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Development of the Neotropical brown stink bug *Euschistus heros* (Fabricius) on strawberry plants and mortality with a product based on *Sophora flavescens*

Desenvolvimento do percevejo-marrom *Euschistus heros* (Fabricius) em morangueiro e mortalidade com produto à base de *Sophora flavescens*

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Highlights

Sophora flavescens extract reduces *Euschistus heros* population on strawberry plants.
Sophora flavescens doses between 3 and 9 mL L^{-1} cause higher mortality of *E. heros.*
Strawberry plants with reproductive structures allow the development of *E. heros.*

Abstract

Euschistus heros is an important pest in soybean crops and has migrated to strawberry plants, causing injuries, mainly to strawberry fruits. This study aimed to assess the development capacity of *E. heros* and its ability to damage strawberry plants, as well as to evaluate its probability of survival when exposed to a commercial product based on *Sophora flavescens*. Two bioassays were conducted. The first was performed in a greenhouse, where plants were placed in pots and enclosed in voile cages to evaluate the longevity of the Neotropical brown stink bug on plants with and without reproductive structures. The viability of nymphs and the time required to reach the adult stage were also assessed. In the second bioassay, conducted in a laboratory, the probability of female survival was evaluated under different

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doses of an insecticide based on *S. flavescens.* The results showed that the Neotropical brown stink bug exhibits greater longevity on strawberry plants with reproductive structures. Only two females reached adulthood, and none was able to reproduce under the experimental conditions. The commercial product based on *S. flavescens* caused mortality in *E. heros* and showed potential for use in managing this pest in strawberry crops, with effectiveness starting at a dose of 3 mL L^{-1}.
Key words: Botanic insecticide. *Fragaria × ananassa* Duchesne. Oximatrine. Pentatomidae.

Resumo

Euschistus heros é uma importante praga na cultura da soja e tem migrado para o morangueiro, causando injúrias, principalmente em pseudofrutos. Os objetivos deste trabalho foram determinar a capacidade de desenvolvimento de *E. heros* e causar danos em plantas de morangueiro, bem como avaliar a probabilidade de sobrevivência do mesmo, quando submetido a um produto comercial a base de *S. flavescens.* Foram feitos dois bioensaios, sendo o primeiro em casa de vegetação, com plantas dispostas em vasos e envoltas em gaiolas de voil, em que se avaliou a longevidade do percevejo-marrom em plantas com estruturas reprodutivas e sem estruturas reprodutivas. Também foram avaliados a viabilidade de ninfas e duração para alcançar a fase adulta. No segundo bioensaio, em laboratório, avaliou-se a probabilidade de sobrevivência de fêmeas do percevejo-marrom, submetidas a diferentes doses do inseticida *S. flavescens.* Observou-se que o percevejo-marrom tem maior longevidade em plantas de morangueiro com estruturas reprodutivas. Apenas duas fêmeas chegaram à fase adulta e nenhuma foi capaz de se reproduzir nas condições do experimento. O produto comercial a base de *S. flavescens* causa mortalidade de E. heros, tendo potencial para uso no manejo deste inseto, na cultura do morango, a partir da dose de 3 mL L^{-1}.
Palavras-chave: *Fragaria × ananassa* Duchesne. Inseticida botânico. Pentatomidae. Oximatrine.

Global strawberry production increased from 7,879,108 t in 2013 to 9 million t in 2022 (Food and Agriculture Organization of the United Nations [FAOSTAT], 2023), representing a 13% growth. One of the main challenges to high production is the incidence of pests in this crop.

Several insects, mites, and mollusks are primary pests that cause economic damage to strawberry plants, including *Tetranychus urticae* Koch (Acari: Tetranychidae), *Phytonemus pallidus* Banks (Acari: Tarsonemidae), and *Neopamera bilobata* Say (Hemiptera: Rhyparochromidae) (Lahiri et al., 2022). However, in recent years, several new pests have been reported for the first time on strawberry plants in Brazil, including *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) (Deprá et al., 2014), *Duponchelia fovealis* (Zeller) (Lepidoptera: Crambidae) (Zawadneak et al., 2016), and *Heliothrips haemorrhoidalis* (Bouché) (Thysanoptera: Thripidae) (Souza et al., 2019). Therefore, studies on potential insects that may become strawberry pests are essential. In this context, no study to date has linked the Neotropical brown stink bug, *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae), to strawberry plants.

