Sphenophorus levis behavior: repellency to insecticide treatment and nocturnal adult activity and location pattern

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Article

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Abstract

Sphenophorus levis is a difficult to control pest in sugarcane that causes great damage to the subterranean part of the plant. Low insect control has been a result of the pesticide application technology adopted but also because of the lack of studies regarding the pest's behavior. This research aimed to examine the repellency of insecticides to *S. levis* adults and to evaluate the activity and location behavior of *S. levis* adults under 24 hour-observations. Repellency studies were conducted in free-choice tests providing treated soil with lambda-cyhalothrin + thiamethoxam and untreated soil as choice options to *S. levis* adults. Insect activity and location behavior studies were assessed by hourly observations of *S. levis* adults in containers with soil and sugarcane plant. Results indicated *S. levis* adults were not repelled nor attracted to soil treated with lambda-cyhalothrin + thiamethoxam. Additionally, insects presented nocturnal behavior with most activities (walking, digging and mating) starting at 18:00 pm until 2:00 am. An average of 20.7% of insects were out of the soil at night while the majority, 79.3%, remained inside the soil. During the day most insects, 95%, remained hidden in the soil. Exposed insects were primarily located on soil surface. According to these results, nocturnal insecticide applications may improve *S. levis* adult control due to greater insect activity and exposure at night.

Introduction

Sphenophorus levis Vaurie, 1978 (Coleoptera: Curculionidae), commonly known as the sugarcane weevil, is an important soil pest in sugarcane (*Saccharum officinarum* L.) causing significant negative impacts to farmers and industry. This pest has specially increased its incidence over the last twenty years in Brazil after the shift from manual burnt sugarcane harvesting system to mechanical sugarcane harvesting without burning. As a consequence of not using fire as a tool to facilitate harvesting, pests like root spittlebug [*Mahanarva fimbriolatta* Stål (Hemiptera; Cercopidae], sugarcane borer [*Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae)] and *S. levis* started to increase its incidence[1].

As a pest that damages the subterranean part of sugarcane, mostly the rhizomes, *S. levis* usually is located underneath the soil which makes pest control extremely difficult to succeed. Among the pest control options currently available for *S. levis* management, the chemical control is the one most used despite its low efficacy. Some authors have reported low insecticide efficacy for a range of products and field conditions[2–4]. Several factors may contribute to insecticides low efficacy, including pesticide application technologies used, active ingredients, environmental and meteorological conditions and insect's behavior.

The interaction of the insecticide active ingredient and the insect behavior is one example of how pest control may be affected. Some authors have reported, for instance, the repellency of insecticides to certain insects, including repellency of pyrethroids and neonicotinoids to Mexican bean beetle (*Epilachna varivestis*) and repellency of imidacloprid to pollinator beetles[5,6]. Hence, if *S. levis* may be repelled by applied insecticides in sugarcane there is a chance of pest control to be reduced. However, no research has investigated, up to date, the repellency or attraction of insecticides to *S. levis*. In fact, in one study evaluating the potential of the entomopathogenic fungi *Beauveria bassiana* for *S. levis* control, the author states the necessity of determining the repellency potential of *B. bassiana* applications and its implications on low control levels[7].

In addition of examining the repellency potential of applied substances for pest control, the understanding of the insect behavior is vital to an effective integrated pest management program. Regarding the behavior of *S. levis*, some authors have reported, for example, the gregarious activity of insects that tend to aggregate in distinct points distributed in the field[8–10]. In a study observing the spatial distribution of *S. levis*, the range of adult incidence in field varied from 28 m to 53 m[9]. Another described behavior of *S. levis* is the low flight capacity of adults, similar to other Curculionidae and *Sphenophorus* species as it was related for hunting billbugs (*Sphenophorus venatus vestitus*) with limited flight ability[11,12,13]. Some reports have also indicated that *S. levis* adults are nocturnal despite no specific studies examining insect activity[14]. Although several Curculionidae species are known for their nocturnal behavior like the banana weevil (*Cosmopolites sordidus*) with peak activity hours ranging from 21:00 to 4:00 am and *S. venatus vestitus* being most active from 00:00 to 4:00, no research has been conducted to evaluate *S. levis* maximum activity hours[15,16]. Pest control methods can, therefore, be enhanced by knowing at what time *S. levis* adults are mostly active and exposed.

Based on the importance of better understanding the *S. levis* behavior regarding its perception and repellency to applied insecticides and its activity throughout the day, the objectives of this study included assessing the repellency to *S. levis* adults of a mixture of two insecticides, lambda-cyhalothrin and thiamethoxam, and evaluating the activity and location of observable *S. levis* adults.

