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Sublethal effect of neonicotinoids and lambda-cyhalothrin on the feeding behavior of *Euschistus heros* Fabricius (Hemiptera: Pentatomidae)

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ABSTRACT

Soybean plants (*Glycine max*) L. Merrill are often attacked by various pests from planting to harvest. Damage can affect the root system, the main stem, leaves, flowers, and pods. Among the pests associated with soybeans, the stink bug group is noteworthy because it includes one of the most economically damaging pests, the Neotropical brown stinkbug, (*Euschistus heros*) Fabricius (Hemiptera: Pentatomidae). The present study aimed to evaluate the mortality and possible sublethal effects of dinotefuran, thiamethoxam, and lambda-cyhalothrin on *E. heros* in soybean pods under laboratory conditions. The first bioassay aimed to assess the sublethal effects on the neotropical brown stink bug estimating the LC_{10} and LC_{25} values. The estimated LC_{10} and LC_{25} for dinotefuran were 1.31 and 5.41 g of active ingredient (a.i.) ha^{-1}; thiamethoxam were 0.37 and 2.74 g a.i. ha^{-1}, and lambda-cyhalothrin were 10.65 and 76.58 g a.i. ha^{-1}. The feeding behavior studies were conducted in Petri dishes consisting of two pods and five adults of *E. heros*. The treatments were applied to the pronotum of each insect using the LC_{10} and LC_{25}. Evaluations were performed by noting the number of insects from each Petri dish present on the pods at 10, 30, 45, 60, 120, and 180 minutes after application. The consumption of the pods of the different treatments was assessed for 180 minutes, evaluating the number of insect punctures, the total feeding time per insect, the feeding time per puncture, and mortality. The low concentrations of dinotefuran and lambda-cyhalothrin (LC_{10} and LC_{25}) reduced the time adults of *E. heros* spent on the pods and their feeding times.

Keywords: Neotropical brown stink bug; Insecticide; Phagodeterrence; Sublethal concentration.

Efeito subletal de neonicotinoides e lambda-cialotrina sobre o comportamento alimentar de *Euschistus heros* Fabricius (Hemiptera: Pentatomidae)

RESUMO

As plantas de soja [*Glycine max* (L.) Merrill] são atacadas por várias pragas desde o plantio até a colheita. Os danos podem afetar o sistema radicular, o caule, as folhas, as flores e as vagens. Dentre as pragas associadas à soja, destaca-se o complexo de percevejos, que inclui um dos insetos-praga mais danoso à cultura, o percevejo-marrom (*Euschistus heros*) Fabricius (Hemiptera: Pentatomidae). O presente estudo teve como objetivo avaliar a mortalidade e os possíveis efeitos subletais dos ingredientes ativos dinotefuran, tiametoxan e lambda-cialotrina sobre *E. heros* em vagens de soja, em condições de laboratório. O primeiro bioensaio teve como objetivo avaliar os efeitos subletais no percevejo-marrom, estimando os valores de CL_{10} e CL_{25}. As concentrações de CL_{10} e CL_{25} para dinotefuran foram de 1,31 e 5,41 g de ingrediente ativo (a.) ha^{-1}; tiametoxan foi de 0,37 e 2,74 g i.a. ha^{-1}, e lambda-cialotrina foi de 10,65 e 76,58 g i.a. ha^{-1}. Os estudos de comportamento alimentar foram realizados em placas de Petri constituídas por duas vagens e cinco adultos de *E. heros*. Os tratamentos foram aplicados no pronoto de cada inseto utilizando a CL_{10} e CL_{25}. As avaliações foram realizadas anotando-se o número de insetos de cada placa de Petri presentes nas vagens aos 10, 30, 45, 60, 120 e 180 minutos após a aplicação. O consumo das vagens dos diferentes tratamentos foi avaliado por um período de 180 minutos, avaliando-se o número de puncturas dos insetos, o tempo total de alimentação por inseto, o tempo de alimentação por puncturas e a mortalidade. As baixas concentrações de dinotefuran e lambda-cialotrina (CL_{10} e CL_{25}) reduziram o tempo de permanência dos adultos de *E. heros* nas vagens e o seu tempo de alimentação.