Euschistus heros has been expanding to other crops and regions of economic interest. Its population is present across most of Brazil and is well adapted to soybean crops due to the extensive cultivated areas that provide abundant food. After soybean harvest, these insects tend to disperse to neighboring crops, such as cotton, causing severe economic damage (Soria et al., 2016). Reports from producers and field observations indicate that *E. heros* primarily migrates to strawberry plants during the soybean harvest period, causing injuries, particularly to berries. These injuries include deformities and, indirectly, an increased incidence of diseases in affected strawberries. The Neotropical brown stink bug has been reported to feed on plants from the families Euphorbiaceae, Fabaceae, Solanaceae, Brassicaceae, and Compositae (Panizzi et al., 2000; Pazinni & Lucini, 2022). Additionally, injuries caused by Euschistus species have been documented in other small fruits, such as blackberries (*Morus alba,* Rosaceae) (Pasini & Lúcio, 2014). Therefore, studying the feeding behavior of this insect on different hosts is essential.

The Neotropical brown stink bug is difficult to control, as only a limited number of chemical insecticides with different modes of action (MoA) are available (Panizzi & Lucini, 2022). For this reason, there is a continuous need to explore new pest control methods using products with low environmental impact. Several plant species exhibit insecticidal potential due to compounds with toxic, repellent, or antifeedant properties, among other effects on insect pests (Isman, 2020). Plants can synthesize aromatic secondary metabolites, most of which are phenols or their derivatives. These compounds may contribute to plant defense mechanisms and exhibit activity against pests.

Essential oils extracted from whole plants or specific plant parts, such as roots, stems, leaves, flowers, fruits, and seeds, are widely used in the preparation of plant-based extracts with pesticidal effects (Isman, 2020). In this context, plant extracts derived from *Sophora flavescens* (Fabaceae) can be utilized for pest control. This plant's extract exhibits acaricidal and insecticidal activity, primarily due to its active ingredient, oxymatrine, a quinolizidine alkaloid (Zanardi et al., 2015). Recently, a patent registration highlighted the potential of a commercial product based on S. flavescens for controlling *E. heros* (Whipple et al., 2022).

This study aimed to assess the ability of the Neotropical brown stink bug to develop and cause damage to strawberry plants, as well as to evaluate its survival probability when exposed to a commercial product based on *S. flavescens.*

The experiments were conducted at two locations: the Paraná Rural Development Institute (IDR-PR) and the Entomology Laboratory of the State University of Londrina (UEL).

Nymphs and adults (male and female) of *E. heros* used in the bioassays were obtained from a stock-rearing system established at the IDR-Paraná Entomology Laboratory. These insects were fed soybeans, peanuts (*Arachis hypogaea,* Fabaceae), and common bean pods (*Phaseolus vulgaris,* Fabaceae) and had no prior contact with strawberry plants.

The first bioassay evaluated the ability of *E. heros* to complete its developmental cycle on strawberry plants grown in a greenhouse. Albion cultivar plants, eight months after transplanting, were grown in pots (4 dm^{-3} capacity) containing substrate 9084H (Carolina Soil do Brasil, Santa Cruz do Sul, Rio Grande do Sul, Brazil). Irrigation was performed twice daily, applied directly to the pot using a hose. The plants were pruned before infestation with the stink bugs to standardize their size.

A completely randomized experimental design was adopted with the following treatments: (T1) plants without stink bug infestation; (T2) adult stink bugs kept on strawberry plants without reproductive structures; (T3) adult stink bugs kept on strawberry plants with reproductive structures; (T4) nymphs kept on strawberry plants with reproductive structures; (T5) adult stink bugs kept with the same standard diet as the stock rearing; and (T6) nymphs kept with the same standard diet as the stock rearing.

Ten replicates were used for treatments involving strawberry plants (T1 to T4). Each replicate consisted of a potted plant enclosed in a cylindrical cage (30 cm in diameter, 50 cm in height) with a side opening secured by a zipper. The cages were attached to the upper edge of the pots using rubber bands and kept suspended by metal frames.

For treatments where stink bugs were maintained on the standard stock-rearing diet (T5 and T6), five replicates were used. Each replicate consisted of an acrylic box with a perforated lid for gas exchange. The boxes contained either a pair of bugs (T2, T3, and T5) or 10 third-instar nymphs (T4 and T6). Water was provided via moistened cotton, and food (soybeans, peanuts, and green beans) was replaced once or twice a week, as needed.