Materials And Methods

Experiment 1 – Insecticide Repellent Activity

An experiment studying the repellency of one insecticide on *S. levis* adults was conducted in 2021 in Jaboticabal, SP, Brazil. The experiment was conducted in a completely randomized design with four replications and was performed in duplicate with first and second experiment replicates on June 4th and June 15th, respectively. A structure of five circular plastic containers, 1 L, with one central container (E) connected by plastic cylindrical hose outlets, 9.53 mm diameter and 10 cm length, to other four containers (A, B, C, D) was build (Fig. 1c) based on previous studies[17–19]. Containers A and B (controls) were arranged diagonally and were filled with 80 g of untreated soil. Containers C and D were filled with 80 g of soil treated with insecticide (Fig. 1a). Insecticide soil treatment was conducted in 50 L pots with ratoon sugarcane plants of CTC 4 variety (Centro de Tecnologia Canavieira S.A., Piracicaba, SP, Brazil) in a pot mixture of soil, sand and manure in a proportion of 3:1:1, respectively. Soil samples were taken for soil analysis at the Soil Fertility Laboratory at UNESP following Raij et al. methodology[20] for organic matter content (14 g dm^{-3}) cation exchange capacity (73 mmolc dm^{-3}), base saturation (81%) and soil pH (6.0). Insecticide treatment in soil consisted of liquid applications in four pots with lambda-cyhalothrin + thiamethoxam (Engeo Pleno™ S, Syngenta, Basel, Switzerland) at 212 + 282 g a.i. ha^{-1}, with application volume of 200 L ha^{-1} using a 10 mL syringe to simulate stream jet nozzles used in ratoon field applications. Four untreated pots were also included. A uniform amount of soil (160 g) was collected from the 5 cm surface of each treated and untreated pot, placed in individual plastic bags and distributed in each circular container. In all four containers A, B, C and D, one sugarcane stalk of CTC 4 variety from sugarcane fields in Jaboticabal, SP, Brazil, was cut in half, 35 g and 10 cm long (Fig. 1a), and was placed on top with cut surface facing the soil. In the central container E, five *S. levis* adults were released in the center (Fig. 1b) and after 24, 48 and 72 h the total number of insects per container was assessed (Fig. 1d). Insects in containers were maintained in controlled conditions in laboratory under 12 h photoperiod, room temperature (22.3°C ± 1.4) and relative humidity (59% ± 2). Room temperature and relative humidity were measured with a digital thermo hygrometer Jprolab (JProlab, São José dos Pinhais, PR, Brazil). The original population of *S. levis* adults was collected between March and May, 2021, in sugarcane fields in Jaboticabal, SP, Brazil with previous infestation history and no insecticide application in the year. The percentage of repellency (PR) was calculated as in research described by Mazzonetto and Vendramin[17] and in Viteri Jumbo et al.[19] following Eq. (1):

$$\mathrm{RI} = \frac{(2 \times \mathrm{T})}{(\mathrm{T} + \mathrm{C})}$$

1

Where RI refers to the repellency index, T represents the percentage of insects in the treated containers and C represents the percentage of insects in the untreated containers. RI values indicating repellency levels ranged from 0 to 2 and results were classified accordingly: when RI < 1 repellence (R) was detected; when RI = 1 neutral activity (N) was detected; when RI > 1 attractivity (A) was detected. To improve RI classification (CL) accuracy, the standard deviation (SD) value of each treatment was considered when classifying repellency. Therefore, each treatment was only considered repellent or attractive when the RI value was out of the neutral RI value within the SD range (out of 1.00 ± SD).

Experiment 2 – Daily Adult Activity Pattern

A study evaluating the activity and location pattern of *S. levis* adults under semi-controlled conditions was conducted in Jaboticabal, SP, Brazil, in 2021. Three observational experiment replicates were conducted in August 11th, August 18th and October 10th, respectively. Sugarcane ratoon plants (60 days after harvest) of CTC 4 variety (Centro de Tecnologia Canavieira S.A., Piracicaba, SP, Brazil) were collected in field with no *S. levis* infestation history on July 7th, 2021, in Jaboticabal, SP, Brazil. Sugarcane plants were carefully collected with most of the rhizome and superficial roots and were inspected for any *S. levis* damage or insect presence to ensure plants were not infested with *S. levis* larvae, pupae or adults. On the same date plants were collected they were transplanted in 4.5 L square containers (26.6 cm x 26.6 cm x 9 cm) containing a 4.1 Kg of soil, sand and manure in a proportion of 3:1:1, respectively (Figure 2a). In the same containers, 35 cm plastic sticks were attached at each container corner and a voile fabric was used to surround it (Figure 2c). *S. levis* adults used in the study were originated from sugarcane field collections in March, April and May, 2021, using baits of sugarcane stalks cut in half (30 cm) that were immersed in 50 L water containers with 10% of melted sugar solution for 24 h following adapted methodology[21]. Containers with sugarcane plants were kept outside under natural light, temperature and relative humidity to simulate field conditions. During the experiment, in case of rainfall events, containers were covered ensuring similar meteorological conditions but allowing the conduction of activity evaluations. The photoperiod, hourly temperature and relative humidity of each experiment replication was recorded. Temperature and relative humidity were measured with a digital thermo hygrometer Jprolab (Jprolab, São José dos Pinhais, PR, Brazil) during hourly insect activity evaluations. Yellow neon acrylic non-toxic paint (Acrilex Tintas Especiais S.A., SP, Brazil) was used on insects to facilitate insect location at night (Figure 2b). One small mark on insect's elytra was done using a paint brush (size 1). Preliminary observations were conducted to ensure the paint used would not affect insect's behavior. Twenty *S. levis* adults were placed in the center of each sugarcane pot, 12 hours before (noon) activity and location pattern observations for insect acclimation. Four containers (replicates) were used. At midnight, activity pattern evaluations started and were conducted every hour during 24 hours. Adults were observed for an average of 3 minutes per container. The location (e.g., soil surface, cane stalk, tiller base, leaf, not visible) and behavior (e.g., walking, digging, mating, inactive) of each *S. levis* adult per container was recorded (Figure 2d). A blacklight lantern WY6548 model (Coquimbo, Shenzhen, China) was used at night to evaluate insects with minimum disturbance. The following day after the third experiment replicate being conducted on October 27th, sugarcane ratoon plants and soil of each container were removed for a visual assessment of the number and location of remaining *S. levis* per container. The conduction of both experiments 1 and 2, and the collection of sugarcane plant material and insects in field were all conducted following relevant institutional, national and international guidelines and legislation.