Palavras-chave: Percevejo marrom; Inseticida; Fagodeterrência; Concentração subletal.

1. Introduction

Because of the high rate of population growth coupled with rising per capita income, an increase in food, fiber, and energy production has become extremely necessary to meet the demands of the world market. Brazil has become essential for food production, as it is one of the few regions where productivity gains are still possible, especially in oilseeds such as soybean crop (*Glycine max*) L. Merrill. Due to climate change and the irregular distribution of rainfall in different regions of the country, as well as attacks by pathogens and pests, grain production has been affected, and yields have become unstable in the short term (FIESP, 2020).

The greatest threat to soybeans is the stink bug complex (Silva et al., 2013; Silva et al., 2014; Souza et al., 2016; Canassa et al., 2017a, b), especially the species *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae). Native to warm regions, the brown stink bug has easily adapted to Brazilian climatic conditions and the agricultural scenario. The country's no-till system predominates, which favors the establishment and further development of these insects' populations (Sosa-Gomez et al., 2020; Defensor et al., 2021).

Euschistus heros has several host plants and its destruction is not restricted to soybeans. Its damage can be seen on Brassicae, Solanaceae, corn, and other legumes (Medeiros and Megier, 2009; Istchuk et al., 2023). Direct damage to soybeans comes from the attack by adults and nymphs, which suck the grains, leaving them shriveled, brittle and/or unviable (Depieri and Panizzi, 2011; Scopel et al., 2016). Indirect damage is related to the entry of pathogens into the seeds and the injection of toxins, which can cause physiological disturbances in the plant (Scopel et al., 2016).

To define the most appropriate control strategy for the brown stink bug, it is necessary to monitor the crop (Guedes et al., 2006; Borges et al., 2011). Two types of stink bug monitoring are used in soybean cultivation: the most common is with the beaten cloth, which considers two stink bugs per linear meter as the level of control for grain production areas and one stink bug per linear meter for seed production areas (Bueno et al., 2013). Another type of monitoring, which is less common, is the use of sex pheromone traps, which are very efficient in the reproductive stages (R1 to R5) of soybeans (Borges et al., 2011).

Another common management method of monitoring brown stink bugs in soybean crops is the chemical method (Belo et al., 2012). Starting from the 1990s onwards, the continuous use of endosulfan and organophosphates to control brown stink bugs resulted in the first cases of population resistance to these products (Sosa-Gomez et al., 2010). To reduce selection pressure, the chemical industries have sought to innovate with new insecticide molecules belonging to different mechanisms of action. Various mixtures based on neonicotinoids and pyrethroids have been developed to manage stink bugs. The mixture composed of thiamethoxam + lambda-cyhalothrin is one example (Ribeiro et al., 2016). Since the first decade of the 2000s, the molecule dinotefuran (neonicotinoid) has appeared in a mixture with a pyrethroid (lambda-cyhalothrin) (Wakita et al., 2003).

Considering the high losses of grains and seeds due to the attack of the *E. heros* and the difficulties related to managing its populations, it is necessary to evaluate the bioactivity of products on this pest. With these problems in mind, this study aimed to evaluate the mortality and possible sublethal effects (feeding deterrence) of chemical products such as dinotefuran, thiamethoxam and lambda-cyhalothrin on *E. heros* in soybeans.

2. Material and Methods

This research was carried out in the Laboratory of Host Plant Resistance to Insects and Botanical Pesticides (LARESPI) of the Department of Crop Protection in the School of Agriculture - São Paulo State University - UNESP, Botucatu city, Brazil, during the year 2023.