After infestation, the cages and acrylic boxes were inspected three times a week. The following parameters were recorded: the number of dead bugs, the number of individuals that reached adulthood, the time required to reach adulthood, and, for treatments with pairs of bugs, the number of eggs laid and their location.

During the evaluation period, the plants and acrylic boxes were randomly arranged on four benches inside a greenhouse equipped with eight reflectors (Hqi Metal Vapor Lamp, 400 W). Analog timers controlled the reflectors, ensuring illumination from 6 to 8 A.M. and from 6 to 8 P.M., maintaining a circadian rhythm of 14 h of photophase and 10 h of scotophase, thus preventing the bugs from entering diapause. At each evaluation, the plants and acrylic boxes were randomized again on the benches.

When no more eggs were laid in the cages containing pairs of stink bugs and all nymphs had reached adulthood, the plants were removed, washed, and subjected to drying with air circulation at 60 ºC until a constant weight was achieved. Subsequently, using a precision scale, the total dry weight of the plant, as well as the dry weight of flowers and fruits, was recorded.

The time required for nymphs to reach adulthood, the longevity of males and females, the total number of eggs, the total dry weight of the plant, and the dry weight of flowers and fruits were compared between treatments using the Kruskal-Wallis test, followed by Dunn's test at a 5% significance level. The number of eggs laid on versus off the strawberry plant was compared using the Mann-Whitney test at a 5% significance level. Statistical analyses were performed using the BioEstat 5.3 program.

The second bioassay aimed to evaluate the efficacy of an insecticide based on *Sophora flavescens* for controlling *E. heros* adults. One-week-old females were exposed to the following concentrations of the insecticide: 0 (water only), 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 9 mL of the commercial product (Matrine®) per liter of water. Five replicates were used for each concentration, with each replicate consisting of an acrylic box with a perforated lid for gas exchange, containing ten insects.

Prior to insecticide application, the stink bugs were kept at a low temperature (10–12 ºC) for two minutes to reduce their mobility and dispersion. The insecticide solutions were applied using hand-held sprayers, calibrated to deliver 1 mL per spray, with a single spray applied from a distance of 30 cm.

After applying the insecticides, the acrylic boxes containing the insects were randomly distributed on a bench and kept at room temperature. Additionally, raw peanuts and green beans were provided in each acrylic box as a food source, while water was supplied using moistened cotton.

Inspections were conducted 3, 24, 48, and 72 h after insecticide application, recording the number of insects that failed to move when stimulated by touch (considered dead) and those that exhibited impaired locomotor function (moribund).

The data were subjected to a non-parametric analysis using the Kaplan-Meier survival test with the Mantel-Cox regression model, assuming proportional hazards at a 95% confidence level. Analyses were performed using the GraphPad Prism 9 program.

In the first bioassay, treatments with either one pair of stink bugs or ten *E. heros* nymphs per plant did not significantly affect the total dry weight, flower and fruit weight, total number of eggs, or the number of eggs laid on versus off the plant ($p > 0.05$). Regarding nymph longevity, the insects had a higher average survival time in acrylic boxes compared to plants with reproductive structures (Table 1). For male and female longevity on strawberry plants, survival time was higher on plants with reproductive structures and in acrylic boxes than on plants without reproductive structures (Table 1).

Table 1
Mean longevity (days) of adults (males and females) and nymphs and number of eggs of *Euschistus heros* on strawberry plants. Londrina, Paraná, Brazil

Treatments	Nymphs longevity*	Adult male longevity	Adult female longevity	Total number of eggs
Plants without reproductive structures	-	9.00 b	11.30 b	13.50 a
Plants with reproductive structures	28.70 b	24.00 a	32.40 a	31.30 a
Acrylic box with insects	43.68 a	39.40 a	35.80 a	42.80 a
p-valor	0.001	0.01	0.001	0.18
H	1200	15.52	13.50	3.37

Means with similar letters are not statistically different by the Kruskal-Wallis test and Dunn's post-hoc test for multiple comparisons at 5% significance level. *Means with similar letters are not statistically different by the Mann-Whitney test at 5% significance.

In the second bioassay, 3 h after treatment application, doses of 3, 4, 5, 6, 7, 8, and 9 mL L^{-1} of *S. flavescens* increased the probability of *E. heros* mortality (6.00%, 15.50%, 11.50%, 15.50%, 10.50%, 15.50%, and 15.50%, respectively) compared to doses of 0.5, 1, and 2 mL L^{-1} (0%, 0%, and 0%, respectively) and the water-only control (0%) (Figure 1).

