mortality, respectively (Table 3). Pyriproxyfen + acetamiprid and bifenthrin reached 87.4 and 80.3% mortality, while etofenprox + acetamiprid and acetamiprid were the most harmful to adults, causing 96.5 and 98.6% mortality (Table 3).

According to IOBC classifications for acute toxicity, buprofezin, cyantraniliprole and spiromesifen are harmless (M_a < 25% = class 1) to nymphs and adults of *M. basicornis*. Acetamiprid, etofenprox + acetamiprid, pyriproxyfen + acetamiprid and bifenthrin are categorized as harmful (M_a > 75% = class 4) to this predator (Tables 2 and 3).

Survival rates for nymphs and adults after 72 h exposure to insecticides showed significant differences among treatments (nymphs: 2 = 686.96, *df* = 7, *p* < 0.001; adults: 2 = 661.1, *df* = 7, *p* < 0.001). Buprofezin, cyantraniliprole and spiromesifen were similar to the control (Table 4).

Table 4. Median lethal time (LT_{50}) in days for third-instar nymphs and adults of *Macrolophus basicornis* after 72 h in contact with insecticide residues on tomato leaves.

Treatment	**LT_{50} (95% CI)**			
	Third-Instar Nymphs		**Adults**	
Control	55.0 (48.8–61.1)	a	26.4 (23.7–29.1)	a
Acetamiprid	1.9 (1.5–2.2)	b	2.2 (1.7–2.5)	b
Bifenthrin	2.1 (1.7–2.5)	b	3.2 (2.6–3.8)	b
Buprofezin	51.1 (45.1–57.1)	a	28.5 (25.1–31.8)	a
Cyantraniliprole	58.9 (52.7–65.2)	a	26.5 (23.3–29.8)	a
Etofenprox + acetamiprid	2.1 (1.7–2.4)	b	2.5 (2.1–2.9)	b
Pyriproxyfen + acetamiprid	1.8 (1.5–2.1)	b	3.2 (2.8–3.6)	b
Spiromesifen	55.4 (49.5–61.3)	a	22.6 (20.5–24.7)	a
χ^2	686.96		661.1	
df	7		7	
p	<0.001		<0.001	

Means followed by the same letter in a column do not differ by the Holm-Sidak test. CI: Confidence interval with 95% probability.

In comparison to the control group, the LT_{50} values of acetamiprid, etofenprox + acetamiprid, pyriproxyfen + acetamiprid and bifenthrin were reduced by the acute toxicity of these active ingredients, ranging from 1.8 to 2.1 days for nymphs and 2.2 to 3.2 days for adults (Table 4). In the survival curves, nymphs (Figure 1) were more vulnerable to the harmful insecticides than adults (Figure 2).

The median lethal concentration (LC_{50}) values are shown in Table 5 for acetamiprid, bifenthrin, etofenprox + acetamiprid and pyriproxyfen + acetamiprid, at 72 h after exposure of the adults to insecticide residues. Acetamiprid and bifenthrin had similar LC_{50} values and etofenprox + acetamiprid and pyriproxyfen + acetamiprid were less toxic and with similar LC_{50} values, with overlapping confidence intervals from 3.28 to 11.25 mg a.i. L^{-1} (Table 5).

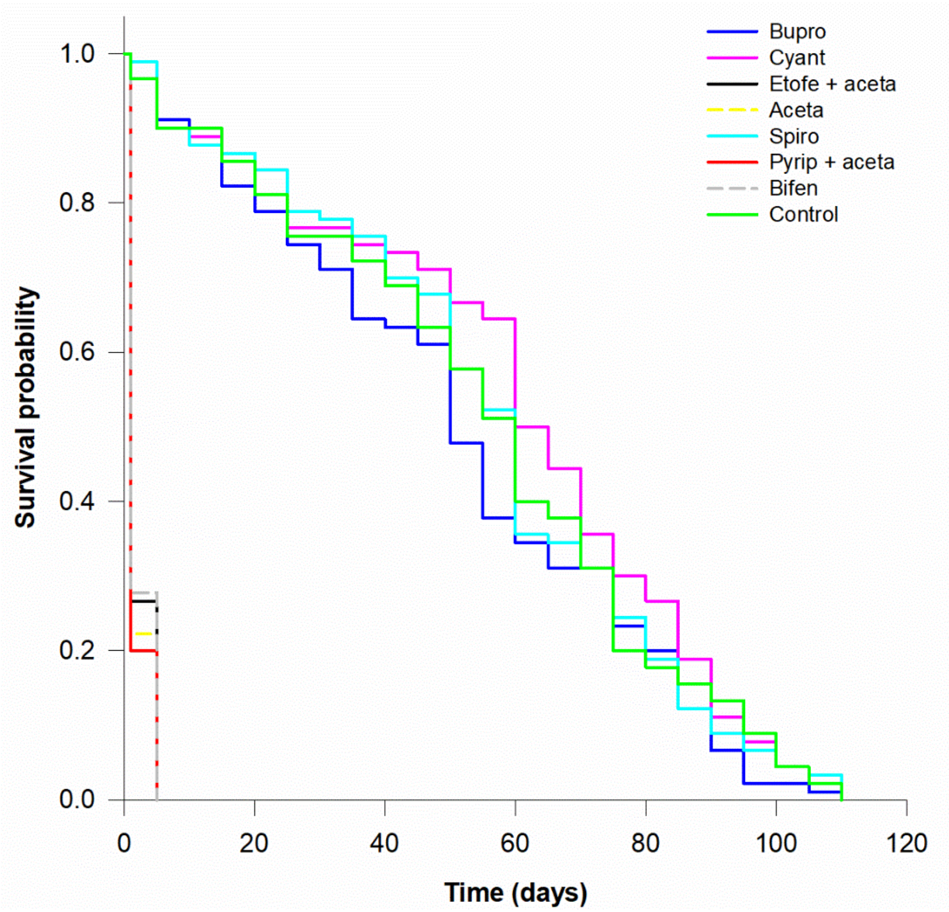

Figure 1. Survival curves for *Macrolophus basicornis* third-instar nymphs exposed to residues of buprofezin (Bupro), cyantraniliprole (Cyant), etofenprox + acetamiprid (Etofe + aceta), acetamiprid (Aceta), spiromesifen (Spiro), pyriproxyfen + acetamiprid (Pyrip + aceta), bifenthrin (Bifen) and control (water). The insects were in contact with the residues on tomato leaves for 72 h in controlled conditions.

Table 5. Median lethal concentration (LC_{50}) of insecticides to adults of *Macrolophus basicornis* after contact with residues on tomato leaves for 72 h.

Insecticides	LC_{50} (95% CI) (mg a.i. L^{-1})	χ2	*df*	RQ	Category*
Acetamiprid	0.26 (0.16 – 0.35)	11.58	4	334.6	2
Bifenthrin	0.38 (0.29 – 0.48)	30.34	7	3.95	1
Etofenprox + acetamiprid	4.80 (3.28 – 6.31)	32.07	5	38.91	1
Pyriproxyfen + acetamiprid	8.71 (6.18 – 11.25)	65.86	4	10.33	1

Data observed and predicted by the binomial model test with log-logistic regression. $p < 0.0001$. CI: confidence interval with 95% probability. * Risk quotient categories according to the values at which the insecticides were classified as safe (RQ < 50), slightly to moderately toxic (50 < RQ ≤ 2500), or dangerously toxic (RQ > 2500) [26].

The RQ values of acetamiprid, bifenthrin, etofenprox + acetamiprid and pyriproxyfen + acetamiprid were 334.6, 3.95, 38.91 and 10.33, respectively. Etofenprox + acetamiprid, pyriproxyfen + acetamiprid and bifenthrin were classified as safe (category 1) and acetamiprid was classified as slightly to moderately toxic (category 2) (Table 5).

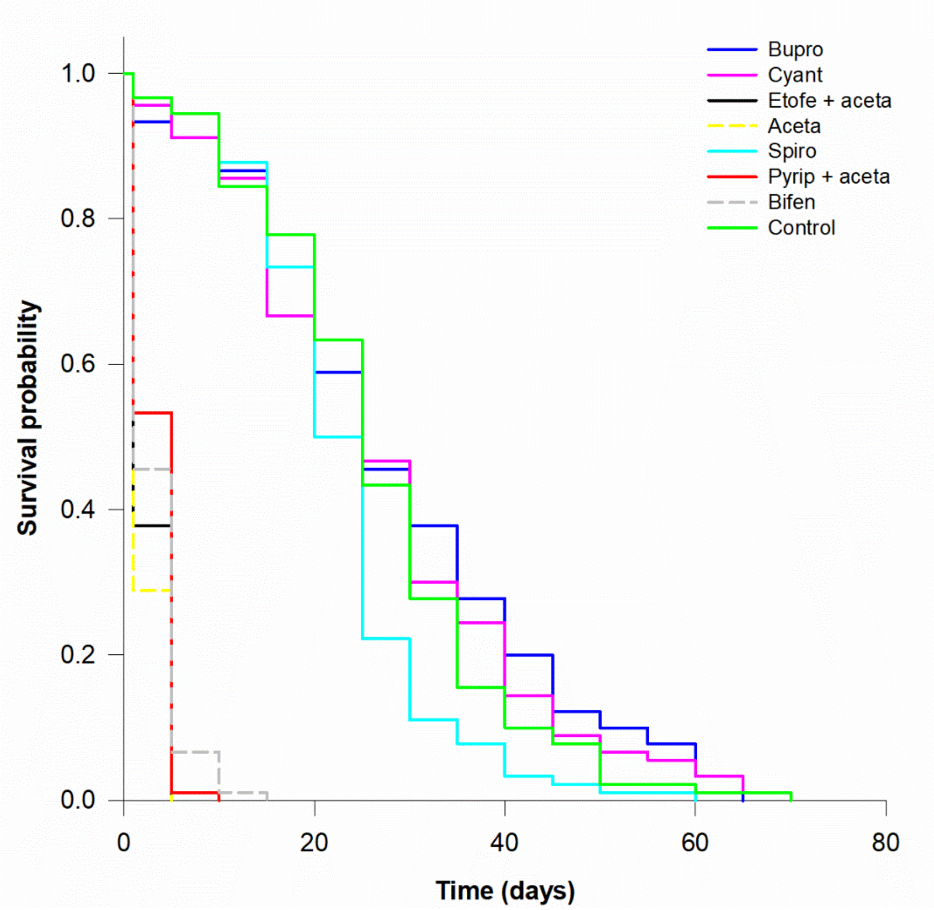

Figure 2. Survival curves for *Macrolophus basicornis* adults exposed to residues of buprofezin (Bupro), cyantraniliprole (Cyant), etofenprox + acetamiprid (Etofe + aceta), acetamiprid (Aceta), spiromesifen (Spiro), pyriproxyfen + acetamiprid (Pyrip + aceta), bifenthrin (Bifen) and control (water). The insects were in contact with the residues on tomato leaves for 72 h in controlled conditions.

2.4. Discussion

Mirid predators can help to manage *B. tabaci* in tomato crops [1, 2]. The mirid *M. basicornis* preys on tomato pests in Brazil and may become a biological control agent for use in IPM programs [3–6, 36, 37]. Insecticides from different chemical groups and active ingredients for control of *B. tabaci* are commercially available. These products range from reduced-risk, which rarely harm natural enemies, to broad-spectrum, which are acutely toxic to natural enemies [38–40], compromising the implementation of IPM programs. To mitigate incompatibility issues, information is needed on the acute toxicity of the insecticides that are most often used to control *B. tabaci* and their effects on natural enemies.

According to our findings, buprofezin, cyantraniliprole and spiromesifen were considered reduced-risk insecticides for *M. basicornis* and classified as harmless according to the IOBC criteria (class 1), with LT_{50} values similar to the control treatment. Buprofezin is an insect growth regulator (IGR) that acts on the immature stage of sucking pests by inhibiting chitin synthesis and consequently the insects cannot molt normally [41,42]. Spiromesifen, which inhibits the acetyl CoA carboxylase, derived from tetronic and tetramic acids, interferes with the development, fecundity and lipid biosynthesis of the pest [43]. The diamide cyantraniliprole can act on nymphs and adults of sucking pests, inhibiting muscle contraction when the molecules bind to ryanodine receptors, resulting in starvation, paralysis and death [44, 45]. In the present case, these insecticides did not cause acute toxicity to the natural enemy and the survival rate was also similar to the control. The results demonstrated that these insecticides are not harmful to *M. basicornis* in controlled conditions.

Similar results were found when residues of parallel insecticides did not cause high levels of acute toxicity to *M. basicornis* adults and nymphs [18–21]. Interestingly, Wanumen et al. [18] showed that spiromesifen was innocuous to adults of *M. basicornis* exposed to residues on an inert substrate, but mortality increased in extended laboratory assays (sprayed on tomato leaves). In the present study, spiromesifen on tomato leaves retained the harmless acute effect in controlled conditions. This was elucidated by differences in the concentrations used, contributing to this negative effect. Therefore, at the semi-field level, it does not cause a lethal effect on this natural enemy [18] and probably will not be lethal in field conditions.

Similarly to the results for *M. basicornis*, buprofezin did not have a lethal effect on adults and nymphs of *Pilophorus typicus* Distant (Hemiptera: Miridae) under controlled conditions [46] or on *Macrolophus caliginosus* Wagner (Hemiptera: Miridae) under field conditions [47]. For the predator *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae), cyantraniliprole was lethal to nymphs and was less toxic to adults [48]. These studies demonstrate the importance of knowing the acute toxicity of insecticides for the integrated use of biological and chemical controls in an IPM program. The physiological effects of reduced-risk insecticides may depend on the sensitivity of a species and its life stages and, therefore, it is important to test each active ingredient on each species of natural enemy [49]. Knowledge of the sensitivity of a species in tropical conditions is important for agriculture, considering that the sensitivity can differ depending on climate, temperature and light incidence [50].

Acetamiprid induces excitation until death, acting on neurons as a competitive modulator of the nicotinic acetylcholine receptor [51, 52]. It is a chlorinated neonicotinoid which mainly acts by ingestion, due to its activity inside the plants, allowing systemic translocation in the sap vessels [53]. When sprayed on tomato leaves, it caused 80% mortality in adults of another mirid predator, *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) after five days of exposure [54]. In addition to acting systemically due to its hydrophobicity, acetamiprid also acts by contact [55, 56]. On inert substrates in controlled conditions, acetamiprid caused 100% mortality in *M. caliginosus* and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) [57]. Because mirid predators are omnivorous [58], systemic insecticides such as acetamiprid may affect this natural enemy by both the contact and ingestion routes of exposure.

Etofenprox and bifenthrin affect insects mainly through contact exposure [59, 60]. Etofenprox and bifenthrin modulate the sodium channel in neuron axons, which keep the insect hyperexcited and, also, cause death [59]. Some pyrethroids tested on piercing-sucking predators (Hemiptera) also caused acute toxicity [61, 62], as did bifenthrin in this study. The active ingredient lambda-cyhalothrin is efficient in controlling the *B. tabaci* pest population in tomatoes, but significantly affected the survival of the mirid predator *N. tenuis* under laboratory conditions [61]. Deltamethrin demonstrated acute toxicity similar to bifenthrin, which caused 70% mortality in *N. tenuis* after contact with residues for more than 72 h [62]. Bifenthrin had a similar effect to our results when tested with a full con-centration series bioassay in the laboratory, proving highly toxic to adults of *Geocoris punctipes* (Say) (Hemiptera: Geocoridae) and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) [63]. Pyrethroids are harmful to *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), with high toxicity to nymphs and adults at the highest recommended rate in soybeans, even when mixed with low-risk insecticides [64].

Most acute toxicology studies do not address the ecological vulnerability of natural enemies to broad-spectrum pesticides and only assess whether they kill the insect, which is insufficient to recommend these pesticides in IPM programs. However, by determining the median lethal concentration (LC_{50}) for the most physiologically harmful insecticides to beneficial insects, it is possible to calculate the risk quotient (RQ) to determine the ecological risks of a given insecticide to a natural enemy. The insecticides evaluated here, especially acetamiprid and bifenthrin, showed quite low LC_{50} values. Calculating the RQ values for each formulation and considering the concentration of the active ingredients, most of the RQ values were classified as safe (RQ < 50), except for acetamiprid, categorized as slightly to moderately toxic (50 < RQ ≤ 2500). These results are important to understand both the physiological and ecological risks together, in order to make decisions for IPM recommendations [65, 66].

This is the first study with *M. basicornis* to assess the acute toxicity and RQ values of the insecticides that are most often used to control *B. tabaci* in tomato crops. Other studies conducted with important natural enemies in different crop systems contributed useful information IPM [26, 27, 35, 67–69]. Nevertheless, the researchers also made clear that certain insecticides tested, although classified as slightly to moderately harmful, should be thoroughly evaluated for inclusion in an IPM program, as they show high acute toxicity to the predator and other species.