A population of *E. heros* was maintained under controlled conditions (14:10 h L:D at 26 ± 2 °C and 65 ± 10% RH) as described in (Canassa et al., 2017a, b). The adults were maintained in plastic containers (8 L, 22 cm diameter and 20 cm in height) covered with organdy ("voile") to allow adequate ventilation. The bottom surfaces of the containers were lined with filter paper to absorb excrement and maintain sanitary conditions. Each container housed 100 adults, which were maintained on a natural diet of green pods [*Phaseolus. vulgaris* (L.)] (10 pods/container) and raw peanuts [*Arachis hypogaea* (L.)] in portions of 50 g/cage, which were deposited in Petri dishes. The food was replaced, and the containers were cleaned every week to avoid fungal contamination.

Cotton moistened with distilled water was placed in the Petri dishes to meet the hydration needs of the stinkbugs and to maintain moisture levels within the containers. Discs of dry cotton were placed equidistantly along the bottom surface of the container to serve as oviposition sites and shelter for the insects. The discs were suspended along the top edge of the cage using hooks fashioned from number two paper clips. To ensure that the eggs were not consumed by the adults (Canassa et al., 2017a, b), the eggs were collected daily and placed in Petri dishes (8.5 cm diameter) lined with moistened filter paper and containing a bean pod as a food source for the insects' first nymphal stage. When the insects reached the second nymphal stage, they were transferred to plastic containers prepared as described previously. To evaluate the mortality and possible sublethal effects of *E. heros* to insecticides, we used the manufacturer's recommended rates which are described in Table 1.

Table 1. Commercial insecticides used to evaluate the mortality and possible sublethal effects of chemical products on *Euschistus heros*

Active ingredient (AI)	Insecticide class (IRAC MoA)	Trade name	AI (%)	Company/manufacturer
Dinotefuran	Neonicotinoid (4A)	Dinno	20	Iharabras Chemical Industry S.A, Sorocaba, SP, Brazil
Thiamethoxam	Neonicotinoid (4A)	Actara	25	Syngenta Crop Protection Ltda, Paulínia, SP, Brazil
Lambda-cyhalothrin	Pyrethroid (3A)	Karatê Zeon	25	Syngenta Crop Protection Ltda, Paulínia, SP, Brazil

This test was carried out to estimate the lethal concentration values at 10% and 25% (LC_{10} and LC_{25}) of the active ingredients dinotefuran, thiamethoxam and lambda-cyhalothrin on *E. heros* adults under laboratory conditions (T = 25 ± 2 °C, RH = 65 ± 10%, and photoperiod = 14 h).

Tests were performed with six or seven concentrations for each active ingredient to establish the concentration mortality curves. To facilitate the application of the treatments, the adults of *E. heros* were kept in a freezer (Electrolux®, Curitiba, PR, Brazil) for 120 s. Preliminary tests showed that the procedure does not affect the survival and behavior of the adults.

To start the tests, five adults were transferred to Petri dishes (9.0 cm Ø), covering the base with filter paper containing a soybean pod ('NA89 5909RG' cultivar) in phenological phase R5/R6 (Fehr and Caviness, 1977). Using a micropipette (2 μL), the treatments were applied to the pronotum of each insect, according to the IRAC-029 methodology (IRAC, 2021). Each Petri dish corresponded to one repetition, for a total of six repetitions in a completely randomized design. The mortality of insects was evaluated three days after application (DAA). The individuals were considered dead when they remained immobile after stimulus with a brush.

The pods were obtained from plants grown in 3 L pots inside a greenhouse, were free of insect infestation, and presented a phenological stage of R5/R6. The pots were sown in stages to provide pods (R5/R6) throughout the test period. As a substrate, we used fertilized soil with the pH adjusted to be suitable for soybean crops.

The tests were conducted in Petri dishes (9.0 cm Ø), covering the base with filter paper. Before starting the tests in the Petri dishes, two soybean pods were placed with five adults of *E. heros* (up to 48 h old). The adults had been fasting for 24 hours prior to being placed in the Petri dish. Using a micropipette (2 μL), the treatments were applied to the pronotum of each insect, according to the IRAC-029 methodology (IRAC, 2021), using the LC_{10} and LC_{25} collected from the previous test.