At 24 h after treatment application, the highest average probability of *E. heros* mortality was observed at doses of 4 to 9 mL L^{-1} of *S. flavescens* (43.67%, 41.00%, 43.67%, 40.43%, 43.67%, and 39.72%, respectively), which was higher than that observed in the water-only treatment (2.67%) (Figure 1).

At 48 h, the highest average probability of *E. heros* mortality was recorded at doses of 3 to 9 mL L^{-1} of S. flavescens (66.44%, 71.83%, 70.50%, 71.83%, 70.17%, 71.83%, and 69.86%, respectively), whereas mortality in the water-only treatment remained lower (8.51%) (Figure 1). At 72 h after application, all treatments resulted in higher *E. heros* mortality probabilities than the water-only treatment (21.32%). The highest mortality rates were observed at doses of 3, 4, 5, 6, 7, 8, and 9 mL L^{-1} of *S. flavescens* (98%, 100%, 100%, 100%, 100%, 100%, and 100%, respectively). Even at the lower doses of 0.5, 1, and 2 mL L^{-1}, *S. flavescens* increased *E. heros* mortality probability to 40.02%, 74.80%, and 88.21%, respectively (Figure 1).

The presence of the Neotropical brown stink bug on strawberry plants did not significantly affect fruit production or biomass. However, in the study on the biology of the insect, male and female longevity was reduced on plants without reproductive structures. Additionally, nymph longevity was shorter under greenhouse conditions compared to nymphs reared on the standard diet used in mass rearing at IDR-PR.

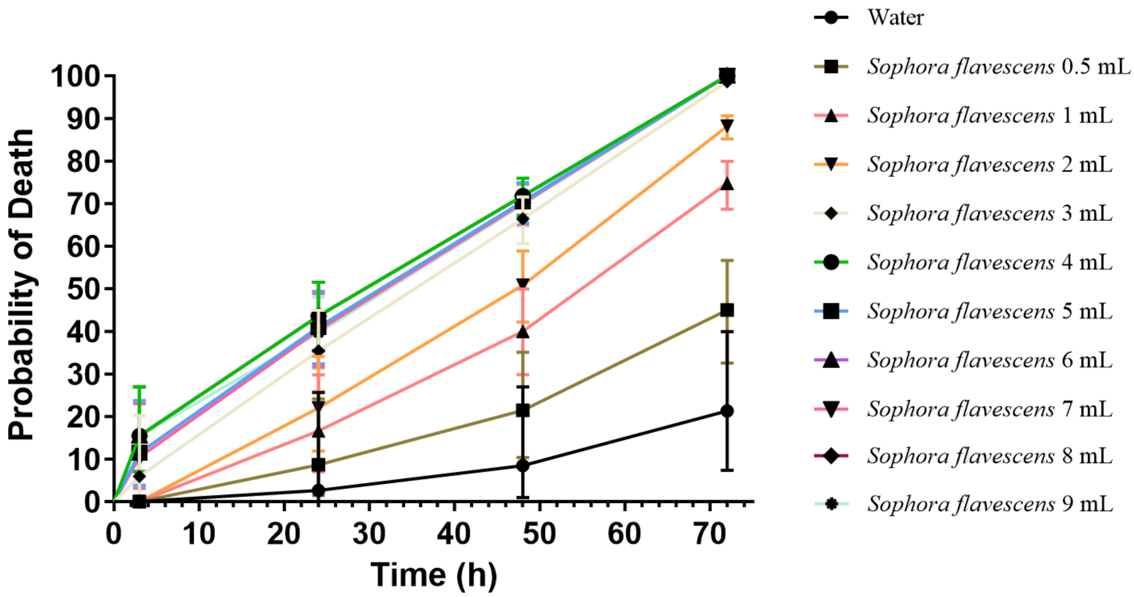

Figure 1. Cumulative probability of death, estimated by the Kaplan-Meier method, in *Euschistus heros* subjected to *Sophora flavescens* doses (0; 0,5; 1; 2; 3; 4; 5; 6; 7; 8 e 9 mL^{-1} per liter of water), after 3, 24, 48 and 72 h after treatment application. Mean ± 95% confidence interval. Londrina-PR, 2022.