Insecticides can act differently in each insect species and it is therefore important to study the pesticide formulations and their effect on the natural enemies that are most frequently found and released in the crops. As an example of the action of the same insecticides on different species, in the case of parasitoids of the genus *Trichogramma*, neonicotinoids and pyrethroids were tested to determine the LC_{50} and to calculate the RQ values [27]. For *Trichogramma dendrolimi* Matsumura, *Trichogramma ostriniae* Pang et Chen and

Trichogramma chilonis Ishii (Hymenoptera: Trichogrammatidae), the LC_{50} values for acetamiprid were 0.32, 1.37 and 0.53 g a.i. ha^{-1} and the RQ values were 188.8, 44.1 and 114.0, respectively. Therefore, acetamiprid was categorized as slightly to moderately harmful to *T. dendrolimi* and *T. chilonis* (class 2) but safe for *T. ostriniae* (class 1) [27]. In the present study, the LC_{50} were also very low for all insecticides tested, similarly to the studies with *Trichogramma* species, but the RQ values differed for acetamiprid, showing that these specific studies must be considered when assessing the insecticides' risks to a new species of natural enemy.

Taken together, the present results support the hypothesis that some of the insecticides tested were physiologically more harmful than others to the natural enemy. In addition, this study elucidated the ecological risks of those that proved to be physiologically harmful. Physiological and ecological risks must be considered when using IPM tools such as chemical and biological controls. If we consider only the physiological hazard, we eliminate all the other factors that can minimize the effect of these chemicals on non-target organisms in the field. These factors can potentially make pesticides more selective, based on, for example, formulation, placement, dosage and timing [64]. If the ecological risks are considered, there is a chance to match the methods to actual conditions in the tomato fields. It may be possible to use these insecticides with temporal and spatial separation [65]. IPM methods provide better results when most of the tools can be implemented in the field of the crop cycle [70].

2.5. Conclusions

The results obtained in controlled conditions for *M. basicornis* nymphs and adults are important to understand the action of insecticides on this natural enemy. Buprofezin, cyantraniliprole and spiromesifen were considered reduced-risk insecticides, but future studies should assess sublethal and transgenerational effects on this beneficial insect. Acetamiprid, bifenthrin, etofenprox + acetamiprid and pyriproxyfen + acetamiprid were harmful and considered broad-spectrum for *M. basicornis*. The physiological and ecological classifications for broad-spectrum insecticides were determined for *M. basicornis* adults and will support future IPM decisions. The RQ data provide insight into the ecological risk assessment for data acquired under more controlled conditions, but this needs to be confirmed with semi-field and field assays. Further studies are necessary to confirm compatibility of the methods with these active ingredients, such as in a greenhouse with regular insecticide spraying, to determine the persistence of the compound residues on tomato plants and the effects on the predator. It is also important to study crop management with these products to gather more accurate information.

References

1. Cassis, G.; Schuh, R. Systematics, biodiversity, biogeography, and host associations of the Miridae (Insecta: Hemiptera: Heteroptera: Cimicomorpha). *Annu. Rev. Èntomol.* **2012**, *57*, 377–404. https://doi.org/10.1146/annurev-ento-121510-133533.
2. Van Lenteren, J.C. The state of commercial augmentative biological control: Plenty of natural enemies, but a frustrating lack of uptake. *BioControl* **2012**, *57*, 1–20. https://doi.org/10.1007/s10526-011-9395-1.
3. Bueno, V.H.P.; Montes, F.C.; Pereira, A.M.C.; Lins, J.C.; van Lenteren, J.C. Can recently found Brazilian hemipteran predatory bugs control *Tuta absoluta*? *IOBC-WPRS Bull.* **2012**, *80*, 63–67.
4. Bueno, V.H.P.; van Lenteren, J.; Lins, J.J.C.; Calixto, A.M.; Montes, F.C.; Silva, D.B.; Santiago, L.D.; Pérez, L.M. New records of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) predation by Brazilian Hemipteran predatory bugs. *J. Appl. Èntomol.* **2013**, *137*, 29–34. https://doi.org/10.1111/jen.12017.
5. Bueno, V.H.P.; Calixto, A.M.; Montes, F.C.; van Lenteren, J.C. Population growth parameters of three Neotropical mirid predators (Hemiptera: Miridae) at five temperatures on tobacco with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as food. *Israel J. Entomol.* **2018**, *48*, 1–22. https://doi.org/10.5281/zenodo.1185333.
6. Bueno, V.H.P.; Montes, F.C.; Sampaio, M.V.; Calixto, A.M.; van Lenteren, J.C. Performance of immatures of three Neotropical Miridae at five different temperatures, reared on *Ephestia kuehniella* eggs on tobacco plants. *Bull. Insectology* **2018**, *71*, 77–87.
7. Silva, W.M.; Berger, M.; Bass, C.; Williamson, M.; Moura, D.M.; Ribeiro, L.M.; Siqueira, H.A. Mutation (G275E) of the nicotinic acetylcholine receptor α6 subunit is associated with high levels of resistance to spinosyns in *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Pestic. Biochem. Physiol.* **2016**, *131*, 1–8. https://doi.org/10.1016/j.pestbp.2016.02.006.
8. van Lenteren, J.C.; Hemerik, L.; Lins, J.C.; Bueno, V.H.P. Functional responses of three Neotropical mirid predators to eggs of *Tuta absoluta* on tomato. *Insects* **2016**, *7*, 34. https://doi.org/10.3390/insects7030034.
9. van Lenteren, J.C.; Bueno, V.H.P.; Smit, J.; Soares, M.A.; Calixto, A.M.; Montes, F.C.; De Jong, P. Predation of *Tuta absoluta* eggs during the nymphal stages of three Neotropical mirid predators on tomato. *Bull. Insectology* **2017**, *70*, 69–74.
10. Castañé, C.; Arnó, J.; Gabarra, R.; Alomar, O. Plant damage to vegetable crops by zoophytophagous mirid predators. *Biol. Control* **2011**, *59*, 22–29. https://doi.org/10.1016/j.biocontrol.2011.03.007.
11. Calvo, F.J.; Lorente, M.J.; Stansly, P.A.; Belda, J.E. Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. *Èntomol. Exp. et Appl.* **2012**, *143*, 111–119. https://doi.org/10.1111/j.1570-7458.2012.01238.x.

12. Lins, J.J.C.; Van Loon, J.J.A.; Bueno, V.H.P.; Lucas-Barbosa, D.; Dicke, M.; van Lenteren, J. Response of the zoophytophagous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* to volatiles of uninfested plants and to plants infested by prey or conspecifics. *BioControl* **2014**, *59*, 707–718. https://doi.org/10.1007/s10526-014-9602-y.
13. De Barro, P.J.; Liu, S.-S.; Boykin, L.M.; Dinsdale, A.B. *Bemisia tabaci*: A statement of species status. *Annu. Rev. Èntomol.* **2011**, *56*, 1–19. https://doi.org/10.1146/annurev-ento-112408-085504.
14. Perring, T.M.; Stansly, P.A.; Liu, T.; Smith, H.A.; Andreason, S.A. Whiteflies: biology, ecology, and management. In *Sustainable Management of Arthropod Pests of Tomato*; Wakil, W., Brust, G.E., Perring, T. M., Eds.; Elsevier: London, UK, 2018; pp. 73–110. https://doi.org/10.1016/B978-0-12-802441-6.00004-8.
15. Desneux, N.; Decourtye, A.; Delpuech, J.-M. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Èntomol.* **2007**, *52*, 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440.
16. Barzman, M.; Bàrberi, P.; Birch, A.N.E.; Boonekamp, P.; Dachbrodt-Saaydeh, S.; Graf, B.; Hommel, B.; Jensen, J.E.; Kiss, J.; Kudsk, P.; et al. Eight principles of integrated pest management. *Agron. Sustain. Dev.* **2015**, *35*, 1199–1215. https://doi.org/10.1007/s13593-015-0327-9.
17. Naranjo, S.E.; Ellsworth, P.C.; Frisvold, G.B. Economic value of biological control in integrated pest management of managed plant systems. *Annu. Rev. Èntomol.* **2015**, *60*, 621–645. https://doi.org/10.1146/annurev-ento-010814-021005.
18. Wanumen, A.C.; Carvalho, G.A.; Medina, P.; Viñuela, E.; Adán, Á. Residual acute toxicity of some modern insecticides toward two mirid predators of tomato pests. *J. Econ. Èntomol.* **2016**, *109*, 1079–1085. https://doi.org/10.1093/jee/tow059.
19. Passos, L.C.; Soares, M.A.; Costa, M.A.; Michaud, J.; Freire, B.C.; Carvalho, G.A. Physiological susceptibility of the predator *Macrolophus basicornis* (Hemiptera: Miridae) to pesticides used to control of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Biocontrol Sci. Technol.* **2017**, *27*, 1082–1095. https://doi.org/10.1080/09583157.2017.1381879.
20. Passos, L.C.; Soares, M.A.; Collares, L.J.; Malagoli, I.; Desneux, N.; Carvalho, G.A. Lethal, sublethal and transgenerational effects of insecticides on *Macrolophus basicornis*, predator of *Tuta absoluta*. *Èntomol. Gen.* **2018**, *38*, 127–143. https://doi.org/10.1127/entomologia/2018/0744.
21. Soares, M.A.; Passos, L.C.; de Campos, M.R.; Collares, L.J.; Desneux, N.; Carvalho, G.A. Side effects of insecticides commonly used against *Tuta absoluta* on the predator *Macrolophus basicornis*. *J. Pest Sci.* **2019**, *92*, 1447–1456. https://doi.org/10.1007/s10340-019-01099-4.
22. Ellsworth, P.C.; Martinez-Carrillo, J.L. IPM for *Bemisia tabaci*: A case study from North America. *Crop. Prot.* **2001**, *20*, 853–869. https://doi.org/10.1016/s0261-2194(01)00116-8.
23. E Naranjo, S. Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. *Crop. Prot.* **2001**, *20*, 835–852. https://doi.org/10.1016/s0261-2194(01)00115-6.
24. Dale, A.G.; Borden, M.A. Evaluation of reduced-risk insecticides to control chilli thrips (Thysanoptera: Thripidae) and conserve natural enemies on ornamental plants. *Fla. Èntomol.* **2018**, *101*, 237–243. https://doi.org/10.1653/024.101.0213.
25. Hassan, S. International Organization for Biological and Integrated Control of Noxious Animals and Plants/Working Group "Pesticides and Beneficial Organisms" 1992. Guidelines for testing the effects of pesticides on beneficial organisms: Description of test methods. *IOBC-WPRS Bull.* **1992**, *XV*, 3.
26. Preetha, G.; Stanley, J.; Suresh, S.; Samiyappan, R. Risk assessment of insecticides used in rice on miridbug, *Cyrtorhinus lividipennis* Reuter, the important predator of brown planthopper, *Nilaparvata lugens* (Stal.). *Chemosphere* **2010**, *80*, 498–503. https://doi.org/10.1016/j.chemosphere.2010.04.070.

27. Cheng, S.; Lin, R.; Wang, L.; Qiu, Q.; Qu, M.; Ren, X.; Zong, F.; Jiang, H.; Yu, C. Comparative susceptibility of thirteen selected pesticides to three different insect egg parasitoid *Trichogramma* species. *Ecotoxicol. Environ. Saf.* **2018**, *166*, 86–91. https://doi.org/10.1016/j.ecoenv.2018.09.050.
28. Wang, Z.; Dai, P.; Yang, X.; Ruan, C.-C.; Biondi, A.; Desneux, N.; Zang, L.-S. Selectivity of novel and traditional insecticides used for management of whiteflies on the parasitoid *Encarsia formosa*. *Pest Manag. Sci.* **2019**, *75*, 2716–2724. https://doi.org/10.1002/ps.5380.
29. Jiang, J.; Liu, X.; Huang, X.; Yu, X.; Zhang, W.; Zhang, X.; Mu, W. Comparative ecotoxicity of neonicotinoid insecticides to three species of *Trichogramma* parasitoid wasps (Hymenoptera: Trichogrammatidae). *Ecotoxicol. Environ. Saf.* **2019**, *183*, 109587. https://doi.org/10.1016/j.ecoenv.2019.109587.
30. Liu, Y.; Li, X.; Zhou, C.; Liu, F.; Mu, W. Toxicity of nine insecticides on four natural enemies of *Spodoptera exigua*. *Sci. Rep.* **2016**, *6*, 39060. https://doi.org/10.1038/srep39060.
31. Arnó, J.; Gabarra, R. Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). *J. Pest Sci.* **2011**, *84*, 513–520. https://doi.org/10.1007/s10340-011-0384-z.
32. Finney, D.J. *Probit Analysis*, 3rd ed.; Cambridge University: London, UK, 1971; 333p.
33. R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. 2020. Available online: https://www.R-project.org/ (accessed on 24 Oct 2021).
34. Püntener, W. *Manual for Field Trials in Plant Protection*, 2nd ed.; Ciba-Geigy, Ltd.: Basel, Switzerland, 1981.
35. Ritz, C.; Baty, F.; Streibig, J.C.; Gerhard, D. Dose-response analysis using R. *PLoS ONE* **2015**, *10*, e0146021. https://doi.org/10.1371/journal.pone.0146021.
36. Silva, D.B.; Bueno, V.H.P.; Van Loon, J.J.A.; Peñaflor, M.F.G.V.; Bento, J.M.S.; Van Lenteren, J.C. Attraction of three mirid predators to tomato infested by both the tomato leaf mining moth *Tuta absoluta* and the whitefly *Bemisia tabaci*. *J. Chem. Ecol.* **2017**, *44*, 29–39. https://doi.org/10.1007/s10886-017-0909-x.
37. van Lenteren, J.C.; Bueno, V.H.P.; Montes, F.C.; Hemerik, L.; De Jong, P.W. Adult lifetime predation of *Tuta absoluta* eggs by three Neotropical mirid predators on tomato. *Bull. Insectology* **2018**, *71*, 179–188.
38. Stansly, P.; Sánchez, P.; RodríguezJ.; Cañizares, F.; Nieto, A.; Leyva, M.; Fajardo, M.; Suárez, V.; Urbaneja, A. Prospects for biological control of *Bemisia tabaci* (Homoptera, Aleyrodidae) in greenhouse tomatoes of southern Spain. *Crop. Prot.* **2004**, *23*, 701–712. https://doi.org/10.1016/j.cropro.2003.11.016.
39. Koss, A.M.; Jensen, A.S.; Schreiber, A.; Pike, K.S.; Snyder, W.E. Comparison of predator and pest communities in Washington potato fields treated with broad-spectrum, selective, or organic insecticides. *Environ. Èntomol.* **2005**, *34*, 87–95. https://doi.org/10.1603/0046-225x-34.1.87.
40. Togni, P.H.B.; Venzon, M.; de Souza, L.M.; Santos, J.P.C.R.; Sujii, E.R. Biodiversity provides whitefly biological control based on farm management. *J. Pest Sci.* **2018**, *92*, 393–403. https://doi.org/10.1007/s10340-018-1021-x.
41. Sohrabi, F.; Shishehbor, P.; Saber, M.; Mosaddegh, M. Lethal and sublethal effects of buprofezin and imidacloprid on *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Crop. Prot.* **2011**, *30*, 1190–1195. https://doi.org/10.1016/j.cropro.2011.05.004.
42. Ullah, F.; Gul, H.; Yousaf, H.K.; Xiu, W.; Qian, D.; Gao, X.; Tariq, K.; Han, P.; Desneux, N.; Song, D. Impact of low lethal concentrations of buprofezin on biological traits and expression profile of chitin synthase 1 gene (CHS1) in melon aphid, *Aphis gossypii*. *Sci. Rep.* **2019**, *9*, 12291. https://doi.org/10.1038/s41598-019-48199-w.