Data analysis

Statistical analysis for both experiments was conducted using the RStudio Version 1.4.1717 software[22]. In the first experiment studying insect repellency, the evaluation period, experiment date and treatment were treated as independent variables and percentage of insects per container was treated as dependent variable. For both studies, goodness of fit of models was assessed by half-normal plots with simulation envelopes using the hnp package[23] in R software and based on Akaike information criterion (AIC) and residual deviance values. Insect repellency results were submitted to an analysis of deviance (type II Wald chi-square tests) and significant differences between treatments were analyzed using the emmeans package[24] with Sidak's test at $p < 0.05$.

In the second study, the number of observable *S. levis* adults were treated as the dependent variable and hour of evaluation was treated as independent variable. Container repetition was treated as random effect. A generalized linear mixed model was adopted using glmmtmb package[25]. Mean number of observed insects were submitted to an analysis of deviance (type II Wald chi-square tests) using the Car package[26] and significant differences between hours of evaluation were analyzed using the emmeans package[24] with Sidak's test at $p < 0.05$. Pearson correlations between the dependent variable of exposed insects per container and both temperature and relative humidity variables observed at each hour were assessed with the correlation function in R software.

Results

Experiment 1 – Insecticide Repellent Activity

There were no significant differences of *S. levis* repellency regardless of experiment date ($p = 0.988$), evaluation period ($p = 0.999$) and treatment ($p = 0.728$). Therefore, no insect preference was observed between soil treated with lambda-cyhalothrin + thiamethoxam and untreated soil, regardless of evaluation period (24, 48 and 72 hours after insect exposure) and experiment date (June 4th and June 15th). At the first evaluation, at 24 h, 50% of insects had moved to untreated soil and 50% to treated soil representing a RI value of 1 and repellency classification of neutral activity (N) as in Figure 3. After 48 h, 55.6% of *S. levis* adults were in containers with untreated soil, with a RI value of 0.88 and neutral activity classification (Figure 3). At 72 h, 52.1% of insects moved to containers with treated soil representing a RI of 1.04 and neutral activity classification (Figure 3).

Experiment 2 – Daily Adult Activity Pattern

Insect activity was significantly affected by time during a 24-hour period for the experiments on August 11th ($p = 0.0147$) and August 17th ($p < 0.0001$) as in Figure 4 and Figure 5. The experiment on October 27th showed no significant effect of time on insect activity ($p = 0.0527$) as in Figure 4 and Figure 5. *S. levis* adults were more active during the night for all experiments. On August 11th, most insects started to be exposed at 19:00 pm until 6:00 am and were mostly hidden from 7:00 am until 18:00 pm. During activity peak at night, insects were either resting, walking, digging or mating (Figure 4) and were mostly located on soil surface and subsurface (Figure 5). Mating was only observed at 20:00 pm on August 11th. Despite activity peak at night, most insects were hidden underneath the soil. During the most active period, at 00:00, 21.2% insects were exposed while 78.8% were hidden for that day. During the day, most insects were hidden, an average of 98.5% of adults. There was a weak positive correlation ($r = 0.22$) between exposed insects and relative humidity and there was a weak negative correlation ($r = -0.20$) between exposed insects and air temperature on August 11th. The sunrise on August 11th was at 06:39 am and the sunset was at 17:57 pm with total day length of 11 hours and 17 minutes.

On August 17th, insect activity was mostly observed from 19:00 pm until 23:00 pm with one exception at 11:00 am in which insect walking was also observed (Figure 4). Mating behavior was observed at 17:00 and 19:00 pm. Most exposed *S. levis* adults were either on the soil surface or subsurface (Figure 5). Most insects were also hidden underneath the soil even at highly active periods. At 20:00 pm, the most active period on August 17th, 25% insects were exposed with 75% hidden. During the day, an average of 90.6% of *S. levis* adults were hidden. A very a weak negative correlation ($r = -0.02$) was observed between the number of exposed insects and relative humidity while a very weak positive correlation (r= 0.03) was seen for the number of exposed insects and air temperature on August 17th. The sunrise of August 17th was at 06:35 am and sunset was at 17:59 pm (11 h 23 min day length).

On October 27th, insect activity was mostly observed from 14:00 pm until 00:00 am but maximum number of exposed insects were observed at 18:00 and 19:00 pm, respectively. Mating was observed at 19:00, 20:00 and 00:00. Most insects were resting on soil surface/subsurface or on sugarcane tiller base. At the period of most active insects, 19:00 pm, 16% of adults were exposed while 84% were hidden. During the day, an average of 96% *S. levis* adults were hidden underneath the soil. There was a weak positive correlation (r= 0.10) between exposed insects and relative humidity and there was a weak negative correlation (r = -0.12) between exposed insects and air temperature on October 27th. The sunrise on October 27th was at 05:34 am and sunset was at 18:20 pm (12 h 46 min day length) and two rainfall events occurred on that day. The first rain happened from 16:30 pm to 17:40 pm and the second rain started at 21:30 pm until 23:50 pm.

During sugarcane removal the following day of experiment 3 at 11:30 am, assessing insect number and location, it was noticed that all insects were located underneath the soil with 92% of adults attached to sugarcane rhizomes and roots (Figure 6, Supplementary Video S1 and Supplementary Video S2) and 8% were freely in the soil (Figure 7).