After soybean harvest, the Neotropical brown stink bug tends to disperse to alternative plants and soybean straw in search of shelter and food. As a polyphagous insect with a broad host range (Panizzi et al., 2000; Panizzi & Lucini, 2022), *E. heros* successfully persists until the next soybean crop cycle, its primary host plant. A study on *E. heros* hosts identified 15 plant species from the families Amaranthaceae, Asteraceae, Brassicaceae, Fabaceae, Lauraceae, Malpighiaceae, Malvaceae, Ranunculaceae, Salicaceae, and Solanaceae as accidental hosts for this insect. Nonetheless, *E. heros* was only able to complete its reproductive cycle on six species belonging to the families Euphorbiaceae and Fabaceae (Smaniotto & Panizzi, 2015). Furthermore, research on Pentatomidae species indicated that forest fragments also serve as refuge sites for adult stink bugs undergoing diapause/oligopause, emphasizing the importance of these habitats in sustaining pest populations around crops (Laterza et al., 2023).

In the present study, the Neotropical brown stink bug exhibited better development on strawberry plants with reproductive structures. The extended development period on these plants may be associated with the nutritional quality of the reproductive structures, which could facilitate better insect development (Silva et al., 2011). However, *E. heros* was unable to complete its life cycle on strawberry plants, suggesting that strawberries serve as accidental hosts. Further studies on the biology of this insect on strawberry plants are needed, particularly during the off-season after harvest.

The Neotropical brown stink bug feeds directly on soybean pods and can cause significant crop damage by injecting toxic saliva (Panizzi & Lucini, 2022). However, in the present study, strawberry plants did not exhibit a reduction in biomass. While this insect showed better development on plants with reproductive structures, no severe deformations or biomass reductions were observed in fruits under the experimental conditions. Nonetheless, slight deformations that could lower the commercial quality and value of the product, as well as physiological changes, were detected (Figure 2). In addition, feeding damage may serve as an entry point for pathogens. In a study with an insect from the same family (Pentatomidae), *Halyomorpha halys* Stål, feeding activity led to a reduction in sugar content, ultimately diminishing fruit quality (Weber et al., 2021).

Figure 2. (A) *Euschistus heros* nymph on green strawberry and feeding on an achene (achenes around with injuries can be observed, signs of feeding visible as black dots on each achene); (B) *E. heros* adult on green strawberry and feeding on an achene (achenes around with injuries can be observed, signs of feeding visible as black dots on each achene); (C) *E. heros* adults mating on the strawberry, with noticeable slight deformation (it is observed that where there is a sign of feeding there is compromise of the achenes and the surrounding area does not develop, possibly due to the decrease in the release of auxins).

In the second bioassay, the *S. flavescens*-based product increased the probability of mortality in the Neotropical brown stink bug across all tested doses at 72 h after application. At doses above 4 mL L^{-1}, the mortality probability reached 100%, while at 3 mL L^{-1}, mortality was 98.66%, though it did not differ significantly from the 4 mL L^{-1} dose. Therefore, a concentration of 3 mL L^{-1} can be recommended for *E. heros* control.

This is the first study to evaluate *E. heros* mortality following treatment with an *S. flavescens*-based product. Previous research on another hemipteran, Diaphorina citri Kuwayama (Hemiptera: Liviidae), demonstrated that *S. flavescens* extract inhibited feeding and oviposition (Zanardi et al., 2015). Moreover, a recent patent was registered for the use of *S. flavescens* in controlling various pests, including the

Neotropical brown stink bug (Whipple et al., 2022), further supporting the present study's findings with the commercial product based on matrine.

For a control method to be incorporated into an integrated pest management program, it must be compatible with other strategies, particularly biological control. When utilizing bioinputs, selectivity is an essential factor to consider.

The Neotropical brown stink bug utilizes strawberry plants as a secondary host while searching for a more suitable plant for its development and reproduction. Although no significant biomass reduction in fruits was observed, feeding by *E. heros* caused deformations (Figure 2C). Therefore, further studies are necessary to better characterize potential economic losses, particularly regarding the role of test bites as entry points for diseases in fruits. Research on other phytophagous pentatomids is also needed to confirm their potential injuries/damage to fruits and their ability to reproduce and persist on strawberry plants.

In summary, the Neotropical brown stink bug has a longer lifespan on strawberry plants with reproductive structures. Among all *E. heros* nymphs monitored in the bioassays, only two (1.3%) reached adulthood, and none was able to reproduce on strawberry plants. Commercial product based on *Sophora flavescens* induces mortality in *E. heros* and shows potential for managing this pest in strawberry crops, starting at a dose of 3 mL L^{-1}.

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