43. Bretschneider, T.; Benet-Buchholz, J.; Fischer, R.; Nauen, R. Spirodiclofen and spiromesifen—novel acaricidal and insecticidal tetronic acid derivatives with a new mode of action. *Chim. Int. J. Chem.* **2003**, *57*, 697–701. https://doi.org/10.2533/000942903777678588.
44. Lahm, G.P.; Selby, T.P.; Freudenberger, J.H.; Stevenson, T.M.; Myers, B.J.; Seburyamo, G.; Smith, B.K.; Flexner, L.; Clark, C.E.; Cordova, D. Insecticidal anthranilic diamides: A new class of potent ryanodine receptor activators. *Bioorganic Med. Chem. Lett.* **2005**, *15*, 4898–4906. https://doi.org/10.1016/j.bmcl.2005.08.034.
45. Sattelle, D.B.; Cordova, D.; Cheek, T.R. Insect ryanodine receptors: Molecular targets for novel pest control chemicals. *Invertebr. Neurosci.* **2008**, *8*, 107–119. https://doi.org/10.1007/s10158-008-0076-4.
46. Nakahira, K.; Kashitani, R.; Tomoda, M.; Kodama, R.; Ito, K.; Yamanaka, S.; Momoshita, M.; Arakawa, R. Side effects of vegetable pesticides on a predatory mirid bug, *Pilophorus typicus* Distant (Heteroptera: Miridae). *Appl. Èntomol. Zoöl.* **2010**, *45*, 239–243. https://doi.org/10.1303/aez.2010.239.
47. Rasdi, M.Z.; Che Salmah, M.R.; Abu Hassan, A.; Hamady, D.; Hamaseh, A.; Fauziah, I. Field evaluation of some insecticides on whitefly (*Trialeurodes vaporariorum*) and predator (*Macrolophus caliginosus*) on brinjal and tomato plants. *Asian J. Agric. Rural Dev.* **2012**, *2*, 302–311.
48. Amarasekare, K.G.; Shearer, P.W. Laboratory bioassays to estimate the lethal and sublethal effects of various insecticides and fungicides on *Deraeocoris brevis* (Hemiptera: Miridae). *J. Econ. Èntomol.* **2013**, *106*, 776–785. https://doi.org/10.1603/EC12432.
49. Müller, C. Impacts of sublethal insecticide exposure on insects—Facts and knowledge gaps. *Basic Appl. Ecol.* **2018**, *30*, 1–10. https://doi.org/10.1016/j.baae.2018.05.001.
50. Daam, M.A.; Chelinho, S.; Niemeyer, J.; Owojori, O.; De Silva, P.M.C.; Sousa, J.P.; van Gestel, C.A.; Römbke, J. Environmental risk assessment of pesticides in tropical terrestrial ecosystems: Test procedures, current status and future perspectives. *Ecotoxicol. Environ. Saf.* **2019**, *181*, 534–547. https://doi.org/10.1016/j.ecoenv.2019.06.038.
51. Casida, J.E.; Durkin, K.A. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annu. Rev. Èntomol.* **2013**, *58*, 99–117. https://doi.org/10.1146/annurev-ento-120811-153645.
52. Simon-Delso, N.; Amaralrogers, V.; Belzunces, L.P.; Bonmatin, J.M.; Chagnon, M.; Downs, C.; Furlan, L.; Gibbons, D.W.; Giorio, C.; Girolami, V.; et al. Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. *Environ. Sci. Pollut. Res.* **2015**, *22*, 5–34. https://doi.org/10.1007/s11356-014-3470-y.
53. Saha, S.; Mondal, R.; Mukherjee, S.; Sarkar, M.; Kole, R.K. Persistence of acetamiprid in paddy and soil under West Bengal agro-climatic conditions. *Environ. Monit. Assess.* **2017**, *189*, 150. https://doi.org/10.1007/s10661-017-5871-0.
54. Mostafiz, M.; Hassan, E.; Shim, J.-K.; Lee, K.-Y. Lethal and sublethal effects of methyl benzoate on the predatory bug *Nesidiocoris tenuis*. *Insects* **2020**, *11*, 377. https://doi.org/10.3390/insects11060377.
55. Yamada, T.; Takahashi, H.; Hatano, R. A Novel insecticide, acetamiprid. In *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*; Yamamoto, I., Casida, J. E., Eds.; Springer: Tokyo, Japan, 1999; pp. 149–176. https://doi.org/10.1007/978-4-431-67933-2_7.
56. Kiriyama, K.; Itazu, Y.; Kagabu, S.; Nishimura, K. Insecticidal and neuroblocking activities of acetamiprid and related compounds. *J. Pestic. Sci.* **2003**, *28*, 8–17. https://doi.org/10.1584/jpestics.28.8.
57. Van de Veire, M.; Tirry, L. Side effects of pesticides on four species of beneficials used in IPM in glasshouse vegetable crops:" worst case" laboratory tests. *IOBC-WPRS Bull.* **2003**, *26*, 41–50.
58. Coll, M.; Guershon, M. Omnivory in terrestrial arthropods: Mixing plant and prey diets. *Annu. Rev. Èntomol.* **2002**, *47*, 267–297. https://doi.org/10.1146/annurev.ento.47.091201.145209.

59. Gammon, D.W.; Liu, Z.; Chandrasekaran, A.; El-Naggar, S.F.; A Kuryshev, Y.; Jackson, S. Pyrethroid neurotoxicity studies with bifenthrin indicate a mixed Type I/II mode of action. *Pest Manag. Sci.* **2019**, *75*, 1190–1197. https://doi.org/10.1002/ps.5300.
60. Rabelo, M.M.; Paula-Moraes, S.V.; Pereira, E.J.G.; Siegfried, B.D. Contrasting susceptibility of lepidopteran pests to diamide and pyrethroid insecticides in a region of overwintering and migratory intersection. *Pest Manag. Sci.* **2020**, *76*, 4240–4247. https://doi.org/10.1002/ps.5984.
61. Soares, M.A.; Carvalho, G.A.; Campos, M.R.; Passos, L.C.; Haro, M.M.; Lavoir, A.; Biondi, A.; Zappalà, L.; Desneux, N. Detrimental sublethal effects hamper the effective use of natural and chemical pesticides in combination with a key natural enemy of *Bemisia tabaci*on tomato. *Pest Manag. Sci.* **2020**, *76*, 3551–3559. https://doi.org/10.1002/ps.5927.
62. Perdikis, D.; Psaroudaki, S.; Papadoulis, G. Compatibility of *Nesidiocoris tenuis* and *Iphiseius degenerans* with insecticides, miticides and fungicides used in tomato crops. *Bull. Insectology* **2020**, *73*, 181–192.
63. Prabhaker, N.; Naranjo, S.; Perring, T.; Castle, S. Comparative toxicities of newer and conventional insecticides: Against four generalist predator species. *J. Econ. Èntomol.* **2017**, *110*, 2630–2636. https://doi.org/10.1093/jee/tox202.
64. Stecca, C.D.S.; Da Silva, D.M.; Bueno, A.D.F.; Pasini, A.; Denez, M.D.; Andrade, K. Selectivity of insecticide use in soybean crop to the predator *Podisus nigrispinus* (Hemiptera: Pentatomidae). *Semina: Ciências Agrárias* **2017**, *38*, 3469–3480. https://doi.org/10.5433/1679-0359.2017v38n6p3469.
65. Collier, R.; Jukes, A.; Daniel, C.; Hommes, M. Ecological selectivity of pesticides and application methods. *IOBC-WPRS Bull.* **2016**, *118*, 94–98.
66. Duso, C.; Van Leeuwen, T.; Pozzebon, A. Improving the compatibility of pesticides and predatory mites: Recent findings on physiological and ecological selectivity. *Curr. Opin. Insect Sci.* **2020**, *39*, 63–68. https://doi.org/10.1016/j.cois.2020.03.005.
67. Zhao, X.; Wu, C.; Wang, Y.; Cang, T.; Chen, L.; Yu, R.; Wang, Q. Assessment of toxicity risk of insecticides used in rice ecosystem on *Trichogramma japonicum*, an egg parasitoid of rice lepidopterans. *J. Econ. Èntomol.* **2012**, *105*, 92–101. https://doi.org/10.1603/ec11259.
68. Wang, Y.; Wu, C.; Cang, T.; Yang, L.; Yu, W.; Zhao, X.; Wang, Q.; Cai, L. Toxicity risk of insecticides to the insect egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). *Pest Manag. Sci.* **2014**, *70*, 398–404. https://doi.org/10.1002/ps.3571.
69. Pazini, J.D.B.; Padilha, A.C.; Cagliari, D.; Bueno, F.A.; Rakes, M.; Zotti, M.J.; Martins, J.F.D.S.; Grützmacher, A.D. Differential impacts of pesticides on *Euschistus heros* (Hem.: Pentatomidae) and its parasitoid *Telenomus podisi* (Hym.: Platygastridae). *Sci. Rep.* **2019**, *9*, 1–10. https://doi.org/10.1038/s41598-019-42975-4.
70. Bueno, A.D.F.; Batistela, M.J.; Bueno, R.C.O.D.F.; França-Neto, J.D.B.; Nishikawa, M.A.N.; Filho, A.L. Effects of integrated pest management, biological control and prophylactic use of insecticides on the management and sustainability of soybean. *Crop. Prot.* **2011**, *30*, 937–945. https://doi.org/10.1016/j.cropro.2011.02.021.

Supplementary material

Include:
Figure S1: Detail of experimental units with flasks used to insert the petiole of each tomato leave to maintain turgidity during the bioassay and the cage covered with voile fabric.

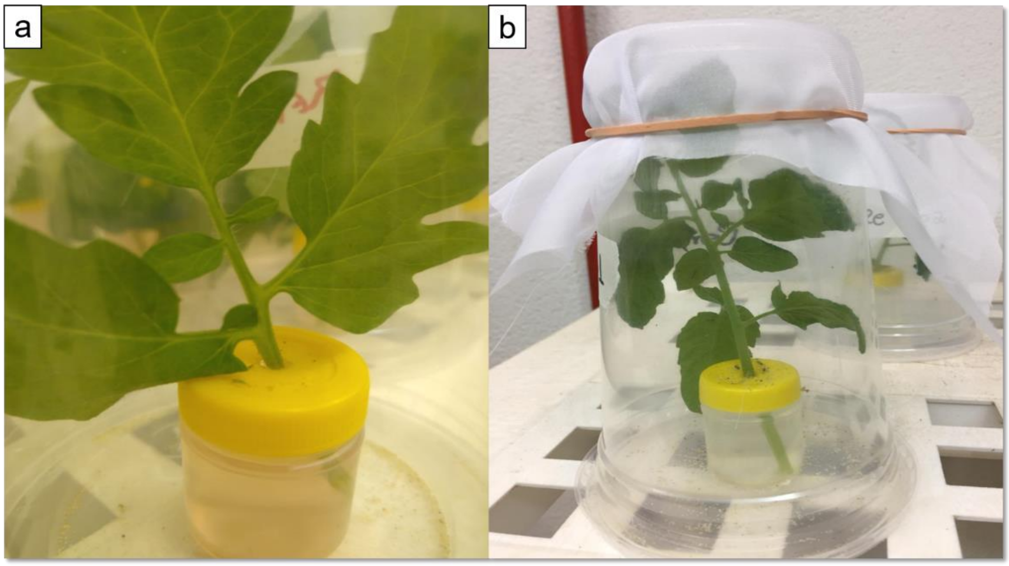

Figure S1 a) Detail of the tomato leave inserted in the orifice of the flask (20 mL) with a lid containing an orifice, previously filled with water to maintain turgidity during the bioassay; b) the flask with the leave inside the cage (12 cm high × 5 cm diameter) covered with voile fabric to prevent accumulation of toxic gases and retain the insects, representing the experimental unit.

3. SUBLETHAL AND TRANSGENERATIONAL EFFECTS OF REDUCED-RISK INSECTICIDES ON *Macrolophus basicornis* (HEMIPTERA: MIRIDAE)

Abstract

Reduced-risk insecticides and mirid predators have been used to control *Bemisia tabaci* (Hemiptera: Aleyrodidae) in tomato crops. However, even when causing low mortality to the beneficial insects, these products might cause side effects. This study investigated the sublethal and transgenerational effects of buprofezin, cyantraniliprole, and spiromesifen on *Macrolophus basicornis* (Hemiptera: Miridae). After 72 h of exposure of third-instar nymphs and adults to residues on tomato leaves, adult couples were formed and kept in cages with residue-free tomato leaves. The leaves were changed every 48 h and the offspring were assessed in 6 different periods. Body size was assessed by measuring the hind-tibia length of adults (F_0) from exposed nymphs and in three different offspring groups. None of the insecticide residues caused reduction of offspring populations or affected the body size of adults in generation F_0. Regardless, buprofezin and spiromesifen reduced the tibia length of adults (F_1) from exposed nymphs assayed in the third mating period. Cyantraniliprole did not affect any parameter and could be recommended for control of *B. tabaci* in association with *M. basicornis* releases. This study may contribute to future field assays of the compatibility of these insecticides with *M. basicornis*.

Keywords: Biological control; chemical control; ecotoxicology; integrated pest management; selectivity; side effects.

3.1. Introduction

Sustainable agricultural practices are a reality in several countries (Xie et al., 2019). With integrated pest management (IPM), the use of chemical and biological controls in combination is possible, considering all factors for which neither tactic seriously harms the other (Barzman et al., 2015). To control the serious pests *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Bemisia tabaci* (Gennadius) Biotype B (Hemiptera: Aleyrodidae) in tomato crops, generalist predatory bugs are used as biological-control agents in conjunction with reduced-risk insecticides (Calvo et al., 2012; Desneux et al., 2022). To assure compatibility of these methods in the field, ecotoxicological studies are necessary to determine the safety of active ingredients of insecticides for each species of natural enemy.

In a study carried out with the whitefly predator *Macrolophus basicornis* (Stal) (Hemiptera: Miridae), the insecticides buprofezin, cyantraniliprole, and spiromesifen did not cause acute toxicity to nymphs or adults and were therefore classified as harmless or reduced-risk (Matioli et al., 2021). Although these insecticides were not toxic to the mirid, investigations of sublethal and transgenerational effects of reduced-risk insecticides on natural enemies have shown that acute toxicity is an insufficient criterion to identify physiological selectivity (Passos et al., 2018; Matioli et al., 2019; Soares et al., 2019; 2020). Effects on reproduction can reduce a predator population over the long term, and morphological effects can contribute to transgenerational problems (Passos et al., 2018). To safely combine insecticides with releases of this natural enemy, it is essential to evaluate their side effects.

Macrolophus basicornis is a whitefly predator native to Latin America and a promising biological-control agent, the subject of several studies in Brazil (Silva et al., 2016; Passos et al., 2017; 2018; Soares et al., 2019; Matioli et al., 2021). Passos et al. (2018) and Soares et al. (2019) reported low acute toxicity but side effects of insecticides used to control *T. absoluta* on nymphs and adults of the predator. The insect growth regulators (IGR) triflumuron, tebufenozide, teflubenzuron, and methoxifenozide decreased the offspring production by exposed insects (Passos et al., 2018; Soares et al., 2019). Also, teflubenzuron and methoxifenozide caused a reduction in body size of adult females of the F_1 generation (Passos et al., 2018).

For another mirid predator, *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae), different application rates of cyantraniliprole caused problems with reproduction (Amarasekare and Shearer, 2013). The number of offspring decreased when the whitefly parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (Drobnjaković and Marčić, 2020) was exposed to buprofezin, and when the pollinator *Bombus terrestris* Linnaeus (Hyminoptera: Apidae) was exposed to spiromesifen (Besard et al., 2010).

A previous study hypothesized that insecticides used to control *B. tabaci* may cause sublethal and transgenerational effects on *M. basicornis*, although their acute toxicity is low (Matioli et al., 2021). This study evaluated the sublethal and transgenerational effects of buprofezin, cyantraniliprole, and spiromesifen on the predator *M. basicornis*, complementing previous evaluations (Matioli et al., 2021). Effects on offspring production and morphological changes in the F_1 generation were evaluated.

3.2. Material and methods

3.2.1. Insects

The rearing colony were maintained at the Integrated Pest Management Laboratory, with a lineage obtained from the Laboratory of Insect Biology at the Entomology and Acarology Department of the “Luiz de Queiroz” College of Agriculture (ESALQ/USP), Piracicaba, Brazil. The insects were originally collected in tobacco (*Nicotiana tabacum* L.) fields in the state of Minas Gerais, Brazil (21_08.5960 S and 45_03.4660 W, 808 m a.s.l.) (Bueno et al., 2013). For the *M. basicornis* rearing colony, adults and nymphs were kept on tobacco plants in acrylic cages (60 × 30 × 30 cm) and fed ad libitum with eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Bueno et al., 2013). The insects were reared in a climate-controlled room at 25 ± 2 °C, 70 ± 10% RH and 12:12 h (L:D). To control age and to obtain insects for the experiments, adults were kept for about 48 h in cages with tobacco plants for oviposition, and were then removed and taken to new plants in a different cage. Both third-instar nymphs and adults (< 3 days old) were obtained by this procedure (Passos et al., 2018).

3.2.2. Insecticides

The insecticides are used in Brazilian tomato crops to control the whitefly *B. tabaci*. They were tested on *M. basicornis* at the highest recommended field doses (Table 1).

Table 1. Active ingredient, trade name, chemical group, mode of action, and field application rate of the insecticides used to control *Bemisia tabaci* in tomato crops in Brazil, for which sublethal and transgenerational effects were evaluated on the predator *Macrolophus basicornis*.
OD (Oil Dispersion); SC (Suspension Concentrate); WP (Wettable Powder); c.p. (commercial product); a.i. (active ingredient).*MAPA (2022).

Active ingredient	Trade name	Chemical group	Exposure route	Mode of action	Field rate* (g or mL100 L^{-1})	
					a.i.	c.p.
Buprofezin	Applaud® 25 WP	Thiadiazinone	Contact	Chitin synthesis inhibitors	50	200
Cyantraniliprole	Benevia® 10 OD	Diamide	Systemic and contact	Ryanodine receptor modulator	12.5	125
Spiromesifen	Oberon® 24 SC	Tetronic and tetramic acid derivatives	Contact and ingestion	Inhibitors of acetyl CoA carboxylase	14.4	60

3.2.3. Exposure of nymphs and adults of *Macrolophus basicornis* to insecticides

The insecticides evaluated for sublethal and transgenerational effects were considered innocuous for third-instar nymphs and adults of *M. basicornis* in previous tests (Matioli et al., 2021). The present bioassays were performed with both nymphs and adults of *M. basicornis*.