Discussion

Experiment 1 – Insecticide Repellent Activity

Sphenophorus levis adults were not attracted nor repelled by treated soil with lambda-cyhalothrin + thiamethoxam which, and according to Mazzonetto and Vendramin classification[18], can be classified as neutral activity. Despite some studies indicating the potential of pyrethroid and neonicotinoid insecticides having a repellent activity on a range of Coleoptera species, with pyrethroid repellency to *E. varivestis*[5] and imidacloprid showing repellency to different species of pollinator beetles[6], both active ingredients used in the present study were not repellent to *S. levis*. However, future studies should include different insecticide concentrations. Studying the repellency of clove and cinnamon essential oils on bean weevil (*Acanthoscelides obtectus*), for example, it has been observed that *A. obtectus* were only repelled by higher dosages of cinnamon oil, specifically those above the lethal dose of 50% (LD_{50})[19]. In addition, other insecticides should also be tested for repellency against *S. levis*.

In practical terms, no insecticide repellency to *S. levis* may be positive if proper pest control is achieved once *S. levis* adults are attracted to sugarcane plants and get in contact with the product. However, optimal *S. levis* control has not been a reality as several authors have reported[2-4]. Moreover, no insecticide repellency to *S. levis* may be necessary when adopting behavioral control methods including the attract-and-kill baiting approach used in sugarcane. In this method, sugarcane stalks are treated with insecticides and distributed across the field aiming to attract and control *S. levis* adults. Studying the attractiveness of vinasse, a sugarcane byproduct, to *S. levis* adults, it has been reported great attraction to sugarcane stalk baits mixed with vinasse[27]. Perhaps treating sugarcane stalks with both vinasse and insecticides could improve the attract-and-kill control method. Additionally, if *S. levis* aggregation pheromones, like the 2-methyl-4-octanol could be identified and synthesized, adding them to sugarcane baits would possibly increase even more its attraction to insects[8]. For instance, a similar attraction response was observed when testing sugarcane baits mixed with aggregation pheromones from *Sphenophorus incurrens* to capture adults in field[28].

Experiment 2 – Daily Adult Activity Pattern

Results of *S. levis* activity indicate a primary nocturnal behavior of *S. levis* adults. Insects were more active at night and, among the behaviors observed, walking, digging and mating were the most common ones. Considering all three experiment dates, most insect activities were seen from 18:00 pm to 2:00 am. The observed nocturnal behavior is in accordance with reported in another study[14]. Other Curculionidae species are also known for their nocturnal behavior such as *C. sordidus* with maximum activity from 21:00 to 4:00 am and other *Sphenophorus* species[15]. Similar results were described for *S. venatus vestitus* in which insects were most active between 00:00 and 4:00 am[16]. In fact, one monitoring option recommended for *S. venatus vestitus* in turfgrass is to scout adults at night[29]. In addition, during preliminary tests of the present study, *S. levis* adults were shown to move away when a light source was present, moving towards dark locations such as the soil, a characteristic of negative phototaxis. In fact, extraretinal photoreceptors like the Hofbauer-Buchner eyelet, are known to be responsible for the light responses and the communication with circadian clocks affecting locomotor and activity behaviors in many insects[30,31]. Regarding the locomotion of observable *S. levis* adults, insects were mostly resting while some were walking on soil. Mating was another observed behavior that was mainly noticed at night, from 19:00 to 00:00 pm, except on August 17th with mating activity also reported at 17:00 pm. Nocturnal mating was also described for *S. venatus vestitus*, with most occurrence from 00:00 to 4:00 am[16]. *S. levis* adults were mainly located underneath the soil but when exposed, were placed mostly on soil surface or subsurface and sometimes were seen on top of sugarcane leaves, stems and tiller base as also related in another study[14].

Despite *S. levis* adults being more active at night, most adults were hidden underneath the soil surface. On average, considering all three experiment dates, 20.7% of insects were exposed at night while 79.3% were hidden inside the soil at night. As all three experiments were conducted in August and October, the low rate of emerged adults from soil (<21%) may be explained by the insect's main distribution in specific months with higher temperatures and moisture. Several authors have reported greater field distribution of *S. levis* adults between October and November and between February and March while larvae distribution being primary observed during June and July[7,11,32,33]. In despite of other physiological factors related to optimum temperature, light and humidity for adult development and behavior, it is hypothesized that adults may be more exposed and active in these specific periods within the year as a consequence of water saturated soils common to months with heavy rainfall. Thus, soil pores occupied by water might act inducing *S. levis* adults to emerge from the soil subsurface. Studying the effect of soil moisture on *S. venatus vestitus*, for example, it has been observed larvae better developed under 20% of total pore space with water[29], which also helps understanding the higher *S. levis* distribution of larvae during dryer periods (June-July). Therefore, it is possible that the percentage of exposed and active *S. levis* adults in the present study were to be higher if conducted during the rainfall season and under high soil moisture. Further studies should be conducted in different periods throughout the year for a better understanding of *S. levis* adult exposure and activity. Additionally, as no strong correlation was noticed for both air temperature and relative humidity in relation to the number of *S. levis* adults exposed, it is assumed that other environmental stimulus, such as soil moisture, are associated with adult behavior and should also be considered in future studies. In contrast, southern corn billbug (*Sphenophorus callosus*) activity was more associated with air temperature than soil temperature and insects were more active during the day, from 12:00 to 14:00 pm[32].