To identify the feeding behavior of each stink bug, each insect was duly marked with yellow, blue, pink, and green dyes and one was left unmarked (BioQuip Products, Rancho Dominguez, California, USA) (Pannuti et al., 2019).

Evaluations were performed by noting the number of insects from each Petri dishes present on the pods at 10, 30, 45, 60, 120 and 180 minutes after application. Attractiveness ratings of the soybean pods were also recorded, using a chronometer, insect consumption of the pods containing different treatments was assessed for a period of 180 minutes by evaluating the number of insect punctures, the total feeding time per insect, and the feeding time per puncture. Determination of the number of insects on pods, total number of punctures, total feeding time, and feeding time per puncture were conducted using visual inspection, which is one of the methods utilized by Canassa et al. (2017a). The feeding time was determined with the aid of a digital timer, which was activated at the beginning of feeding by each insect and stopped at the time when the stinkbug withdrew its stylet from the pod. At the end of the observation period, the mortality was verified, by counting the number of *E. heros* adults that were dead in each Petri dish. After that, the dead insects from each Petri dish were discarded.

The mortality data of *E. heros* was subjected to Probit analysis to determine the mortality dose curves for each treatment, using the procedure PROC PROBIT (SAS Institute, 2011). Curves were obtained with a probability of accepting the null hypothesis (that the data have Probit distribution) greater than 0.05 by the χ^2 test. Lethal doses (LC_{10} and LC_{25}) and their respective confidence intervals at 95% probability (CI_{95}) were obtained from the curves.

The data from the number of insects on pods, total number of punctures, total feeding time, feeding time per puncture, and mortality were submitted to an analysis of variance by F-test. The normality was verified by the Shapiro-Wilk test and homogeneity was verified by the

Levene test. The significance of the treatment effects was determined using Fisher's Least Significant Difference (LSD) to compare the means. For the analysis, we used the statistical package PROC MIXED-SAS 9.2 (SAS Institute 2011).

3. Results and Discussion

Applied insecticide concentrations typically degrade to low and sublethal concentrations due to field degradation and plant growth, resulting in frequent exposure of pests to low or sublethal concentrations (Desneux et al., 2005). Low or sublethal insecticide concentrations ultimately affect the physiological and behavioral traits of exposed insects, such as lifespan, developmental period, fecundity, host finding, and feeding activity (Desneux et al., 2007; Gul et al., 2023). Therefore, in-depth information about the impact of low or sublethal concentrations of thiamethoxam, dinotefuran, and lambda-cyhalothrin on the feeding behavior could be crucial for managing *E. heros*.

The treatments presented progressive insecticidal activity as the concentration increased on *E. heros* adults via topical application. The concentrations of these treatments necessary to cause 10% and 25% mortality in the populations ranged from 0.37 to 10.65 g. a.i. ha^{-1}, and 2.74 to 76.58 g. a.i. ha^{-1}, respectively (Table 2).

The thiamethoxam was more toxic than dinotefuran and lambda-cyhalothrin, with the lowest values at LC_{10} (0.37 g i.a. ha^{-1}) and LC_{25} (2.74 g i.a. ha^{-1}), respectively. At the LC_{10} level thiamethoxam was 3.54 times more toxic than dinotefuran, and 28.78 more toxic than lambda-cyhalothrin. For the LC_{25}, thiamethoxam was 1.97 times more toxic than dinotefuran, and 27.94 more toxic than lambda-cyhalothrin.