The insecticides and control treatment (distilled water) were sprayed until the run-off point (~50 mL per plant) on six 5-week-old tomato plants (cv. Santa Clara), using a hand-held sprayer (Light Sprayer—Breeze, 500-mL capacity; Guarany, São Paulo, Brazil) in a greenhouse. The leaves were allowed to dry for 2 h and then collected and transferred to the laboratory under controlled conditions [(25 ± 2 °C, 70 ± 10% RH and 12:12 h (L: D)]. To maintain the turgidity of the leaves during the bioassay, the petioles were inserted into a flask (20 mL) filled with water, through a hole in the lid of the flask. Then, each flask was transferred to a cage (12 cm high × 5 cm diameter) (PET crystal, 500 mL; Copozan, Orleans, Brazil), with each unit representing one repetition (Matioli et al., 2021). Fifteen third-instar nymphs or 15 adults of *M. basicornis* (< 3 days old) were released into each cage, which was covered with voile fabric to prevent the insects from escaping and allow air circulation. As an alternative food source for *M. basicornis*, eggs of *E. kuehniella* (~ 0.4 g) were offered per unit. The design was completely randomized, with 6 replicates per treatment. The insects were kept for 72 h in the cages to allow contact with the insecticide residues.

3.2.3.1. Effects on offspring number of nymphs and adults

At the end of the 72-h period, the insects were removed from the cages to assess sublethal effects. In the assay of adults, the couples were formed immediately after the 72-h residue contact period. In the assay of adults developed from exposed nymphs, as soon as the third-instar nymphs began to hatch, they were counted and then separated into new cages for development to adulthood. To assess the offspring over time, 15 couples per treatment were placed in cages containing an insecticide-free tomato leaf for oviposition. Every 48 h, these leaves were replaced to assess the number of nymphs per couple in this time of oviposition. In this trial, six evaluations per couple were made over time, as follows: evaluation 1 = offspring from 0 to 48 h; evaluation 2 = offspring from 48 to 96 h; evaluation 3 = offspring from 96 to 144 h; evaluation 4 = offspring from 144 to 192 h; evaluation 5 = offspring from 192 to 240 h; and evaluation 6 = offspring from 240 to 288 h.

3.2.3.2. Effects on offspring body size

As soon as the nymphs began to hatch, they were counted and then separated into new cages so that they could develop to adulthood. To evaluate the effect on the body size of individuals from the F_1 generation in both bioassays, as soon as the adults were formed, 15 females and 15 males were randomly collected at three different times of the maternal generation (F_0) oviposition throughout the entire experiment. The adults (F_1) were collected from: period 1 (0 to 96 h), period 2 (96 to 192 h), and period 3 (192 to 288 h). For the bioassay with third-instar nymphs, 15 females and 15 males (F_0) that had contact with the insecticides were also evaluated. To estimate the sublethal and transgenerational effects on the insects caused by the insecticides, the right hind legs of adults were measured, following the method of Querino and Zucchi (2011) as modified by Matioli et al. (2019). The tibia size is proportional to the body size and transgenerational effects can be observed in the fitness of biological-control agents after insecticide residues contact (Thorne et al., 2006; Passos et al., 2018;

Soares et al., 2019; Matioli et al., 2019).Tibias were mounted on glass slides and measured with a Zeiss light microscope at 40 × magnification (Carl Zeiss do Brasil Ltd., São Paulo, SP, Brazil), coupled to a digital length-measuring device (Wild MMS 235).

3.2.4. Statistical analysis

The offspring data were analyzed by means of generalized linear models (GLM), assuming a Poisson distribution, and when overdispersion was detected, and the standard errors were corrected using a Quasi-Poisson model. In this study, all fitted models showed overdispersion. The significance level considered was 5% probability. The analyses were performed using the R 4.1.1 (2021) software, and the models were adjusted using the “glm” function of the “stats” package.

The data from tibia measurements were checked for normality and homoscedasticity, using Shapiro-Wilk’s and Bartlett’s tests. The assumptions of ANOVA were not met, so we used the Kruskal-Wallis test as non-parametric one-way ANOVA, with the dunn.test package, with Bonferroni correction post-hoc (*P* = 0.05), always using R 4.1.1 (R Development Core Team, 2021) software with the “ExpDes”, “easyanova”, “dunn.test” packages.

3.3. Results

The insecticides did not cause effects on offspring from *M. basicornis* adults or third-instar nymphs that had had contact with the residues. In all six evaluations, the mean numbers of nymphs in the control and insecticide treatments were similar to the control treatment and did not differ significantly (Tables 2 and 3).

Table 2. Numbers (means ± SE) of *Macrolophus basicornis* offspring (F_1) of adults of parental generation (F_0) that as third-instar nymphs had been exposed to insecticide residues for 72 h, in six evaluations.

Treatment	**Evaluations***					
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**
Control	4.0 ± 1.4	9.4 ± 1.7	11.3 ± 1.9	12.4 ± 2.0	9.1 ± 1.8	6.1 ± 1.2
Buprofezin	2.7 ± 1.0	7.2 ± 1.5	10.8 ± 1.8	10.9 ± 1.5	8.2 ± 2.4	5.7 ± 1.6
Cyantraniliprole	2.3 ± 1.0	7.2 ± 1.7	7.1 ± 1.6	9.7 ± 1.9	12.9 ± 2.6	7.8 ± 1.6
Spiromesifen	3.4 ± 1.4	7.1 ± 2.0	5.0 ± 1.5	11.1 ± 2.0	9.1 ± 1.7	8.1 ± 2.1
F	0.394	0.387	2.786	0.344	0.849	0.517
p	0.758^{ns}	0.763^{ns}	0.051^{ns}	0.794^{ns}	0.474^{ns}	0.673^{ns}

Means in the columns did not differ significantly, using generalized linear models (GLM) with Poisson distribution ($p < 0.05$).
* Evaluations: 1st = 0–48 h; 2nd = 48–96 h; 3rd = 96–144 h; 4th = 144–192 h; 5th = 192–240 h; 6th = 240–288 h, after couples were formed.
^{ns} Non-significant at 5% probability of error by the *F* test.

Table 3. Numbers (means ± SE) of *Macrolophus basicornis* offspring (F_1) of adults of parental (F_0) generation that had been exposed to insecticide residues for 72 h, in six evaluations.

Treatment	**Evaluations***					
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**
Control	11.2 ± 1.0	11.3 ± 0.9	10.8 ± 1.3	6.1 ± 1.3	10.7 ± 1.0	3.5 ± 0.9
Buprofezin	11.8 ± 0.8	11.7 ± 1.4	8.5 ± 1.1	6.3 ± 1.0	7.4 ± 1.3	4.5 ± 1.4
Cyantraniliprole	11.7 ± 0.8	12.1 ± 0.7	8.4 ± 1.1	7.5 ± 1.2	8.5 ± 1.2	3.9 ± 1.1
Spiromesifen	10.7 ± 0.6	11.3 ± 1.4	9.7 ± 1.1	5.7 ± 0.9	10.7 ± 1.2	2.3 ± 1.1
F	0.342	0.100	0.888	0.425	1.741	0.702
p	0.795^{ns}	0.959^{ns}	0.453^{ns}	0.736^{ns}	0.169^{ns}	0.555^{ns}

Means in the columns did not differ significantly, using generalized linear models (GLM) with Poisson distribution ($p < 0.05$).
* Evaluations: 1st = 0–48 h; 2nd = 48–96 h; 3rd = 96–144 h; 4th = 144–192 h; 5th = 192–240 h; 6th = 240–288 h, after couples were formed.
^{ns} Non-significant at 5% probability of error by the *F* test.

The insecticides did not reduce the tibia length of adults that had contacted the residues in the third instar (Table 4). However, spiromesifen caused a reduction in tibia length of female offspring ($\chi^2 = 11.091$; $p = 0.011$; $df = 3$), and buprofezin in male offspring ($\chi^2 = 11.145$; $p = 0.011$; $df = 3$) at period 3 of oviposition (Table 5).

Table 4. Tibia length (mean ± SE) (mm) of adult *Macrolophus basicornis* (F_0) exposed as third-instar nymphs to insecticide residues for 72 h.

Gender	Treatments				χ^2	*p*
	Control	Buprofezin	Cyantraniliprole	Spiromesifen		
Females	1.86 ± 0.02	1.85 ± 0.02	1.89 ± 0.02	1.89 ± 0.02	2.451	0.482
Males	1.98 ± 0.03	2.07 ± 0.03	2.11 ± 0.04	2.08 ± 0.04	3.392	0.335

Means in the lines did not differ significantly using the Kruskal-Wallis test as non-parametric one-way ANOVA with Dunn with Bonferroni correction post-hoc ($p < 0.05$).

Table 5. Tibia length (mean ± SE) (mm) of offspring (F_1) of adults of *Macrolophus basicornis* that had been exposed as third-instar nymphs (F_0) to insecticide residues for 72 h, in three different periods.

Treatments	[a]Period 1		[b]Period 2		[c]Period 3	
	Females	Males	Females	Males	Females	Males
Control	1.84 ± 0.02	2.03 ± 0.01	1.81 ± 0.02	2.01 ± 0.03	1.86 ± 0.02 a	2.05 ± 0.03 a
Buprofezin	1.84 ± 0.02	2.00 ± 0.02	1.87 ± 0.01	2.02 ± 0.02	1.88 ± 0.03 a	1.95 ± 0.02 b
Cyantraniliprole	1.83 ± 0.02	2.07 ± 0.02	1.86 ± 0.01	2.02 ± 0.02	1.85 ± 0.01 a	2.00 ± 0.02 ab
Spiromesifen	1.89 ± 0.03	2.00 ± 0.02	1.88 ± 0.03	2.06 ± 0.03	1.73 ± 0.03 b	2.05 ± 0.02 a
χ^2	2.735	7.261	3.287	1.168	11.091	11.145
p	0.434 ^{ns}	0.064 ^{ns}	0.349 ^{ns}	0.761 ^{ns}	0.011	0.011

Means followed by the same letters in the columns do not differ significantly using the Kruskal-Wallis test as non-parametric one-way ANOVA with Dunn with Bonferroni correction post-hoc ($p < 0.05$).
[a]Period 1: offspring from couples mated 0 to 96 h after exposure to the treatments.
[b]Period 2: offspring from couples mated 96 to 192 h after exposure to the treatments.
[c]Period 3: offspring from couples mated 192 to 288 h after exposure to the treatments.

The insecticides affected the morphology of offspring of previously exposed adults. Cyantraniliprole and spiromesifen caused an increase in the body size of female offspring ($\chi^2 = 34.839$; $p < 0.001$; $df = 3$) and male offspring ($\chi^2 = 40.171$; $p < 0.001$; $df = 3$) at period 1 (Table 6). Also, male offspring showed a significant difference ($\chi^2 = 12.931$; $p = 0.005$; $df = 3$) caused by cyantraniliprole at period 2 (Table 6).

Table 6. Tibia length (mean ± SE) (mm) of offspring (F_1) of adults of *Macrolophus basicornis* (F_0) that had been exposed to insecticide residues for 72 h, in three different periods.

Treatments	**[a]Period 1**		**[b]Period 2**		**[c]Period 3**	
	Females	Males	Females	Males	Females	Males
Control	1.71 ± 0.02 b	1.75 ± 0.03 b	1.76 ± 0.02	1.91 ± 0.02 b	1.81 ± 0.02	1.98 ± 0.02
Buprofezin	1.64 ± 0.03 b	1.80 ± 0.02 b	1.76 ± 0.03	1.90 ± 0.03 b	1.86 ± 0.02	2.02 ± 0.02
Cyantraniliprole	1.82 ± 0.01 a	1.98 ± 0.01 a	1.83 ± 0.02	2.01 ± 0.02 a	1.82 ± 0.02	1.93 ± 0.03
Spiromesifen	1.88 ± 0.02 a	2.02 ± 0.02 a	1.84 ± 0.02	1.98 ± 0.02 ab	1.85 ± 0.02	1.97 ± 0.02
χ^2	34.839	40.171	7.475	12.931	2.817	2.215
p	< 0.001	< 0.001	0.058^{ns}	0.005	0.421^{ns}	0.529^{ns}

Means followed by the same letters in the columns do not differ significantly using the Kruskal-Wallis test as non-parametric one-way ANOVA with Dunn with Bonferroni correction post-hoc ($p < 0.05$).
[a]Period 1: offspring from couples mated 0 to 96 h after exposure to the treatments.
[b]Period 2: offspring from couples mated 96 to 192 h after exposure to the treatments.
[c]Period 3: offspring from couples mated 192 to 288 h after exposure to the treatments.

3.4. Discussion

The insecticides buprofezin, cyantraniliprole, and spiromesifen were previously evaluated according to their acute toxicity to *M. basicornis*, and all were considered reduced-risk (Matioli et al., 2021). However, to determine whether these products, which appeared to be physiologically safe in laboratory conditions, can be used when these insects are released as an IPM tool in the field, sublethal and transgenerational effects of the residues on this natural predator were investigated.

Contact of specimens with the insecticide residues did not compromise the offspring population over time, and there were no differences in the adult tibia length of nymphs exposed to treated tomato leaves. However, in the nymphs' offspring, spiromesifen and buprofezin residues caused a reduction in tibia length in adults from the third mating period (192 to 288 h). These parameters were evaluated because, even though these insecticides did not directly kill *M. basicornis* (Matioli et al., 2021), they can cause reductions in fecundity and fertility as well as problems in embryogenesis (Ishaaya et al., 1988; Wang et al., 2017; Bensafi-Gheraibia et al., 2021). Transgenerational effects are often assessed in reduced-risk insecticides as a change in the size of the hind tibia of the insects (Passos et al., 2018; Soares et al., 2019; Matioli et al., 2019), because the tibia is proportional to the body size and body size can affect the fitness of biological-control agents (Thorne et al., 2006).

Buprofezin is an insect growth regulator (IGR) that acts on sucking insects (Hemiptera) by inhibiting chitin biosynthesis (Izawa et al., 1985), and can also affect embryogenesis, oviposition, and egg fertility (Ishaaya et al., 1988). Its sublethal effects, such as offspring reduction, have been reported in natural enemies such as the beetle *Chilocorus* *bipustulatus* L. (Coleoptera: Coccinellidae) (Mendel et al., 1994) and the whitefly parasitoid *E. formosa* (Drobnjaković and Marčić, 2020). In the present study, the number of offspring was not reduced. However, a reduction occurred in the body size of the male offspring from nymphs that came into contact with the insecticide. It is possible that buprofezin caused a lipid maldistribution in the oocyte at the time of formation of male eggs (Ziegler & Van Antwerpen, 2006; Cremonez et al., 2017). As a result of the lipid misallocation, the insects did not obtain enough energy to reach a size similar to those in the control treatment.

Cyantraniliprole is a diamide that activates the ryanodine receptors (RyR) of sucking pests, which dysregulates the intracellular calcium release, inhibiting muscle contraction (Selby et al., 2013). We observed no sublethal effects caused by cyantraniliprole on *M. basicornis* offspring. Similar results have been detected in reproduction of the parasitoid *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae) (Abbes et al., 2015). Nevertheless, several studies have reported sublethal effects of cyantraniliprole on natural enemies (Amarasekare et al., 2016; Mills et al., 2016; Jiang et al., 2019; Hasan et al., 2020). Fecundity and fertility were reduced when the predatory hemipteran *D. brevis* was exposed to different rates of the diamide (Amarasekare and Shearer, 2013). Cyantraniliprole may act in different ways depending on the species of natural enemy. Detoxification of xenobiotics allows many insects to tolerate more contact with insecticides than others (Lu et al., 2021). This may be one of the reasons that *M. basicornis* offspring was not affected by cyantraniliprole in the parameters evaluated here.

Spiromesifen is an insecticide and acaricide, a tetronic and tetramic acid derivative. Its action inhibits the acetyl-CoA carboxylase, which can affect fecundity, development, and lipid biosynthesis (Bretschneider et al., 2003). Body size is related to the biochemical composition of proteins, carbohydrates, and lipids. Interference with lipid biosynthesis changes the morphology of the insect (Bouabida et al., 2017). This may explain the reduction in tibia size caused by spiromesifen in the *M. basicornis* F_1 generation from the third mating period when the third instar nymphs had contacted the residues. Although this compound did not affect the reproduction of this predator, Besard et al. (2010) observed that the pollinator *B. terrestris* produced fewer offspring when the insects were exposed orally via sugar water. This difference probably occurred because of the route of exposure.