During daylight the low exposure of *S. levis* adults and their activity was even more significant. Considering the three experiment dates, on average, 95% of insects were hidden in soil during the day. As previously discussed, *S. levis* can be classified as negatively phototactic, moving away from the light towards dark locations, specifically the soil. As a result of it, most *S. levis* adults are located underneath the soil during daylight usually coinciding with the period of pesticide applications for its control in sugarcane. Although most insecticides registered for *S. levis* control are considered systemic with some residual effect and should provide some pest protection over time, most applications have been insufficient and ineffective to control *S. levis*[2-4]. In addition to the difficulty of pesticide application technologies proper depositing the pesticide's active ingredient in the soil/rhizome close to the pest, current diurnal applications are possibly missing most of potential exposed *S. levis* adults due to its nocturnal behavior.

Based on current results, therefore, nocturnal insecticide applications could significantly increase the chance of reaching *S. levis* adults and could possibly contribute to better control the insect. Despite of only 20.7% of adults, on average, being exposed at night in the present study, the possibility of reaching adults during nocturnal applications is up to 4 times higher than diurnal applications, where only 5% of adults, on average, were exposed. The benefit of pesticide applications at night is reported in a study evaluating the effect of application timing on fall armyworm (*Spodoptera frugiperda*) control with most effective applications conducted at 20:00, 00:00 and 4:00[33]. Another study described similar benefits regarding nocturnal applications, in which the authors observed satisfactory control levels of burrower bug (*Cyrtomenus mirabilis*) in peanut (*Arachis hypogaea* L.) with different insecticides applied at night[34]. As most insecticide applications targeting *S. levis* are directed towards the soil, usually with a full jet nozzle, nocturnal applications should also include one even flat fan nozzle for band applications towards the plant base to improve spray coverage and deposit on exposed and active *S. levis* adults. For instance, one study compared two application methods for *S. levis* control, using a standard soil application method with one nozzle directed to the soil and another application method with two nozzles where one nozzle sprayed 30% of the application volume in the soil and second nozzle sprayed 70% of the application volume towards the sugarcane base[35]. According to the author[35], the application method with two spray nozzles should be recommended during the rainfall periods due to greater adult distribution but, according to current results, including this application method at night may improve is efficiency even more. In addition, as previously discussed, during peak populational periods (October/November and February/March) the number of exposed and active *S. levis* adults may probably increase in comparison with these observed results of August and October showing 20.7% of exposed adults at night. Hence, future studies should evaluate the potential of nocturnal applications of insecticides for *S. levis* control.

Moreover, if future studies show a strong correlation between the behavior of reared/contained *S. levis* adults with the behavior of field *S. levis* adults, a direct monitoring system could then be developed for better pest management decisions. Such system could be used, for example, to monitor contained *S. levis* adults' activity in real time providing site-specific information about period of exposure and consequently, the best recommended time for insecticide applications targeting exposed adults. In fact, low-cost portable locomotion activity monitor systems have been developed to track field and laboratory insect activity, including circadian rhythm, locomotion and feeding behavior[36].

In addition to semi-controlled studies, such as the present behavior experiment, new studies under field conditions should also be conducted considering the possibility of distinct and more accurate insect behavior in real field conditions.

Finally, during sugarcane and insect removal of each container for insect number and location assessment, it was noticed that all insects were located underneath the soil with 92% of adults attached to sugarcane rhizomes and roots (Figure 6, Supplementary Video S1 and Supplementary Video S2) and with 8% of adults freely in the soil (Figure 7). Several authors have reported the gregarious behavior of *S. levis*[8-10]. Such behavior is induced by aggregation pheromones like the 2-methyl-4-octanol[8]. Additionally, *S. levis* are known to have a slow spatial distribution capacity, ranging from 5.2 to 6.6 m month^{-1} in part because of its rare flying behavior but also because of its aggregation activity[10,32]. Regarding some evident sugarcane damage and openings made by adults on plants from each container, most of it was seen bellow the ground (Figure 6) as described in another study[14] in which authors observed 90% of damage and openings done bellow the soil surface.

Despite important findings observed in the present study, further research should be conducted to better elucidate *S. levis* behavior and biology and consequently improve pest control in sugarcane.

Conclusion

Soil treated with lambda-cyhalothrin and thiamethoxam were not repellent nor attractive to *S. levis* adults. Insects presented nocturnal behavior as most activities and number of *S. levis* adults out of the soil were observed between 18:00 pm and 2:00 am. Despite the nocturnal behavior, most insects remained inside the soil (79.3%) at night and some were either active or inactive on the soil surface, subsurface, plant base and cane stem (20.7%). During the day the vast majority of *S. levis* adults were underneath the soil (95%) aggregating near or attached to the sugarcane rhizome. Based on these results, nocturnal applications of insecticides may improve *S. levis* control in sugarcane

Declarations

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Author contributions statement

P.H.U.F. performed the study, designed and validated the experiment, analyzed the data and wrote the manuscript. M.C.F. supervised the study. All authors reviewed and edited the manuscript.

Additional information

Competing interests

The authors declare no competing interests.

Data Availability

Additional data information is available from the corresponding author, P.H.U.F., upon reasonable request such as datasets generated and/or analyzed during the study.