When analyzing the data obtained using different active ingredients, all three showed progressive activity, responding to the increase in concentration on *E. heros* via graduate topical application. In the definitions of LC_{10} and LC_{25} of the active ingredients, thiamethoxam was found to be more toxic than dinotefuran, and dinotefuran was more toxic than lambda-cyhalothrin. Although the difference in efficiency among the active neonicotinoid ingredients (dinotefuran and thiamethoxam) is large and should not be ignored, the product mixtures may be of greater value when targeting *E heros.* This is based on the different modes of action, the possible sublethal effects, and the possible synergies between the products.

The neonicotinoids represent the fastest-growing class of insecticides introduced onto the market since the commercialization of pyrethroids (Nauen and Bretschneider, 2002). Like the naturally occurring alkaloid nicotine, all neonicotinoids act selectively on the insect central nervous system as agonists of the post-synaptic nicotinic acetylcholine receptors (Bai et al., 1991; Salgado, 2016; Zhang et al., 2000), their molecular target site. In this connection, neonicotinoids are very effective ligands for structural investigations and facilitate the understanding of functional properties of insect nicotinic acetylcholine receptors. As a result of the efficient mode of action, neonicotinoids have each more time replacing pyrethroids, chlorinated hydrocarbons, organophosphates, carbamates, and several other chemical classes of insecticides used to control insect pests on major crops (Denholm et al., 2002; Nauen and Denholm, 2005).

Despite the potential risks to the environment associated with the use of neonicotinoids, the application of such compounds remains the most prevalent control practice for managing sucking insect pests of soybean (e.g., stinkbugs, whiteflies and aphids). For instance, most cases of neonicotinoid application in the South American soybean-growing regions aim at controlling the Neotropical brown stink bug (Sosa-Gómez and Silva, 2010; Hegeto et al., 2015; Tuelher et al., 2018). The reason for this frequent targeting of *E. heros* is because these insects are dominant competitors among other stink bugs (Souza et al., 2016; Tuelher et al., 2016) and are capable of compromising soybean seed quality and yield (Souza et al., 2014; Hegeto et al., 2015).

Dinotefuran is a third-generation neonicotinoid insecticide that is widely used to control various harmful pest species, including stink bugs, plant hoppers, and leafhoppers (Watanabe et al., 2011). The characteristic group, the tetrahydro-3-furylmethyl moiety makes dinotefuran possess a high insecticidal activity over a wide spectrum through a unique mode of action that differs from one-generation and two-generation neonicotinoids (Wakita et al., 2003). With the advantages above, dinotefuran is expected to be used as a new-generation pesticide to control pests with piercing-sucking mouthparts.

Synthetic pyrethroids are compounds whose structures are derived from the naturally occurring pyrethrin esters, which were isolated from the chrysanthemum flower [*Chysanthemum cinerariaefolium* (L.) (Asteraceae)]. The synthetic pyrethroids are grouped in two separate classes - type I and type II. This bifurcation is determined by the chemical structure and the acute poisoning symptoms they elicit. Type I pyrethroids contain an esterified primary alcohol moiety and typically elicit tremors in rodents. In contrast, type II pyrethroids contain ester bonds that incorporate a secondary alcohol moiety which usually possesses a cyano group at a carbon position of the alcohol (Ross, 2011).

Table 2. Estimates of LC_{10} and LC_{25} (g.a.i. ha^{-1}) and confidence interval for the insecticides dinotefuran, thiamethoxam, and lambda-cyhalothrin on adults of *Euschistus heros*. Botucatu-SP, 2021.

Treatments	N[1]	LC10 (95% CI)[2]	LC25 (95% CI)[2]	Slope (± EP)	χ2 ([3])	P
Dinotefuran	330	1.31 (0.52 – 17.42)	5.41 (6.84 – 48.91)	0.9870 ± 0.2321	1.2566	0.8687
Thiamethoxam	330	0.37 (0.03 – 5.34)	2.74 (1.82 – 22.37)	0.6974 ± 0.1818	11.2910	0.9359
Lambda-cyhalothrin	330	10.65 (0.33-109.07)	76.58 (118.01-474370)	0.7086 ± 0.3074	0.1386	0.9868

[1]N=number of stink bugs; [2] CI=confidence interval; [3]Chi-square test.