In contrast to all these results, the insecticides cyantraniliprole and spiromesifen caused an increase in body size of the offspring of adults exposed to the residues in the first and second mating period. Pesticides can cause stress tolerance and environmental adaptation in insects (Lee & Gelembiuk, 2008). Consequently, sublethal insecticide exposure leads to positive effects over generations (Margus et al., 2019). Positive effects have been reported in predators and parasitoids exposed to genetically modified (GMO) crops (Lövei et al., 2009) and to pesticides (Guedes & Cutler, 2014). Although we found these results, it is essential to understand how the insecticides caused the increase in body size.

Few studies have assessed sublethal and transgenerational effects of insecticides on *M. basicornis* with different active ingredients used in Brazil to control *T. absoluta* (Passos et al., 2018; Soares et al., 2019). These are the first data on reduced-risk insecticides used to control *B. tabaci*, and may contribute to future IPM decisions. The assays conducted here are for the purpose of recognizing whether the products tested might cause negative effects. We can affirm that almost no transgenerational effects were observed in the evaluations. Importantly, these laboratory tests were performed under different conditions from those possibly found in the field. The results in field conditions will likely not be more drastic than those in this study; on the contrary, they are likely to be milder. The actual effects on this natural enemy could be completely different in the field, but the data in controlled conditions presented here are reliable and will certainly be used as a reference. We suggest that future bioassays should be performed with these insecticides on *M. basicornis* in tomato fields, to assure the safety of this natural enemy and efficient control of *B. tabaci*.

3.5. Conclusions

Among the insecticides evaluated, buprofezin and spiromesifen caused reduction in tibia size of offspring (F_1) in specific maternal mating periods (F_0). On the other hand, cyantraniliprole and spiromesifen caused positive effects on the F_1 generation, increasing the body size of adults in the first maternal mating period (F_0). Considering these parameters, cyantraniliprole can be recommended to control *B. tabaci* in combination with *M. basicornis* releases. Although the insecticides buprofezin and spiromesifen do not cause acute toxicity, they did cause side effects in controlled conditions. However, these insecticides require further study, mainly field assays, to confirm these results.

References

Abbes, K., Biondi, A., Kurtulus, A., Ricupero, M., Russo, A., Siscaro, G., Chermiti, B., Zappalà, L., 2015. Combined non-target effects of insecticide and high temperature on the parasitoid *Bracon nigricans*. PLoS One 10, e0138411. https://doi.org/10.1371/journal.pone.0138411

Amarasekare, K.G., Shearer, P.W., 2013. Laboratory bioassays to estimate the lethal and sublethal effects of various insecticides and fungicides on *Deraeocoris brevis* (Hemiptera: Miridae). J. Econ. Entomol. 106, 776–785. https://doi.org/10.1603/EC12432

Amarasekare, K.G., Shearer, P.W., Mills, N.J., 2016. Testing the selectivity of pesticide effects on natural enemies in laboratory bioassays. Biol. Control 102, 7–16. https://doi.org/10.1016/j.biocontrol.2015.10.015

Barzman, M., Bàrberi, P., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J.E., Kiss, J., Kudsk, P., Lamichhane, J.R., Messéan, A., Moonen, A.C., Ratnadass, A., Ricci, P., Sarah, J.L., Sattin, M., 2015. Eight principles of integrated pest management. Agron. Sustain. Dev. 35, 1199–1215. https://doi.org/10.1007/s13593-015-0327-9

Bensafi-Gheraibia, H., Kissoum, N., Hamida, Z.C., Farine, J.P., Soltani, N., 2021. Topical bioassay of Oberon® on *Drosophila melanogaster* pupae: delayed effects on ovarian proteins, cuticular hydrocarbons and sexual behaviour. Invertebr. Reprod. Dev. 65, 35–47. https://doi.org/10.1080/07924259.2020.1862315

Besard, L., Mommaerts, V., Vandeven, J., Cuvelier, X., Sterk, G., Smagghe, G., 2010. Compatibility of traditional and novel acaricides with bumblebees (*Bombus terrestris*): A first laboratory assessment of toxicity and sublethal effects. Pest Manag. Sci. 66, 786–793. https://doi.org/10.1002/ps.1943

Bouabida, H., Tine-djebbar, F., Tine, S., Soltani, N., 2017. Activity of a lipid synthesis inhibitor (spiromesifen) in *Culiseta longiareolata* (Diptera: Culicidae). Asian Pac. J. Trop. Biomed. 7, 1120–1124. https://doi.org/10.1016/j.apjtb.2017.10.015

Bretschneider, T., Benet-Buchholz, J., Fischer, R., Nauen, R., 2003. Spirodiclofen and spiromesifen - Novel acaricidal and insecticidal tetronic acid derivatives with a new mode of action. Chimia 57, 697–701. https://doi.org/10.2533/000942903777678588

Bueno, V.H.P., van Lenteren, J.C., Lins, J.C., Calixto, A.M., Montes, F.C., Silva, D.B., Santiago, L.D., Pérez, L.M., 2013. New records of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) predation by Brazilian Hemipteran predatory bugs. J. Appl. Entomol. 137, 29–34. https://doi.org/10.1111/jen.12017

Calvo, F.J., Lorente, M.J., Stansly, P.A., Belda, J.E., 2012. Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. Entomol. Exp. Appl. 143, 111–119. https://doi.org/10.1111/j.1570-7458.2012.01238.x

Cremonez, P.S.G., Pinheiro, D.D.O., Falleiros, Â.M.F., Neves, P.M.O.J., 2017. Performance of reproductive system of *Dichelops melacanthus* (Hemiptera: Pentatomidae) subjected to buprofezin and pyriproxyfen: morphological analysis of ovarioles and testes. Semin. Cienc. Agrar. 38, 2279–2292. https://doi.org/10.5433/1679-0359.2017v38n4Supl1p2279

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M.R., Chailleux, A., Guedes, R.N.C., Karimi, J., Konan, K.A.J., Lavoir, A.-v., Luna, M.G., Perez-Hedo, M., Urbaneja, A., Verheggen, F.J., Zappalà, L., Abbes, K., Ali, A., Bayram, Y., … Biondi, A., 2022. Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. J. Pest Sci. 95, 17–39. https://doi.org/10.1007/s10340-021-01442-8

Drobnjaković, T., Marčić, D., 2020. Acute toxity and sublethal effects of pymetrozine on the whitefly parasitoid *Encarsia formosa* Gahan. Pestic. Phytomed. (Belgrade) 35, 81–95. https://doi.org/10.2298/pif2002081d

Guedes, R.N.C., Cutler, G.C., 2014. Insecticide-induced hormesis and arthropod pest management. Pest Manag. Sci. 70, 690–697. https://doi.org/10.1002/ps.3669

Hasan, F., Mahboob, S., Al-Ghanim, K.A., Al-Misned, F., Dhillon, M.K., Manzoor, U., 2020. Ecotoxicity of neonicotinoids and diamides on population growth performance of *Zygogramma bicolorata* (Coleoptera: Chrysomelidae). Ecotoxicol. Environ. Saf. 203, 110998. https://doi.org/10.1016/j.ecoenv.2020.110998

Ishaaya, I., Mendelson, Z., Melamed-Madjar, V., 1988. Effect of Buprofezin on embryo genesis and progeny formation of sweet potato whitefly (Homoptera: Aleyrodidae). J. Econ. Entomol. 81(3), 781–784. https://doi.org/10.1093/jee/81.3.781

Izawa, Y., Uchida, M., Sugimoto, T., Asai, T., 1985. Inhibition of chitin biosynthesis by buprofezin analogs in relation to their activity controlling *Nilaparvata lugens* Stål. Pestic. Biochem. Physiol. 24, 343–347. https://doi.org/10.1016/0048-3575(85)90145-2

Jiang, J., Liu, X., Zhang, Z., Liu, F., Mu, W., 2019. Lethal and sublethal impact of sulfoxaflor on three species of *Trichogramma* parasitoid wasps (Hymenoptera: Trichogrammatidae). Biol. Control 134, 32–37. https://doi.org/10.1016/j.biocontrol.2019.04.001

Lee, C.E., Gelembiuk, G.W., 2008. Evolutionary origins of invasive populations. Evolut. Appl. 1, 427–448. https://doi.org/10.1111/j.1752-4571.2008.00039.x

Lövei, G.L., Andow, D.A., Arpaia, S., 2009. Transgenic insecticidal crops and natural enemies: A detailed review of laboratory studies. Environ. Entomol. 38, 293–306. https://doi.org/10.1603/022.038.0201

Lu, K., Song, Y., Zeng, R., 2021. The role of cytochrome P450-mediated detoxification in insect adaptation to xenobiotics. Curr. Opin. Insect Sci. 43, 103–107. https://doi.org/10.1016/j.cois.2020.11.004

MAPA, 2022. Ministério da Agricultura, Pecuária e Abastecimento, Brazil. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons, accessed on 10 March 2022.

Margus, A., Piiroinen, S., Lehmann, P., Tikka, S., Karvanen, J., Lindström, L., 2019. Sublethal pyrethroid insecticide exposure carries positive fitness effects over generations in a pest insect. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-019-47473-1

Matioli, T.F., da Silva, M.R., Pazini, J. de B., Barroso, G., Vieira, J.G.A., Yamamoto, P.T., 2021. Risk assessment of insecticides used in tomato to control whitefly on the predator *Macrolophus basicornis* (Hemiptera: Miridae). Insects 12, 1092. https://doi.org/10.3390/insects12121092

Matioli, T.F., Zanardi, O.Z., Yamamoto, P.T., 2019. Impacts of seven insecticides on *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). Ecotoxicology 28, 1210–1219. https://doi.org/10.1007/s10646-019-02129-8

Mendel, Z.., Blumberg, D., Ishaaya I., 1994. Effects of some insect growth regulators on natural enemies of scale insects (*Hom.: Coccoidea*). Entomophaga 39, 199–209.

Mills, N.J., Beers, E.H., Shearer, P.W., Unruh, T.R., Amarasekare, K.G., 2016. Comparative analysis of pesticide effects on natural enemies in western orchards: A synthesis of laboratory bioassay data. Biol. Control 102, 17–25. https://doi.org/10.1016/j.biocontrol.2015.05.006

Passos, L.C., Soares, M.A., Collares, L.J., Malagoli, I., Desneux, N., Carvalho, G.A., 2018. Lethal, sublethal and transgenerational effects of insecticides on *Macrolophus basicornis*, predator of *Tuta absoluta*. Entomol. Gen. 38, 127–143. https://doi.org/10.1127/entomologia/2018/0744

Passos, L.C., Soares, M.A., Costa, M.A., Michaud, J.P., Freire, B.C., Carvalho, G.A., 2017. Physiological susceptibility of the predator *Macrolophus basicornis* (Hemiptera: Miridae) to pesticides used to control of *Tuta absoluta* (Lepidoptera: Gelechiidae). Biocontrol Sci. Technol. 27, 1082–1095

Querino, R., Zucchi, R.A., 2011. Guia de identificação de *Trichogramma* para o Brasil. Brasília, DF: Embrapa Informação Tecnológica. 103 p.

R Development Core Team, 2021. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Available online: https://www.R-project.org/ (accessed on 15 January 2022).

Selby, T.P., Lahm, G.P., Stevenson, T.M., Hughes, K.A., Cordova, D., Annan, I.B., Barry, J.D., Benner, E.A., Currie, M.J., Pahutski, T.F., 2013. Discovery of cyantraniliprole, a potent and selective anthranilic diamide ryanodine receptor activator with cross-spectrum insecticidal activity. Bioorg. Med. Chem. Lett. 23, 6341–6345. https://doi.org/10.1016/j.bmcl.2013.09.076

Silva, W.M., Berger, M., Bass, C., Williamson, M., Moura, D.M.N., Ribeiro, L.M.S., Siqueira, H.A.A., 2016. Mutation (G275E) of the nicotinic acetylcholine receptor α6 subunit is associated with high levels of resistance to spinosyns in *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Pestic. Biochem. Physiol. 131, 1–8. https://doi.org/10.1016/j.pestbp.2016.02.006

Soares, M.A., Carvalho, G.A., Campos, M.R., Passos, L.C., Haro, M.M., Lavoir, A.V., Biondi, A., Zappalà, L., Desneux, N., 2020. Detrimental sublethal effects hamper the effective use of natural and chemical pesticides in combination with a key natural enemy of *Bemisia tabaci* on tomato. Pest Manag. Sci. 76, 3551–3559. https://doi.org/10.1002/ps.5927

Soares, M.A., Passos, L.C., Campos, M.R., Collares, L.J., Desneux, N., Carvalho, G.A., 2019. Side effects of insecticides commonly used against *Tuta absoluta* on the predator *Macrolophus basicornis*. J. Pest Sci. 92, 1447–1456. https://doi.org/10.1007/s10340-019-01099-4

Thorne, A.D., Pexton, J.J., Dytham, C., Mayhew, P.J., 2006. Small body size in an insect shifts development, prior to adult eclosion, towards early reproduction. Proc. R. Soc. B Biol. Sci. 273, 1099–1103. https://doi.org/10.1098/rspb.2005.3416

Wang, R., Zhang, W., Che, W., Qu, C., Li, F., Desneux, N., Luo, C., 2017. Lethal and sublethal effects of cyantraniliprole, a new anthranilic diamide insecticide, on *Bemisia tabaci* (Hemiptera: Aleyrodidae) MED. Crop Prot. 91, 108–113. https://doi.org/10.1016/j.cropro.2016.10.001

Xie, H., Huang, Y., Chen, Q., Zhang, Y., Wu, Q., 2019. Prospects for agricultural sustainable intensification: A review of research. Land 8, 1–27. https://doi.org/10.3390/land8110157

Ziegler, R., Van Antwerpen, R., 2006. Lipid uptake by insect oocytes. Insect Biochem. Mol. Biol. 36, 264–272. https://doi.org/10.1016/j.ibmb.2006.01.014

4. PERSISTENCE OF BROAD-SPECTRUM INSECTICIDES ON *Macrolophus basicornis* (HEMIPTERA: MIRIDAE) IN SEMI-FIELD

Abstract

Broad-spectrum insecticides are used to control *Bemisia tabaci* (Hemiptera: Aleyrodidae) in tomato. However, these products are harmful to the predator *Macrolophus basicornis* (Hemiptera: Miridae). The present study evaluated the persistence and toxicity of four broad-spectrum insecticides on this predator under semi-field conditions. The products were classified according to the criteria of the International Organization for Biological Control (IOBC). We also assessed insect behavior by video recording and quantification of acetamiprid residues by HPLC-UV. Treatments were applied in tomato plants in the maximum field recommended concentrations (MFRC) until run-off. For the analysis, tomato leaves were collected at 0, 5, 15 and 31 days after spraying (DAS). The results showed that bifenthrin was short life (class 1), etofenprox + acetamiprid and pyriproxyfen + acetamiprid were slightly persistent (class 2), and acetamiprid was persistent (class 4). The treatments caused no behavioral effects concerning to walking, resting, and cleaning time after each day evaluated. Acetamiprid residues over time were 30.80 mg a.i. L^{-1} (0 DAS), 29.97 mg a.i. L^{-1} (5 DAS), 21.56 mg a.i. L^{-1} (15 DAS) and 15.45 mg a.i. L^{-1} (31 DAS). Acetamiprid toxicity was proportional to the residues concentration over time. The results found can be used to define integrated pest management (IPM) tactics for the use of *M. basicornis* releases in periods when insecticides probably will not cause harmful effects under semi-fields conditions.

Keywords: Biological control, ecotoxicology, HPLC-UV, residual toxicity, predator.

4.1. Introduction

Macrolophus basicornis (Stal) (Hemipteran: Miridae) is a promising biological control agent of *Bemisia tabaci* (Gennadius) Biotype B (Hemiptera: Aleyrodidae) in Brazilian tomato crops. Its predation capacity is similar to the European mirids used to control pests in greenhouse (Bueno et al., 2013; Silva et al., 2016). In Brazil, the main way of controlling the whitefly in tomato plants is through insecticide applications, which are mostly broad-spectrum (MAPA, 2022). In addition to causing acute toxicity to natural enemies (Desneux et al., 2007), these insecticides can be persistent at the semi-field and field conditions (Wanumen et al., 2016; Passos et al., 2017). In the laboratory, some insecticides were harmful to *M. basicornis* (Matioli et al., 2021) and should be studied for their persistence in semi-field conditions.

One of the premises of integrated pest management (IPM) is the methods compatibilization for control the pests in a crop (Barzman et al., 2015). Normally, broad-spectrum insecticides are harmful to most arthropods, including natural enemies (Naranjo, 2001). It is known that the use of insecticides in time and in the right way can reduce the impacts on non-target organisms (Biondi et al., 2012; Carvalho et al., 2019). Therefore, studying the persistence of the chemicals, it is possible to predict the best time in which either chemical or biological controls could be used in the culture (Barzman et al., 2015). In order for the broad-spectrum insecticides used to control *B. tabaci* not to reach the released population of *M. basicornis*, persistence tests are essential to decide whether these methods can be used together in semi-field.