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Figures

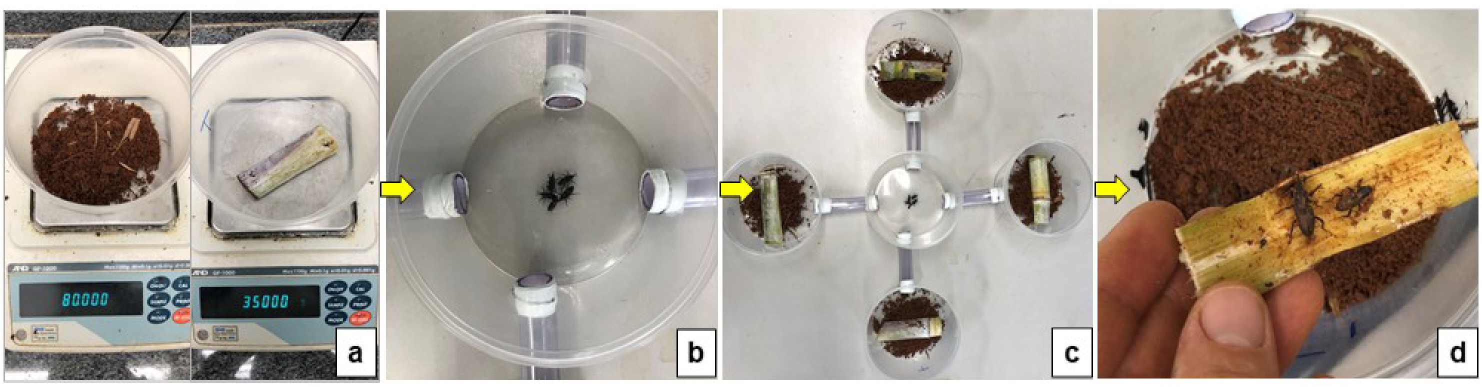

Figure 1

Methodology steps for the insecticide repellency activity including treated soil and sugarcane placement (a) in containers; placement of *S. levis* adults in the central container (b); study apparatus with five containers (c) and repellency evaluations after 24, 48 and 72 h (d).

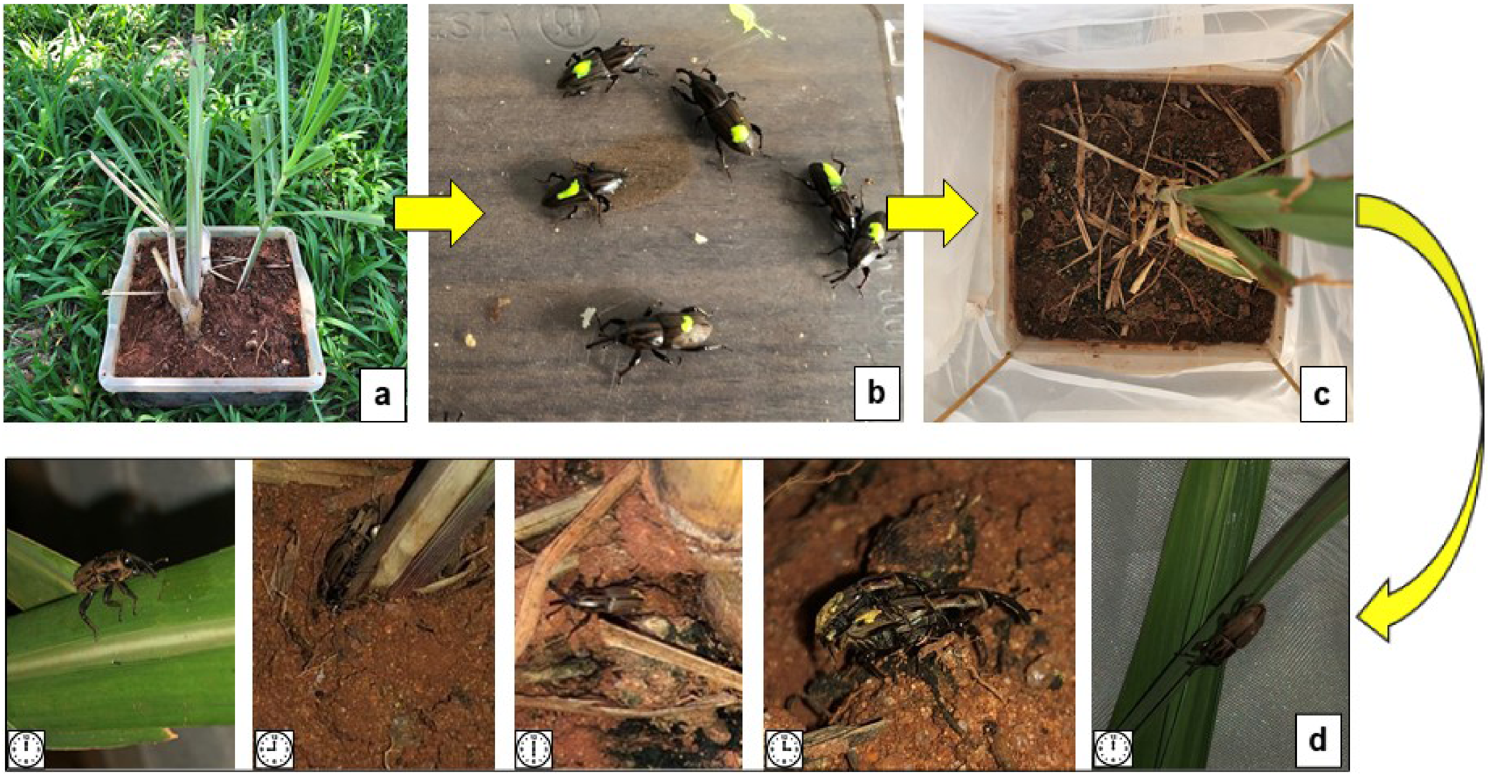

Figure 2

Methodology steps for the *S. levis* adult activity pattern study including ratoon sugarcane in containers (a); placement of 20 marked *S. levis* adults (b) in containers under natural weather conditions (c); and hourly insect activity evaluations recording adult location and behavior (d).