Most poisoning symptoms elicited by type II pyrethroids are derived from the effects on the central nervous system, whereas type I pyrethroids cause nerve dysregulation in both central and peripheral nervous systems. The primary molecular target of pyrethroids in insects and vertebrates, is the voltage-sensitive sodium channels (VSSCs) that are embedded in neuronal membranes. Pyrethroids bind to VSSCs and alter the kinetics of sodium ion influx into the cell, which depending on the class of pyrethroid involved, causes repetitive action-potential firing. Pyrethroids elicit their acute toxic effects by direct interaction of the parent chemical with binding sites on VSSCs (Frank et al., 2018; Mukherjee et al., 2010; Ross, 2011).

Regarding the number of brown stinkbugs feeding during different evaluation periods, all the periods evaluated showed statistically significant differences using the LC_{10} and LC_{25} with the exception of the first evaluation (10 minutes). This revealed that even at low concentrations, these active ingredients interfere with the feeding behavior of *E. heros* (Table 3). In the second evaluation (30 minutes), only the LC_{25} of lambda-cyhalothrin differed from the control (2.83 insects), with fewer insects feeding (1.0 insect) (Table 3). In the 45-minutes evaluation, the concentrations based on dinotefuran and thiamethoxam differed from the control population (0.33 insects), but with a higher mean number of *E. heros* feeding, ranging from 2.50 to 3.83 insects (Table 3). At 60 minutes after the release of the insects, the LC_{25} of dinotefuran and lambda-cyhalothrin differed from the control (2.50 insects), with a mean of 1.17 and 0.83 stinkbugs, respectively (Table 3). LC_{25} of thiamethoxam differed from the control group, but with a higher mean number of *E. heros*. In the 120-minute evaluation, the LC_{10} and LC_{25} of dinotefuran and lambda-cyhalothrin differed from the control (3.17 insects), showing lower mean numbers of *E. heros* feeding, with values ranging from 0.33 to 0.50 (Table 3). In the last evaluation (180 minutes), the concentrations of all active ingredients differed from the control (3.67 insects), with a low mean number of insects feeding, ranging from 0.00 to 1.17 insects (Table 3).

Table 3. Mean number (± EP) of *Euschistus heros* adults feeding of soybean pods (R5) in different periods after insecticides spraying. Botucatu-SP, 2021.

Treatments	g a.i. ha^{-1}	Mean number of *Euschistus heros* feeding pods[1]					
		10 min	30 min	45 min	60 min	120 min	180 min
Check control	---	1.83 ± 0.31	2.83 ± 0.31 ab	0.33 ± 0.21 c	2.50 ± 0.22 bc	3.17 ± 0.17 ab	3.67 ± 0.33 a
LC_{10} dinotefuran	1.31	2.17 ± 0.17	2.67 ± 0.21 ab	3.17 ± 0.17 ab	2.83 ± 0.17 ab	0.33 ± 0.21 d	0.17 ± 0.17 bc
LC_{25} dinotefuran	5.41	1.83 ± 0.31	3.17 ± 0.17 ab	2.50 ± 0.22 b	1.17 ± 0.31 d	0.33 ± 0.21 d	0.00 ± 0.00 c
LC_{10} thiamethoxam	0.37	2.17 ± 0.17	2.50 ± 0.22 ab	2.67 ± 0.21 ab	3.33 ± 0.21 ab	3.33 ± 0.21 a	1.17 ± 0.31 c
LC_{25} thiamethoxam	2.74	2.67 ± 0.21	3.33 ± 0.21 a	3.83 ± 0.31 a	3.83 ± 0.31 a	2.33 ± 0.21 bc	0.33 ± 0.21 bc
LC_{10} lambda-cyhalothrin	10.65	2.67 ± 0.21	2.17 ± 0.17 b	1.17 ± 0.31 c	1.50 ± 0.22 cd	1.83 ± 0.17 c	0.83 ± 0.31 bc
LC_{25} lambda-cyhalothrin	76.58	2.17 ± 0.31	1.00 ± 0.37 c	1.00 ± 0.37 c	0.83 ± 0.31 d	0.50 ± 0.22 d	0.17 ± 0.17 bc
P		0.1039	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1] Means followed by the same lower-case letter per column do not differ by Fishers Least Significant Difference (LSD) ($P \leq 0.05$).