The methodologies proposed by the International Organization for Biological and Integrated Control of Noxious Plants and Animals (IOBC) are the most used for toxicological and persistence tests in semi-field conditions (Hassan et al., 1998). The broad-spectrum insecticides tested on *M. basicornis* were mostly under laboratory conditions (Wanumen et al., 2016; Passos et al., 2017; Passos et al., 2018; Soares et al., 2019a; Matioli et al., 2021) and few studies evaluated the persistence of these compounds. For example, abamectin, indoxacarb and chlorfenapyr were considered slightly persistent, and imidacloprid was persistent to *M. basicornis*, under greenhouse conditions (Wanumen et al., 2016; Passos et al., 2017). The persistence tests of broad-spectrum insecticides are important to know the possible effects of insecticides on the predator. However, other tests can be done in conjunction with acute toxicity to assess the behavior effects and the quantitative degradation of the product over time.

Broad-spectrum insecticides mostly act on the central nervous system of insects (Barreto et al., 2020). Therefore, many studies have evaluated the behavior of insects after being exposed to neurotoxic compounds (Ritzmann and Büschges, 2007; Büschges and Gruhn, 2007). Natural enemies were affected on locomotion in general, walking, cleaning, plant feeding, and predation capacity (Hassani et al., 2008; Martinou et al., 2014; Soares et al., 2019b; Passos et al., 2022). Other researchers observed that the degradation kinetics were strongly related to the toxicity of the neurotoxic insecticides to beneficial insects (Morales et al., 2019; Rakes et al., 2021). The residual foliar persistence of spinosad, dimethoate and imidacloprid in tomato, quantified by liquid chromatography, was proportionally associated to their toxicological effects on the mirid predator *Engytatus varians* (Distant) (Hemiptera: Miridae) (Morales et al., 2019).

In this study, we assessed the persistence and toxicological effects of four broad-spectrum insecticides (acetamiprid, bifenthrin, etofenprox + acetamiprid and pyriproxyfen + acetamiprid) on *M. basicornis* adults, their behavior effects and the residues quantification of acetamiprid over time. We hypothesized that these insecticides have a long residual period on tomato leaves and that they affect the behavior of insects that come into contact with the residues. The main objective is to understand how these insecticides act in semi-field conditions on the predator contributing to their implementation in IPM strategies in tomato crops.

4.2. Material and methods

4.2.1. Insects

The *M. basicornis* rearing colony was obtained from a strain (more than eight generation-old) originally of the municipalities of Ribeirao Vermelho and Lavras, Minas Gerais State, Brazil (21°08.596′S and 045°03.466′W, 808 m) (Bueno et al., 2013). The insects were kept in climate-controlled room at 25 ± 2 °C, 70 ± 10% RH and 12:12 h (L:D) at Integrated Pest Management Laboratory, in the Department of Entomology and Acarology, “Luiz de Queiroz” College of Agriculture (ESALQ/USP), Piracicaba, Brazil. Nymphs and adults were fed with eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) offered ad libitum and kept on tobacco plants in acrylic cages (60 × 30 × 30 cm). To obtain many specimens throughout the experiment, adults were placed in new tobacco plants every 48 h for oviposition. Approximately eight cages were maintained with insects in different ages to have enough adults with the same age to be used in the bioassay.

4.2.2. Insecticides

Insecticides tested for persistence and toxicological effects are those that were physiologically harmful to the predator in laboratory tests (Matioli et al., 2021). The concentrations used were the highest recommended field doses to control *B. tabaci* in tomato fields (Table 1).

Table 1. Active ingredient, trade name, chemical group, exposure route, mode of action and field application rate of the tested insecticides used to control *Bemisia tabaci* in tomato crops in Brazil.

Active ingredient	**Trade Name**	**Chemical Group**	**Exposure Route**	**Mode of Action**	**Field Rate* (g or mL 100 L^{-1})**	
					a.i.	**c.p.**
Acetamiprid	Mospilan WG	Neonicotinoid	Systemic	Competitive modulator of nicotinic acetylcholine receptors	21.8	30
Bifenthrin	Seizer® 10 EC	Pyrethroid	Contact and ingestion	Sodium channel modulator	1.5	15
Etofenprox+ acetamiprid	Eleitto® 30 + 16,7 OD	Pyrethroid + Neonicotinoid	Systemic and contact	Sodium channel modulator + competitive modulator of nicotinic acetylcholine receptors	12 + 6.8	40
Pyriproxyfen+ acetamiprid	Privilege® 10 + 20 OD	Pyridyloxypropyl ether + Neonicotinoid	Contact, ingestion, translaminar and systemic	Juvenile hormone mimics + Nicotinic acetylcholine receptor (NACHR) competitive modulators	3 + 6	30

EC (Emulsifiable Concentrate); OD (Oil Dispersion); WG (Water-dispersible Granules); c.p. (commercial product); a.i. (active ingredient). *MAPA (2022).

4.2.3. Persistence and toxicological effects of insecticides on *Macrolophus basicornis*

Tomato plants (cv. Santa Clara) were grown in plastic pots with a volume of 5 L (24.5 cm diameter × 18.8 cm high × 16.5 cm deep) containing a mix of soil plus substrate, and 200 g of NPK 4-14-8. With 12 expanded leaves, 15 plants per treatment were sprayed with a hand-held sprayer (Light Sprayer—Breeze, 500 mL capacity; Guarany; São Paulo, Brazil) until the run-off point. The control treatment consisted of distilled water only. The plants were maintained throughout the experiment in greenhouse conditions with plastic-covered, which reduced the visible radiation and UV radiation. The daily temperature of the greenhouse was among the maximum of 36 °C and minimum of 22 °C, and around 40% of relative humidity. The plants were used both for residue analysis and for leaves exposure to *M. basicornis* specimens for toxicity assessment.

To assess mortality over time, leaves were collected at 5, 15 and 31 days after spraying (DAS) and then transferred to controlled-room conditions for insect exposure to residues [temperature 25 ± 2 ºC, 70 ± 10% RH and 12:12 h (L: D)]. Each leaf was inserted in a flask (20 mL) filled with water to maintain the turgidity during the bioassay. Then, each tube was transferred to a cage (12 cm high × 5 cm diameter) (PET crystal, 500 mL; Copozan, Orleans, Brazil), with each unit representing of one repetition (Matioli et al., 2021). The cages were covered with voile fabric to prevent toxic gases accumulation and the escape of insects. In each unit were released 10 adults of *M. basicornis* (< 5 days-old). As an alternative food source for *M. basicornis*, eggs of *E. kuehniella* (~ 0.4 g) were offered per unit. The design was completely randomized with 6 replicates per treatment. The insects were kept for 72 h in the cages to have contact to the insecticides residues and the mortality was assessed every 24 h.

4.2.3.1. Behavior effects

To assess locomotion activity in each evaluation day, after 24 h of contact with residues on tomato leaves, 10 insects from each treatment were randomly collected and individually transferred to Petri dishes (7 cm in diameter × 1 cm in height). Each plate, constituting one repetition, contained a qualitative filter paper (7 cm in diameter) to serve as a base and facilitate filming. In an air-conditioned room with a temperature of 25 ± 2 °C and artificial fluorescent light, a video of each repetition lasting 10 minutes was recorded. Cell phone cameras coupled to a support with a numbered base were used to ensure the correct scale of the evaluated parameters. The videos were visually analyzed to determine the average time (in seconds) for walking, resting, and cleaning. The behaviors that were performed under 5 s was not counted.

4.2.4. Residue extraction

The residues quantification was made only for the insecticide acetamiprid (Mospilan WG) which was the one that showed the greatest persistence in terms of toxicity overtime. The extracts were analyzed at the Chemical Analysis Laboratory of the Exact Sciences Department at Esalq/USP. Samples were collected 0, 5, 15 and 31 days after spraying (DAS) from acetamiprid treatment with maximum field recommended concentration (MFRC) of 218 mg a.i. L^{-1} and from control treatment with water. In total, 10 leaflets per repetition was used in 4 repetitions per treatment. The samples were kept at a temperature of -80 °C until all extractions had been carried out. The extraction procedure and analysis in the HPLC-UV equipment followed the methodology of Jankulovska et al. (2019), with modifications.

For the extraction procedure the tomato leaves were weighed to use 6 g of the collected sample. The leaves were macerated, transferred to a glass tube (20 cm high × 2.5 cm in diameter) and were added 15 mL of acetone (99.5% purity). In an ultrasound equipment (Cristófoli Biossegurança, Campo Mourão, Brazil), the samples were left for about an hour, being manually shaken every 15 min. The liquid-solid separation was performed by vacuum filtration with filter paper, and at the end, 10 mL of acetone was added to clean the glassware, ensuring that most of the material to be detected remained in the sample. After that, the sample was transferred to a small volumetric flask and the solvent was evaporated using a rotary evaporator (IKA RV 10 digital, Campinas Brazil). A liquid-liquid partition was made by adding 5 mL of ultrapure water plus 3.5 mL of ethyl acetate (process made twice). The aqueous solution was collected and passed through the rotary evaporator again for 15 to 30 min to remove all the ethyl acetate. Methanol (2.5 mL) was added to the sample, vacuum filtered again, and the volumetric flask was cleaned with another 2.5 ml of methanol and a final vacuum filtration was performed. The sample was transferred to smaller flasks for drying in Speedvac (Savant SC210A, Waltham, United States). After drying, the sample was stored in a refrigerator until HPLC-UV analysis.

4.2.4.1. Residue determination

To quantify the insecticidal residues of acetamiprid present in tomato leaves, after spraying, analyzes were performed using liquid chromatography - High performance Liquid Chromatography - with an ultraviolet detector (HPLC-UV, Agilent 1100 Series, Santa Clara, United States). The negative control treatment, containing only water, was used as a "blank" for the quantification analyses. Before HPLC-UV analysis, the extracts were redissolved in 1 mL of a mixture of water and acetonitrile (1:1) and filtered through syringes with a 0.45 µm ISO-DISC PTFE filter. In order to be analyzed, the extracts were transferred to specific tubes for HPLC. The mobile phase of the analysis was performed with acetonitrile and water (50/50) and the stationary phase with a LiChrospher 60 RP – select B column (250 x 4 mm, 5µm). According to Jankulovska et al. (2019), the detection band of acetamiprid is in the ultraviolet band of 240 nm and retention time of 3.4 min.

4.2.5. Experimental design and data analysis

All data were checked for normality and homoscedasticity using the Shapiro–Wilk and Bartlett tests. The assumptions of ANOVA were not met for any of the data, therefore we analyzed with Kruskal–Wallis non-parametric one-way ANOVA with Dunn with Bonferroni correction post-hoc ($P < 0.05$) through the "ExpDes", "easyanova" and "dunn.test" packages in the R software (R Development Core Team, 2021).

The mortality percentage values after 72 h were corrected according to the Schneider–Orelli formula: Ma (%) = [(Mt - Mc)/ (100 - Mc)] × 100, where M_a is the corrected mortality, M_t the mortality observed in the treatment and M_c the control mortality (Püntener, 1981).

In order to classify the insecticides according to persistence classes proposed by the International Organization for Biological Control (IOBC) working group 'Pesticides and Beneficial Organisms', when the products reduce insect mortality by less than 30% compared to the control treatment, the categories proposed include: class 1 = short life (< 5 days); class 2 = slightly persistent (5 – 15 days); class 3 = moderately persistent (16 – 30 days) and 4 = persistent (> 31 days) (Hassan et al., 1998).

4.3. Results

4.3.1. Persistence and toxicological effects of insecticides on *Macrolophus basicornis*

The insecticides showed different persistence and toxicity over time. At 24h of 5 DAS, bifenthrin, etofenprox + acetamiprid, and pyriproxyfen + acetamiprid caused low mortality. After 48 h, the number of live insects decreased for etofenprox + acetamiprid and pyriproxyfen + acetamiprid, causing more than 30% mortality, and continued decreasing after 72 h of exposure. Bifenthrin did not differ from control treatment, causing less than 30% mortality, which was classified as short life (class 1). Acetamiprid was the most harmful insecticide causing 100% mortality in the 24 h evaluation (Table 2).

Table 2. Number of live adults (mean ± SE) of *Macrolophus basicornis* 24, 48 and 72 h after contact with tomatoes leaves 5 days after spraying (DAS), corrected mortality (M_a) after 72 h and IOBC classification of insecticides.

Treatment	Number of live insects (n = 10)			M_a (%)*	Class[1]
	24 h	48 h	72 h		
Control	10.0 ± 0.0 a	10.0 ± 0.0 a	9.8 ± 0.2 a	-	-
Acetamiprid	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d	100	-
Bifenthrin	9.0 ± 0.6 ab	8.6 ± 0.8 ab	8.0 ± 0.7 ab	18.4	1
Etofenprox + acetamiprid	8.4 ± 0.8 ab	6.2 ± 1.2 bc	5.2 ± 1.3 bc	46.9	-
Pyriproxyfen + acetamiprid	7.2 ± 0.7 bc	5.0 ± 0.7 c	3.0 ± 0.5 c	69.4	-
χ^2	17.082	19.161	20.328	-	-
p	0.001	< 0.001	< 0.001	-	-

Data followed by the same letter in a column do not differ by the Bonferroni test. * Corrected mortality (M_a) by the Schneider–Orelli formula (Püntener, 1982). [1] Persistence toxicological class according to IOBC ("International Organization for Biological and Integrated Control of Noxious Animals and Plants,West Palearctic Regional Section") in which: class 1 = short life (< 5 days); class 2 = slightly persistent (5 – 15 days); class 3 = moderately persistent (16 – 30 days) and 4 = persistent (> 31 days).

In the exposure at 15 DAS, the insecticides etofenprox + acetamiprid and pyriproxyfen + acetamiprid were similar to the control treatment in each day of evaluation, causing 20.5% and 15.9% of mortality after 72 h in contact with residues. For that, the mixture insecticides were classified as slightly persistent (class 2). Acetamiprid continued causing high mortality at 15 DAS resulting in 93.2% mortality (Table 3).

Table 3. Number of live adults (mean ± SE) of *Macrolophus basicornis* 24, 48 and 72 h after contact with tomatoes leaves 15 days after spraying (DAS), corrected mortality (M_a) after 72 h and IOBC classification of insecticides.

Treatment	Number of live insects (n = 10)			M_a (%)*	Class[1]
	24 h	48 h	72 h		
Control	9.4 ± 0.2 a	8.8 ± 0.3 a	8.8 ± 0.3 a	-	-
Acetamiprid	3.8 ± 1.5 b	1.2 ± 0.7 b	0.6 ± 0.5 b	93.2	-
Etofenprox + acetamiprid	8.8 ± 0.5 a	7.8 ± 0.7 a	7.0 ± 1.2 a	20.5	2
Pyriproxyfen + acetamiprid	9.4 ± 0.4 a	7.6 ± 0.5 a	7.4 ± 0.6 a	15.9	2
χ^2	10.728	12.368	11.881	-	-
p	0.013	0.006	0.008	-	-

Data followed by the same letter in a column do not differ by the Bonferroni test. * Corrected mortality (M_a) by the Schneider–Orelli formula (Püntener, 1982). [1] Persistence toxicological class according to IOBC ("International Organization for Biological and Integrated Control of Noxious Animals and Plants,West Palearctic Regional Section") in which: class 1 = short life (< 5 days); class 2 = slightly persistent (5 – 15 days); class 3 = moderately persistent (16 – 30 days) and 4 = persistent (> 31 days).

There was statistical difference of the number of live adults at 31 DAS in the acetamiprid treatment in relation to the control treatment and more than 30% mortality was observed. After 24 h of exposure, the live percentage of adults was more than 70%. In the next evaluations, the insecticide kept the high toxicity which classified it as persistent (class 4) (Table 4).

Table 4. Number of live adults (mean ± SE) of *Macrolophus basicornis* 24, 48 and 72 h after contact with tomatoes leaves 31 days after spraying (DAS), corrected mortality (M_a) after 72 h and IOBC classification of insecticides.

Treatment	Number of live insects (n = 10)			M_a (%)*	Class[1]
	24 h	48 h	72 h		
Control	10.0 ± 0.0 a	9.4 ± 0. a	9.4 ± 0.4 a	-	-
Acetamiprid	7.2 ± 1.2 b	5.8 ± 1.3 b	4.2 ± 1.8 b	65.2	4
χ^2	5.581	3.378	3.377	-	-
p	0.018	0.050	0.052	-	-

Data followed by the same letter in a column do not differ by the Bonferroni test. * Corrected mortality (M_a) by the Schneider–Orelli formula (Püntener, 1982).
[1] Persistence toxicological class according to IOBC (“International Organization for Biological and Integrated Control of Noxious Animals and Plants,West Palearctic Regional Section”) in which: class 1 = short life (< 5 days); class 2 = slightly persistent (5 – 15 days); class 3 = moderately persistent (16 – 30 days) and 4 = persistent (> 31 days).