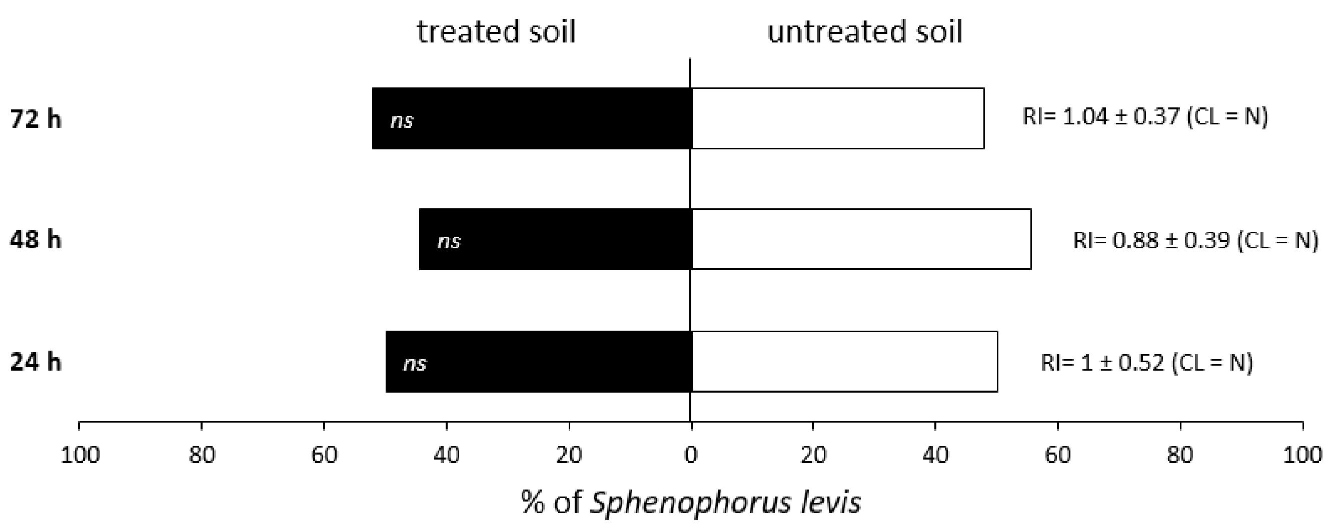

Figure 3

Percentage of *S. levis* adults that moved to soil treated with lambda-cyhalothrin + thiamethoxam and to untreated soil per evaluation period. Each bar represents the mean results of four replicates of two study repetitions. Repellency index (RI) with SD and RI classification (CL) are provided. *ns* – no significant differences were observed at α = 0.05. N – neutral activity.