Regarding the feeding time of the brown stinkbugs (Table 4), there was no statistical difference between the different concentrations of the active ingredients evaluated for the number of punctures at 180 minutes after application, with mean values ranging from 3.67 to 8.33 punctures. In the evaluation of the total feeding time per insect, the LC_{10} and LC_{25} of dinotefuran and lambda-cyhalothrin differed from the control (112.03 minutes), with means of feeding time ranging from 30.93 to 49.33 minutes, proving to be efficient at reducing feeding time of *E. heros* on soybean pods (Table 4). In the assessment of feeding time per puncture, the LC_{10} and LC_{25} of dinotefuran and lambda-cyhalothrin differed from the control (87.07 minutes), with means ranging from 19.03 to 42.33 minutes (Table 4). Regarding the mortality of *E. heros* after 180 minutes, LC_{25} of dinotefuran proved to be the fastest treatment in controlling the insect, differing from the control population (5.00 insects).

In terms of feeding behavior, *E. heros* causes damage by penetrating its stylet into the plant tissues at different depths and injecting its saliva. The enzymes present in the salivary secretions facilitate digestion, degrade tissues, and induce darkening.

Feeding on immature pods and seeds can result in plant deformations, premature abscission, formation of wrinkled or empty seeds. It can also delay maturation and decrease seed vigor (Panizzi et al., 2012; Zerbino and Panizzi, 2019). Thus, the lower the population density of *E. heros* feeding on pods and seeds, the less damage caused. In our study, lambda-cyhalothrin and dinotefuran were the treatments that resulted in the lowest mean numbers of insects feeding on the pods. In the field, using these products can be reflected in a lower population density of *E. heros* feeding on soybeans and reducing yield.

In the first evaluation (10 minutes), the LC_{10} and LC_{25} of the products did not differ statistically from the control. This may be related to the previous fasting to which the stinkbugs were subjected, which stimulated feeding in the first few minutes of evaluation. This may also be associated with the low doses of the treatments and the time frame needed to influence the behavior of the insects.

In general, the LCs of thiamethoxam did not differ from the control population regarding the number of punctures, feeding time per insect, and feeding time/puncture. This suggests that this insecticide did not affect the feeding behavior of *E. heros* until 3 hours of observation. Indeed, previous studies suggested that sublethal concentrations of neonicotinoid insecticides have positive effects on the physiology of *E. heros* (Haddi et al., 2016; Santos et al., 2016). On the other hand, LC_{10} and CL_{25} of dinotefuran and lambda-cyhalothrin reduced feeding time per insect by 2.2 to 3.6 times compared to the control, and this application reduced feeding per puncture by 2.0 to 4.4 times. Evaluating the sublethal effects of insecticides on insects is very important for integrated pest management programs. Doses that do not cause kill the insect can negatively interfere with biological characteristics, reduce populations in subsequent generations, and reduce the potential for crop damage (Cremonez et al., 2022; Wu et al., 2022). For the active ingredient lambda-cyhalothrin, this reduction in feeding time may be associated with the characteristic paralyzing effect of the pyrethroid group because lambda-cyhalothrin acts quickly, causing the insect to "knock down," from the rapid shock action that leads to paralysis and/or death (Ross, 2011).

Table 4. Mean number (± EP) of number of punctures, total feeding time per insect, feeding time per puncture and mortality of *Euschistus heros* adults in soybean pods (R5) until 180 minutes after spraying. Botucatu-SP, 2021.