4.3.1.1. Behavior effects

It was possible to evaluate the behavior effects caused by bifenthrin, pyriproxyfen + acetamiprid, etofenprox + acetamiprid, and control treatment at 5 DAS. The averages in each parameter evaluated for all treatments were similar to the control (Figure 1). The insects spent most time resting (χ^2 = 4.0; *df* = 3; *p* = 0.26) followed by walking (χ^2 = 1.68; *df* = 3; *p* = 0.64) and cleaning (χ^2 = 1.84; *df* = 3; *p* = 0.61).

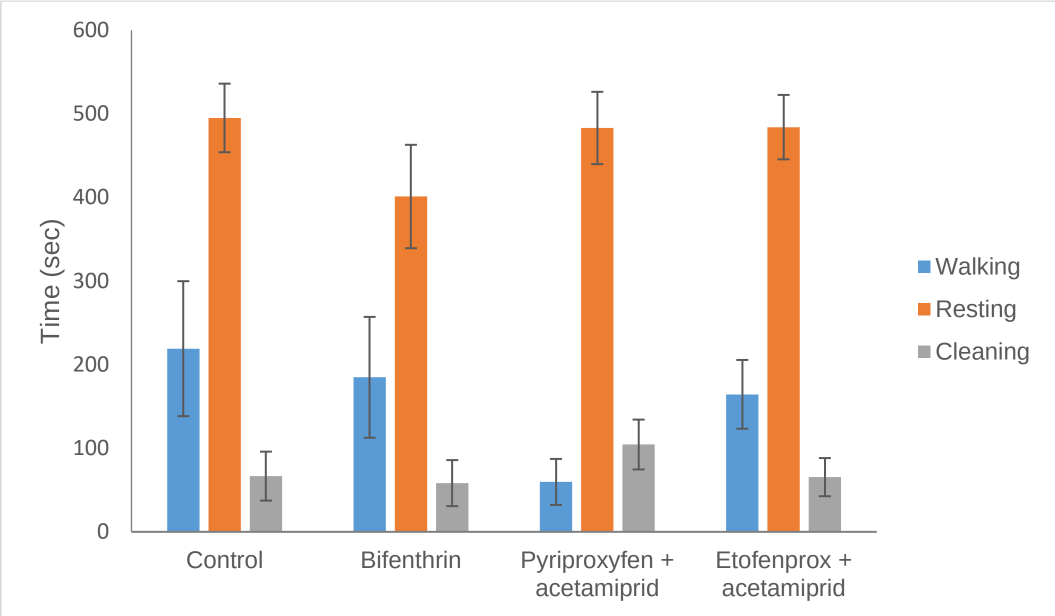

Figure 1. Time spent (seconds) in walking, resting and cleaning (mean ± SE) by *Macrolophus basicornis* adults after contact with plants treated at 5 days after spraying (DAS) for 24 h, in a period of 10 min for each repetition.

In the 15 DAS, the behavior of the insects exposed to the treatments pyriproxyfen + acetamiprid, etofenprox + acetamiprid, acetamiprid and control were evaluated after 24 h of contact with the residues. Similar to the results at 5 DAS, the insecticides did not cause alterations in parameters and likewise the insects spent more time resting (χ^2 = 3.27; *df* = 3; *p* = 0.35), walking (χ^2 = 1.60; *df* = 3; *p* = 0.66), and cleaning (χ^2 = 0.96; *df* = 3; *p* = 0.62), respectively.

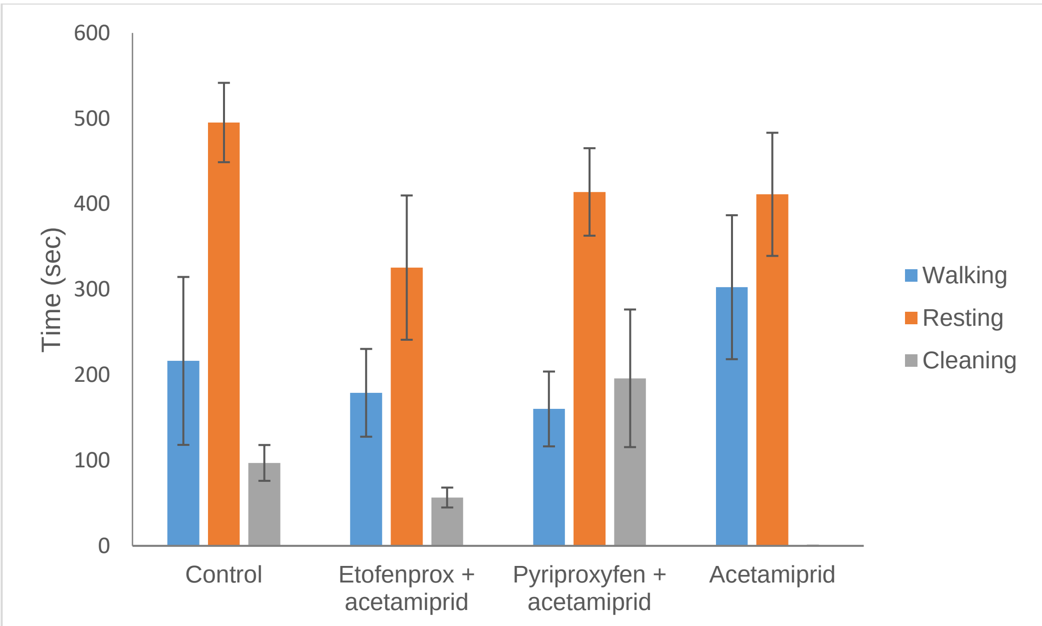

Figure 2. Time spent (seconds) in walking, resting and cleaning (mean ± SE) by *Macrolophus basicornis* adults after contact with plants treated 15 days after spraying (DAS) for 24 h, in a period of 10 min for each repetition.

In the last evaluation, at 31 DAS, even with high toxicity to the *M. basicornis* adults (Table 4), there was no difference in the parameters analyzed comparing the insecticide with the control treatment (Figure 3). Differently to 5 and 15 DAS, the average behaviors showed that the insects spent more time walking (χ^2 = 0.009; *df* = 1; *p* = 0.93), followed by resting (χ^2 = 1.31; *df* = 1; *p* = 0.25) and cleaning (χ^2 = 0.12; *df* = 1; *p* = 0.73).

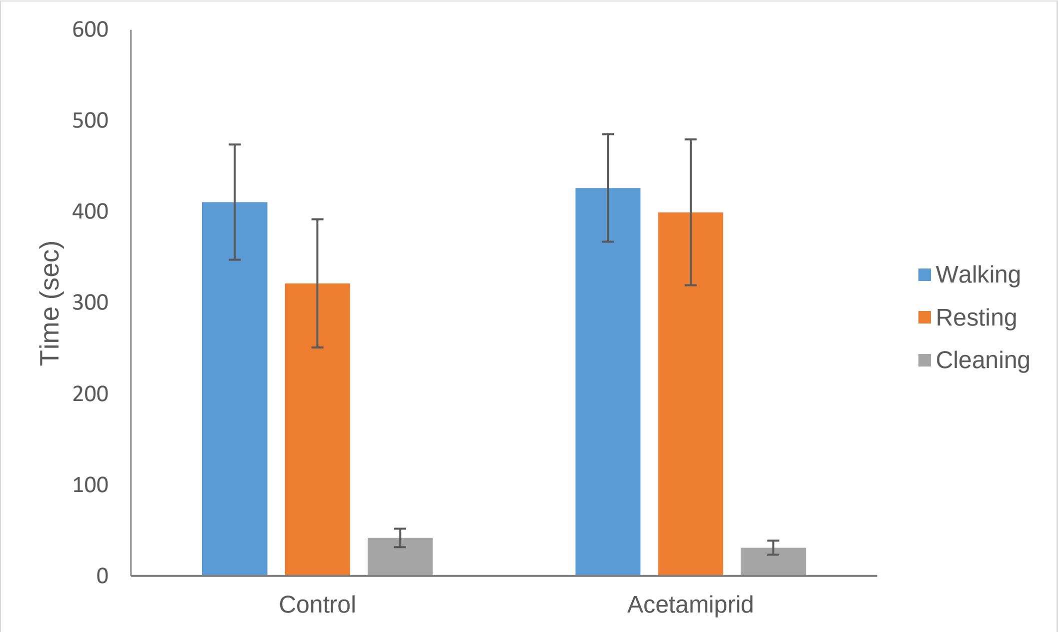

Figure 3. Time spent (seconds) in walking, resting and cleaning (mean ± SE) by *Macrolophus basicornis* adults after contact with plants treated 31 days after spraying (DAS) for 24 h, in a period of 10 min for each repetition.

4.3.2. Residue determination for acetamiprid

The concentration of acetamiprid in tomato leaves analyzed in HPLC-UV gradually decreased over time between days 0 to 31 after spraying (χ^2 = 8.27; *df* = 3; *p* = 0.04). Day 0 and day 5 had similar amounts of the active ingredient while day 15 was between day 5 and day 31, and in the day 31 the concentration was reduced (Figure 4).

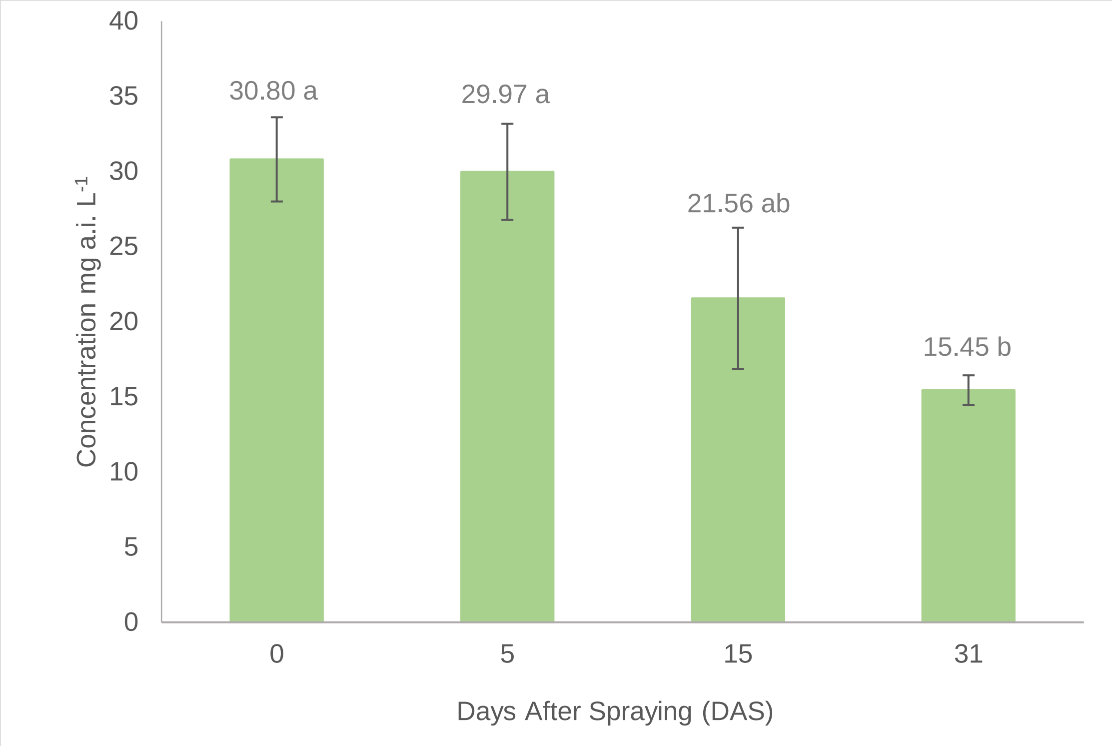

Figure 4. Concentration (mg a.i. L^{-1}) over time (days) of acetamiprid in tomato leaves after spraying until run-off, analyzed with High performance Liquid Chromatography - with an ultraviolet detector (HPLC-UV). The data followed by the same letter do not differ by the Bonferroni test.

4.4. Discussion

The broad-spectrum insecticides acetamiprid, bifenthrin, and the mixtures etofenprox + acetamiprid and pyriproxyfen + acetamiprid are considered harmful for *M. basicornis* under laboratory assays (Matioli et al., 2021). In this study, we evaluated the residual activity of these insecticides under greenhouse conditions and their toxicity, according to IOBC classifications (Hassan, 1998). The toxicological effects of the insecticides overtime are important to estimate the safety releases of zoophytophagous predators in the tomato fields (Morales et al., 2019). It was observed that the insecticides had different toxicity persistence to adults of *M. basicornis* with bifenthrin as short life, etofenprox + acetamiprid and pyriproxyfen + acetamiprid as slightly persistent, and acetamiprid as persistent.

Bifenthrin belongs to the third generation pyrethroids chemical group and acts by contact and ingestion exposure on the sodium channel nervous cells, which increase its permeability maintaining the channels opened (Brown, 2005; Mansoor et al., 2016). Although in laboratory conditions bifenthrin was harmful to the predator *M. basicornis* (Matioli et al., 2021), with semi-field conditions, its persistence was considered short life (class 1). Probably, the insecticide molecules may have photodegraded within the first few days after application in tomato leaves (Xi et al., 2021) and, therefore, the persistence of toxicity was low for *M. basicornis*. Morais et al. (2016) classified bifenthrin as moderately persistent (class 3) when the insecticide was tested on adults of the parasitoid *Ageniaspis citricola* Longvinovskaya (Hymenoptera: Encyrtidae). This variation may have been due to the different components of formulation are from distinct companies and also because the insects are from different orders. In addition to this information, it has been proven that bifenthrin residues do not remain for more than 10 days in in brinjal and chili (Chaudhary et al., 2022), therefore, we might infer that the insecticide has a rapid degradation in tomato leaves.

The ready-mix insecticides etofenprox + acetamiprid and pyriproxyfen + acetamiprid were more persistent than bifenthrin, categorized as slightly persistent (class 2). It is common for the farmers the use of mixtures or more than one active ingredient in the tank for multiple-targeting (Larson et al., 2014; Pazini et al., 2019). In the case of tomato crops, the use of mixtures or ready-mix insecticides are intended to avoid the *B. tabaci* resistance to chemical control (Basit et al., 2013). Both insecticides have acetamiprid in the formula, but one has pyrethroid added and the other a juvenile hormone mimic. Even with this difference, the insecticides acted very similarly due to the presence of neonicotinoid and also because there is a high chance that the active ingredients and the surfactants had a synergistic effect (Gill et al., 2012; Li et al., 2019). In previous studies, some authors have shown that combine insecticides mixtures can cause greater problems for natural enemies (Gill et al., 2012; Larson et al., 2014; Gandini et al., 2020), including *M. basicornis* (Soares et al., 2019a). Besides, the synergic effects also may increase the toxicity persistence to *M. basicornis* in semi-field conditions.

The concentration residue found of acetamiprid over time explains the persistence for the active ingredient and for the ready-mix containing acetamiprid on *M. basicornis* adults. The difference in persistence between the insecticide with acetamiprid alone and in ready-mix was due to the percentage of active ingredient in the formulation. Since that the former there was 72.5% and the others had 16.7% (etofenprox + acetamiprid) and 20% (pyriproxyfen + acetamiprid) of the neonicotinoid. The applied amount MFRC of 218 mg a.i. L^{-1} per plant was detected in 6 g of tomato leaves 0, 5, 15 and 31 DAS under semi-field conditions with concentrations of 30.80, 29.97, 21.56 and 15.45 mg a.i. L^{-1}, respectively. In laboratory conditions, the median lethal concentration (LC_{50}) of acetamiprid detected for adults of *M. basicornis* was 0.26 mg a.i. L^{-1} per insect (Matioli et al., 2021). In this study, the mortality caused by this active ingredient was proportional to the concentration amount found on tomato leaves over time. The high toxicity possibly happened due to the low insecticide degradation after a long period of application.

Acetamiprid is a neonicotinoid insecticide with systemic properties, and causes nerve cell blockage by acting as nicotinic acetylcholine receptors (Tomizawa; Casida, 2005). Due to the fact that the neonicotinoids are systemic, it is likely that the molecules manage to remain in the leaves for all this time or more. Under field conditions, at different dates, the insecticide caused reduction and persisted for up 15 days on populations of *Coccinella undecimpunctata* Linnaeus (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Syrphus corolla* Fabricius (Diptera: Syrphidae) (Abd-Ella, 2015). Another neonicotinoid, imidacloprid, was detected for more than 120 days in tomato plants, caused 50% mortality at 40 DAS in adults of *E. varians*, and it was also classified as persistent (class 4) (Morales et al., 2019). In addition, mirids are zoophytophagous insects (Castañé et al., 2011), which makes them more vulnerable to acetamiprid, as they can access plant sap where most of the residues may have accumulated.

Concerning the analysis of behavioral effects, the results showed no differences between treatments even when acetamiprid was compared to the control treatment 31 DAS. Filming was made 24 h after the contact of the insects with the residues and the total number of insects evaluated were 10 per treatment. This may have influenced the similar behavior of insects and also the parameters evaluated may be not a differential for effects caused by insecticides. However, it is important to mention that the insects that had contact with acetamiprid were, at different times, more moribund than the other treatments. All the insecticides tested in this study act on the nervous system of insects, thereby, it would be possible that *M. basicornis* adults could have some neurological alterations that influence in behavior causing problems to the predatory capacity (Ritzmann and Büschges, 2007; Büschges and Gruhn, 2007).