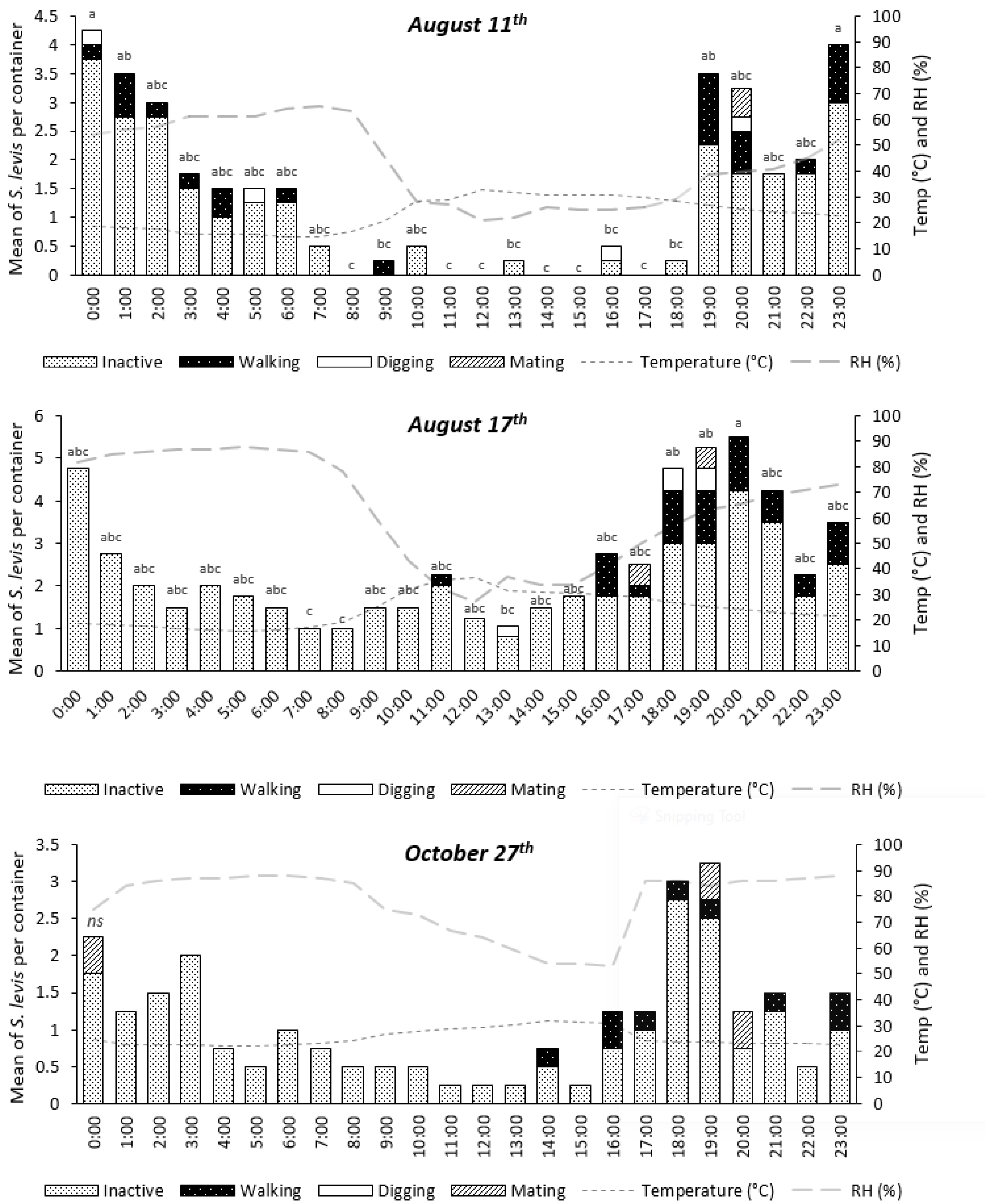

Figure 4

Number of exposed *S. levis*adults per container at every hour in different dates considering insect activity, temperature (temp) and relative humidity (RH%). Bars with mean values followed by same letter are not different at α = 0.05. *ns* – no significant differences were observed at α = 0.05

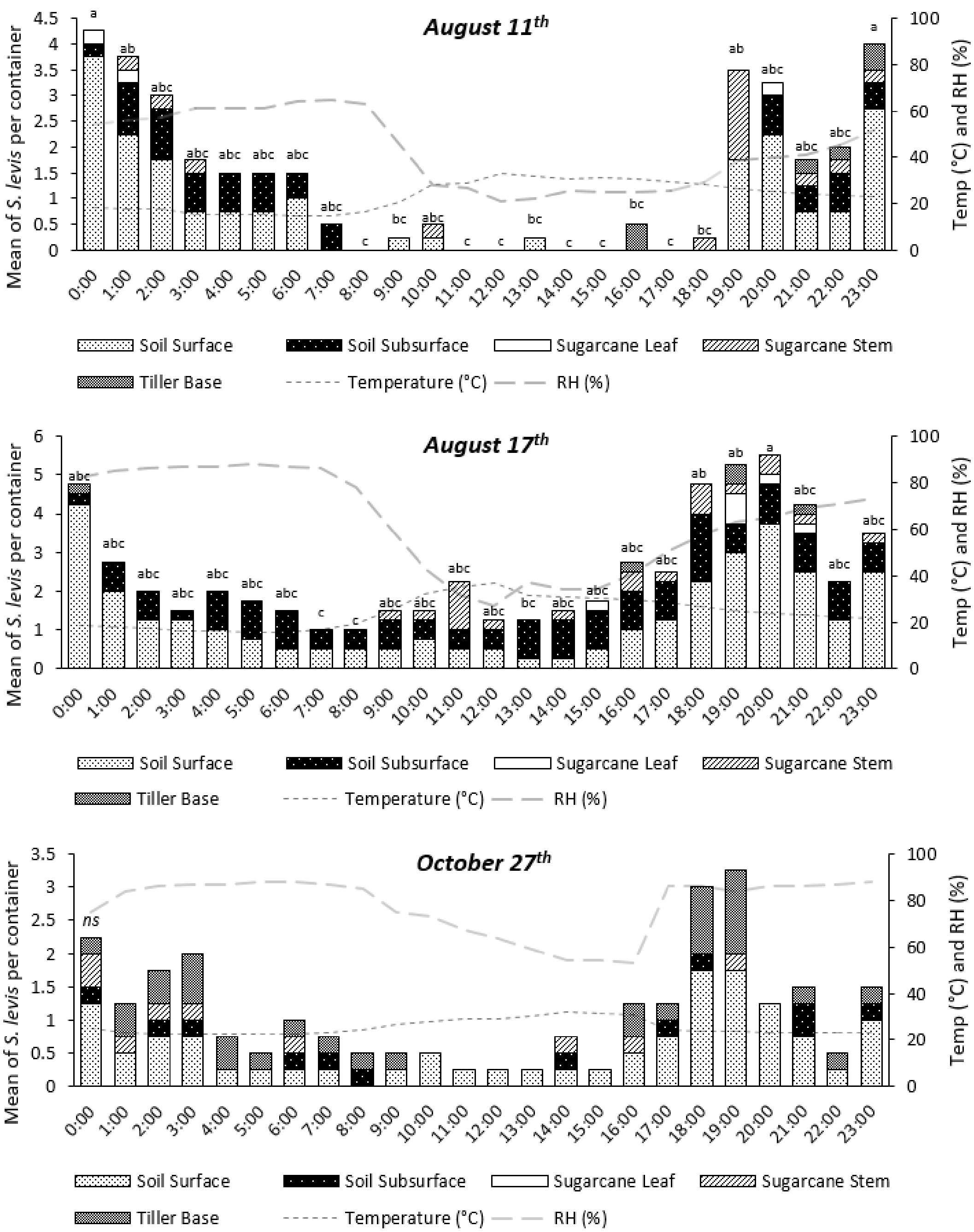

Figure 5

Number of exposed *S. levis*adults per container at every hour in different dates considering insect location, temperature (temp) and relative humidity (RH%). Bars with mean values followed by same letter are not different at α = 0.05. *ns* – no significant differences were observed at α = 0.05

Figure 6

Sphenophorus levis adults attached to sugarcane rhizomes and roots.

Figure 7

Sphenophorus levis adults found freely on soil and not attached to sugarcane rhizomes and roots.

Supplementary Files

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