Treatments	a.i. (g ha^{-1})	Number of punctures¹	Feeding time/insect (min)¹	Feeding/puncture (min)¹	Mortality¹ (180 min)
Check control	---	7.33 ± 1.78	112.03 ± 9.87 a	87.07 ± 14.06 a	500 ± 0.00 a
LC_{10} dinotefuran	1.31	5.00 ± 0.37	49.33 ± 4.53 bc	42.33 ± 3.47 bc	4.67 ± 0.20 a
LC_{25} dinotefuran	5.41	3.67 ± 0.61	38.63 ± 6.72 c	37.40 ± 6.39 c	3.33 ± 0.20 b
LC_{10} thiamethoxam	0.37	5.83 ± 0.54	99.07 ± 10.16 a	86.70 ± 12.48 a	4.50 ± 0.21 a
LC_{25} thiamethoxam	2.74	5.33 ± 0.61	88.23 ± 14.20 ab	79.87 ± 12.18 ab	4.33 ± 0.20 a
LC_{10} lambda-cyhalothrin	10.65	8.33 ± 1.73	49.03 ± 12.27 bc	25.30 ± 6.77 c	5.00 ± 0.00 a
LC_{25} lambda-cyhalothrin	76.58	5.83 ± 1.40	30.93 ± 9.48 c	19.03 ± 4.70 c	5.00 ± 0.00 a
P		0.1342	<0.0001	<0.0001	<0,0001

¹ Means followed by the same lower-case letter per column do not differ by Fisher's Least Significant Difference (LSD) (P ≤ 0.05).

In our study, the low concentrations of dinotefuran and lambda-cyhalothrin (LC_{10} and LC_{25}) reduced the time spent on the pods and the feeding time of *E. heros* adults. In the field, the active ingredients may not reach the amount needed to kill the target, due to drift, drops on the ground, and degradation. The low concentrations, allow the insects to feed less and cause less damage to the soybeans although they do not kill the adults of *E. heros*.

By the IPM view, it's interesting to note that thiamethoxam, which was the most toxic active ingredient in the study, can be associated with *Telenomus podisi* parasitism to control *E. heros* (Abbate et al., 2022). On the other hand, associations between these ingredients showed high impact in the parasitoid development, where lambda-cyhalothrin + dinotefuron had higher effectiveness against *T. podisi* than in controlling *E. heros*, as well as lambda-cyahalothrin + thiamethoxam behavior (Pazini et al., 2024).

Insecticides efficiency had been decreased from 2021/2022 season to 2022/2023 season in *E. heros* control, where the use of isolated active ingredients is not recommended, mainly lambda-cyalothrin (Moreira et al., 2024). Even more producers are adopting associations between these ingredients, so that behavior against the insects can be modified depending on the chemical interactions envolved.

The results obtained in this study contribute to understanding the interaction between some insecticides, the neotropical brown stink bug, and the soybean plant. Research related to repellency, phagodeterrence/ovideterrence using sublethal concentrations should be the subject of future investigations. The major goal of this recommended research would be to offer new perspectives on the use of different active ingredients already on the market, as well as possible associations between them.

4. Conclusions

The low concentrations of dinotefuran and lambda-cyhalothrin (LC_{10} and LC_{25}) reduced the time spent on the pods and the feeding time of *E. heros* adults. These low concentrations, allow the insects to feed less and cause less damage to the soybeans although they do not kill the adults of *E. heros*.

Authors' Contribution

Adilson Massami Nakaghi contributed to data collection and organization, Vinícius Fernandes Canassa wrote the manuscript and assisted with statistical analysis, Aline Marques Pinheiro contributed to data collection and interpretation of results, Rodrigo Donizeti Faria contributed to data collection, Felipe Savieto Furquim de Souza contributed to data collection; Carlos Gilberto Raetano and Caio Antonio Carbonari conceptualized and designed the study.

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