Other authors also have studied the behavior changes in natural enemies by neurotoxic insecticides. Acetamiprid increased the locomotor activity and water-induced proboscis extension reflex after thoracic application in *Apis mellifera* Linnaeus (Hymenoptera: Apidae) (Hassani et al., 2008). Thiacloprid, another neonicotinoid, changed the behavior of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) when cleaning, resting and plant feeding (Martinou et al., 2014). In a similar way, lambda-cyhalothrin and chlorpyrifos affected the behavior of the mirid predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) in different concentrations (Soares et al., 2019b; Passos et al., 2022). Our results suggested that the neurotoxic insecticides tested did not disturb the behavior of *M. basicornis* adults after contact with the residues. However, it is clear that even with no behavior effect, they caused mortality, except for bifenthrin 5 DAS, which did not cause considered behavior or toxic effects on the predator.

4.5. Conclusions

In this report through the semi-field studies of insecticides toxicity persistence on the predator *M. basicornis* over time, the results showed different effects of the chemicals. The acute toxicity after 72 h for each assessment day suggests that bifenthrin is short life, etofenprox + acetamiprid and pyriproxyfen + acetamiprid are slightly persistent, and acetamiprid is persistent, according to IOBC. Acetamiprid concentration over time was quantified in tomato leaves and remained quite high up to 31 DAS, which explain the high toxicity to *M. basicornis* adults. This was the first study to correlate acetamiprid quantification and natural enemy mortality over time after application on tomato leaves. Interestingly, the insecticides had no effect on the behavior of the insects, nevertheless, we suggest that further studies be carried out to substantiate these results. We also suggest that the same tests have to be conducted at field level. The results obtained in these bioassays are important to contribute to IPM tactics for the implementation of *M. basicornis* as a biological control agent of *B. tabaci*.

References

Abd-Ella, A.A., 2015. Effect of several insecticides on pomegranate aphid, *Aphis punicae* (Passerini) (Homoptera: Aphididae) and its predators under field conditions. EPPO Bulletin, 45, 90-98. https://doi.org/10.1111/epp.12192

Barreto, Y.C., Rosa, M.E., Zanatta, A.P., Borges, B.T., Hyslop, S., Vinadé, L.H., Dal Belo, C. A., 2020. Entomotoxicity of jaburetox: Revisiting the neurotoxic mechanisms in insects. Journal of Venom Research, 10, 38.

Barzman, M., Bàrberi, P., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J.E., Kiss, J., Kudsk, P., Lamichhane, J.R., Messéan, A., Moonen, A.C., Ratnadass, A., Ricci, P., Sarah, J.L., Sattin, M., 2015. Eight principles of integrated pest management. Agron. Sustain. Dev. 35, 1199–1215. https://doi.org/10.1007/s13593-015-0327-9

Basit, M., Saeed, S., Saleem, M.A., Sayyed, A.H., 2013. Can resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) be overcome with mixtures of neonicotinoids and insect growth regulators? Crop Protection, 44, 135-141. https://doi.org/10.1016/j.cropro.2012.10.021

Biondi, A., Desneux, N., Siscaro, G., Zappalà, L., 2012. Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. Chemosphere, 87, 803-812. https://doi.org/10.1016/j.chemosphere.2011.12.082

Brown, A.E., 2005. Mode of action of insecticide and related pest control chemicals for production agriculture, Ornamentals and Turf, Department of entomology, College Park. MD 20742, 405e3913.

Bueno, V.H.P., van Lenteren, J.C., Lins, J.C., Calixto, A.M., Montes, F.C., Silva, D.B., Santiago, L.D., Pérez, L.M., 2013. New records of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) predation by Brazilian Hemipteran predatory bugs. J. Appl. Entomol. 137, 29–34. https://doi.org/10.1111/jen.12017

Büschges, A., Gruhn, M., 2007. Mechanosensory feedback in walking: from joint control to locomotor patterns. Advances in insect physiology, 34, 193-230. https://doi.org/10.1016/S0065-2806(07)34004-6

Carvalho, G.A., Grützmacher, A.D., Passos, L.C., Oliveira, R.L.D., 2019. Physiological and ecological selectivity of pesticides for natural enemies of insects. In Natural enemies of insect pests in Neotropical agroecosystems. Springer, Cham. 469-478. https://doi.org/10.1007/978-3-030-24733-1_37

Castañé, C., Arnó, J., Gabarra, R., Alomar, O., 2011. Plant damage to vegetable crops by zoophytophagous mirid predators. Biological control, 59, 22-29. https://doi.org/10.1016/j.biocontrol.2011.03.007

Chaudhary, R., Singh, R., Singh, M., Mogha, N.K., Kumari, P., Paliwal, G., Singh, P.P., Das, M., 2022. LC-MS/MS method for the simultaneous quantification of pyriproxyfen and bifenthrin and their dissipation kinetics under field conditions in chili and brinjal. Journal of Food Science. 87, 1331-1341. https://doi.org/10.1111/1750-3841.16070

Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol., 52, 81-106. https://doi.org/10.1146/annurev.ento.52.110405.091440

El Hassani, A.K., Dacher, M., Gary, V., Lambin, M., Gauthier, M., Armengaud, C., 2008. Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). Arch Environ Contam Toxicol, 54, 653-661. https://doi.org/10.1007/s00244-007-9071-8

Gandini, E.M.M., Costa, E.S.P., dos Santos, J.B., Soares, M.A., Barroso, G. M., Corrêa, J. M., Carvalho, A.G., Zanuncio, J. C., 2020. Compatibility of pesticides and/or fertilizers in tank mixtures. J. Cleaner Prod., 268, 122152. https://doi.org/10.1016/j.jclepro.2020.122152

Gill, R.J., Ramos-Rodriguez, O., Raine, N.E., 2012. Combined pesticide exposure severely affects individual-and colony-level traits in bees. Nature, 491, 105-108. https://doi.org/10.1038/nature11585

Hassan, S.A., Hafes, B., Degrande, P.E., Herai, K., 1998. The side-effects of pesticides on the egg parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae), acute dose-response and persistence tests. J. Appl. Entomology, 122, 569-573. https://doi.org/10.1111/j.1439-0418.1998.tb01547.x

Jankulovska, M.S., Velkoska-Markovska, L., Petanovska-Ilievska, B., Ilievski, U., 2019. Application of high performance liquid chromatography for determination of metalaxyl, acetamiprid and azoxystrobine in tomato samples. J. Analytical Chem., 74, 339-344. https://doi.org/10.1134/S1061934819040075

Larson, J.L., Redmond, C.T., Potter, D.A., 2014. Impacts of a neonicotinoid, neonicotinoid–pyrethroid premix, and anthranilic diamide insecticide on four species of turf-inhabiting beneficial insects. Ecotoxicology, 23, 252-259. https://doi.org/10.1007/s10646-013-1168-4

Li, B.X., Liu, Y., Zhang, P., Li, X.X., Pang, X.Y., Zhao, Y. H., Li, H., Liu, F., Lin, J., Mu, W., 2019. Selection of organosilicone surfactants for tank-mixed pesticides considering the balance between synergistic effects on pests and environmental risks. Chemosphere, 217, 591-598. https://doi.org/10.1016/j.chemosphere.2018.11.061

Mansoor, M.M., Afzal, M.B.S., Basoalto, E., Raza, A.B.M., Banazeer, A., 2016. Selection of bifenthrin resistance in cotton mealybug *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae): Cross-resistance, realized heritability and possible resistance mechanism. Crop Protection, 87, 55-59. https://doi.org/10.1016/j.cropro.2016.04.026

MAPA, 2022. Ministério da Agricultura, Pecuária e Abastecimento, Brazil. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons, accessed on 20 March 2022.

Martinou, A.F., Seraphides, N., Stavrinides, M.C., 2014. Lethal and behavioral effects of pesticides on the insect predator *Macrolophus pygmaeus*. Chemosphere, 96, 167-173. https://doi.org/10.1016/j.chemosphere.2013.10.024

Matioli, T.F., da Silva, M.R., Pazini, J. de B., Barroso, G., Vieira, J.G.A., Yamamoto, P.T., 2021. Risk assessment of insecticides used in tomato to control whitefly on the predator *Macrolophus basicornis* (Hemiptera: Miridae). Insects 12, 1092. https://doi.org/10.3390/insects12121092

Morais, M.R., Zanardi, O.Z., Rugno, G.R., Yamamoto, P. T., 2016. Impact of five insecticides used to control citrus pests on the parasitoid *Ageniaspis citricola* Longvinovskaya (Hymenoptera: Encyrtidae). Ecotoxicology, 25, 1011-1020. https://doi.org/10.1007/s10646-016-1658-2

Morales, S.I., Martínez, A.M., Figueroa, J.I., Campos-García, J., Gómez-Tagle, A., Lobit, P., Smagghe, G., Pineda, S., 2019. Foliar persistence and residual activity of four insecticides of different mode of action on the predator *Engytatus varians* (Hemiptera: Miridae). Chemosphere, 235, 76-83. https://doi.org/10.1016/j.chemosphere.2019.06.163

Naranjo, S.E., 2001. Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. Crop protection, 20, 835-852. https://doi.org/10.1016/S0261-2194(01)00115-6

Passos, L.C., Soares, M.A., Collares, L.J., Malagoli, I., Desneux, N., Carvalho, G.A., 2018. Lethal, sublethal and transgenerational effects of insecticides on *Macrolophus basicornis*, predator of *Tuta absoluta*. Entomol. Gen. 38, 127–143. https://doi.org/10.1127/entomologia/2018/0744

Passos, L.C., Soares, M.A., Costa, M.A., Michaud, J.P., Freire, B.C., Carvalho, G.A., 2017. Physiological susceptibility of the predator Macrolophus basicornis (Hemiptera: Miridae) to pesticides used to control of *Tuta absoluta* (Lepidoptera: Gelechiidae). Biocontrol Sci. Technol. 27, 1082–1095. https://doi.org/10.1080/09583157.2017.1381879

Passos, L.C., Ricupero, M., Gugliuzzo, A., Soares, M.A., Desneux, N., Carvalho, G.A., Zappalà, L., Biondi, A., 2022. Does the dose make the poison? Neurotoxic insecticides impair predator orientation and reproduction even at low concentrations. Pest Manag. Sci. 78, 1698-1706. https://doi.org/10.1002/ps.6789

Pazini, J.B., Padilha, A.C., Cagliari, D., Bueno, F.A., Rakes, M., Zotti, M.J., Martins, J.F.S, Grützmacher, A. D., 2019. Differential impacts of pesticides on *Euschistus heros* (Hem.: Pentatomidae) and its parasitoid *Telenomus podisi* (Hym.: Platygastridae). Scientific reports, 9, 1-10. https://doi.org/10.1038/s41598-019-42975-4

Püntener,W. Manual for Field Trials in Plant Protection, 2nd ed.; Ciba-Geigy, Ltd.: Basel, Switzerland, 1981.

R Development Core Team, 2021. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Available online: https://www.R-project.org/ (accessed on 20 March 2022).

Rakes, M., Pasini, R.A., Morais, M.C., Zanella, R., Prestes, O.D., Bernardi, D., Grützmacher, A. D., 2021. Residual effects and foliar persistence of pesticides used in irrigated rice on the parasitoid *Telenomus podisi* (Hymenoptera: Platygastridae). J. Pest Sci., 1-13. https://doi.org/10.1007/s10340-021-01436-6

Ritzmann, R.E., Büschges, A., 2007. Adaptive motor behavior in insects. Current opinion in neurobiology, 17, 629-636. https://doi.org/10.1016/j.conb.2008.01.001

Silva, W.M., Berger, M., Bass, C., Williamson, M., Moura, D.M.N., Ribeiro, L.M.S., Siqueira, H.A.A., 2016. Mutation (G275E) of the nicotinic acetylcholine receptor α6 subunit is associated with high levels of resistance to spinosyns in *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Pestic. Biochem. Physiol. 131, 1–8. https://doi.org/10.1016/j.pestbp.2016.02.006

Soares, M.A., Campos, M.R., Passos, L.C., Carvalho, G.A., Haro, M.M., Lavoir, A.V., Biondi, A., Zappalà, L., Desneux, N., 2019b. Botanical insecticide and natural enemies: a potential combination for pest management against *Tuta absoluta*. J. Pest Sci. 92, 1433-1443. https://doi.org/10.1007/s10340-018-01074-5

Soares, M.A., Passos, L.C., Campos, M.R., Collares, L.J., Desneux, N., Carvalho, G.A., 2019a. Side effects of insecticides commonly used against *Tuta absoluta* on the predator *Macrolophus basicornis*. J. Pest Sci. 92, 1447–1456. https://doi.org/10.1007/s10340-019-01099-4

Tomizawa, M., Casida, J.E., 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol., 45, 247-268. https://doi.org/10.1146/annurev.pharmtox.45.120403.095930

Wanumen, A.C., Carvalho, G.A., Medina, P., Vinuela, E., Adán, Á., 2016. Residual acute toxicity of some modern insecticides toward two mirid predators of tomato pests. J. Econ. Entomology, 109, 1079-1085. https://doi.org/10.1093/jee/tow059

Xi, N., Li, Y., Chen, J., Yang, Y., Duan, J., Xia, X., 2020. Elevated temperatures decrease the photodegradation rate of pyrethroid insecticides on spinach leaves: Implications for the effect of climate warming. Environ. Sci. Tech., 55, 1167-1177. https://doi.org/10.1016/j.chemosphere.2022.133941

5. FINAL CONSIDERATIONS

Seven insecticides commonly used to control *B. tabaci* were tested on its predator *M. basicornis* following the toxicological tests under laboratory and semi-field conditions proposed by the International Organization for Biological Control (IOBC) and also the tests were evaluated according to statistical tests (Table 1).

The first tests under laboratory showed that buprofezin, cyantraniliprole and spiromesifen did not cause acute toxicity (IOBC class 1) and the insects survived as control treatment. Acetamiprid, bifenthrin, etofenprox + acetamiprid, and pyriproxyfen + acetamiprid were harmful (IOBC class 4) to the insects and their median lethal concentration (LC_{50}) were evaluated. With the LC_{50} it was possible to calculate the risk quotient (RQ), to understand the ecological selectivity, which showed that acetamiprid it is slightly to moderately toxic (Table 1). The others were considered safe according to the calculated RQ.

In the second section, it was possible to understand if the harmless insecticides could cause sublethal or transgenerational effects. Buprofezin and spiromesifen caused reduction of offspring body size after residues exposure on adults for 72 h. Cyantraniliprole did not cause effects on *M. basicornis* and could be recommended to control *B. tabaci* when releasing predator individuals in a tomato area.

In the tests under semi-field conditions, acetamiprid, bifenthrin, etofenprox + acetamiprid, and pyriproxyfen + acetamiprid were analyzed after sprayed on tomato leaves. Bifenthrin was short life (IOBC class 1), etofenprox + acetamiprid, and pyriproxyfen + acetamiprid were slightly persistent (IOBC class 2), and acetamiprid was persistent (IOBC class 4) (Table 1). The insecticides were also evaluated for behavior effects and were not observed any difference compared to the control treatment. Acetamiprid residues in tomato leaves were quantified by HPLC-UV and it was possible to observe a high amount of the active ingredient after 31 days after spraying.

These tests were important to study the compatibilization of seven insecticides commonly used to control *B. tabaci*. Cyantraniliprole is the most compatible insecticide with *M. basicornis*, and acetamiprid is the least compatible. It is important that these insecticides are field tested because compatibility may differ due to conditions.

Table 1. Effects of insecticides used to control *Bemisia tabaci* on the predator *Macrolophus basicornis* under laboratory and semi-field conditions.

Treatments	IOBC classifications		RQ[3]	Sublethal effects
	Laboratory[1]	Semi-field[2]		
Acetamiprid	4	4	2	No
Bifenthrin	4	1	1	No
Buprofezin	1	-	-	Yes
Cyantraniliprole	1	-	-	No
Etofenprox + acetamiprid	4	2	1	No
Pyriproxyfen + acetamiprid	4	2	1	No
Spiromesifen	1	-	-	Yes

[1] Toxicological class for extended laboratory according to IOBC ("International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section") in which: class 1 = harmless (M_a < 25%); class 2 = slightly harmful (25 ≤ M_a ≤ 50%); class 3 = moderately harmful (51 ≤ M_a ≤ 75%); class 4 = harmful (M_a > 75%).
[2] Persistence toxicological class according to IOBC in which: class 1 = short life (< 5 days); class 2 = slightly persistent (5 – 15 days); class 3 = moderately persistent (16 – 30 days) and 4 = persistent (> 31 days).
[3] Risk quotient categories according to the values the insecticides were classified as safe (RQ < 50), slightly to moderately toxic (50 < RQ ≤ 2500), and dangerously toxic (RQ > 2500).