UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

EFFECT OF LIQUID AND SOLID INSECTICIDE APPLICATIONS AND INSECT BEHAVIOR ON *Sphenophorus levis* VAURIE, 1978 (COLEOPTERA: CURCULIONIDAE) CONTROL IN SUGARCANE

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TÍTULO DA TESE: EFFECT OF LIQUID AND SOLID INSECTICIDE APPLICATIONS AND INSECT BEHAVIOR ON *Sphenophorus levis* VAURIE, 1978 (COLEOPTERA: CURCULIONIDAE) CONTROL IN SUGARCANE

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PEDRO HENRIQUE URACH FERREIRA was born in Ribeirão Preto, SP, Brazil, on September 18th, 1991, son of Tatiana Maria Urach Ferreira and Moacyr Ferreira da Silva Filho, with one brother, Guilherme Urach Ferreira. He completed the high school in 2008 in Ribeirão Preto and started his academic career in 2009 in the University of São Paulo, Campus of Escola Superior de Agricultura "Luiz de Queiroz" – ESALQ, in Piracicaba, SP, Brazil. During his undergraduate course, he participated in a study group of sugarcane supervised by Prof. Dr. Edgar de Beauclair and in a study group of pesticide application technology supervised by Prof. Dr. Casimiro Dias Gadanha Jr. In 2013 he participated in the Science Without Borders Program from CNPq with a scholarship to study English in the Institute of Continuing & TESOL Education – ICTE, at the University of Queensland and to study Agriculture Science in the same university at the Gatton Campus during 2014. He conducted his undergraduate thesis with Dr. Andrew Hewitt as his advisor studying pesticide applications in wind tunnel at the Center for Pesticide Application and Safety – CPAS located in Gatton, Qld, Australia and presented his undergraduate thesis in USP – ESALQ under advisory of Prof. Dr. Casimiro Dias Gadanha Jr. In 2015 he started working as a Technical Assistant of Market Development for Syngenta in sugarcane. In 2017 he started his Master's degree as a graduate assistant at Northwest Missouri State University, MO, USA advised by Prof. Dr. Connor Ferguson until transferring to the Mississippi State University, MS, USA under the Plant and Soil Science program with the same advisor, Prof. Dr. Connor Ferguson, and studying pesticide application technologies for weed control in soybean. In 2019 he started his PhD degree as a graduate assistant in the Agriculture Entomology program at Unesp, FCAV, Campus of Jaboticabal, SP, Brazil, advised by Prof. Dr. Marcelo da Costa Ferreira to study application methods for *Sphenophorus levis* control in sugarcane among other pesticide application technologies.

“Embora ninguém possa voltar atrás e fazer um novo começo,
qualquer um pode começar agora e fazer um novo fim”

Chico Xavier

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SUMÁRIO

EFFECT OF LIQUID AND SOLID INSECTICIDE APPLICATIONS AND INSECT BEHAVIOR ON *Sphenophorus levis* VAURIE, 1978 (COLEOPTERA: CURCULIONIDAE) CONTROL IN SUGARCANE

ABSTRACT – Insecticide adoption and current application methods for *Sphenophorus levis* Vaurie, 1978 (Coleoptera: Curculionidae) control have not been sufficient to reduce insect population due to low insecticide concentrations near the insect as a consequence of its location underneath the soil. Few information regarding the insect's behavior is currently available and further research must be conducted to assist *S. levis* management in sugarcane. In addition, conventional control methods are still limited to liquid insecticide applications despite solid applications have proven effective for several soil insects and crops due to gradual insecticide release with a prolonged effect against pests. Therefore, it is extremely important to better understand *S. levis* behavior and to investigate the potential of solid insecticide applications for *S. levis* control in sugarcane. The study aimed to assess the repellency of *S. levis* adults from insecticides and to evaluate adults' activity and location. In addition, the study's objective also included evaluating the efficacy of solid and liquid application methods of different insecticides under semi-controlled conditions and field conditions during sugarcane planting and ratoon treatment. The study investigating the insect's behavior effect was conducted in 2021 and consisted of hourly evaluations of *S. levis* adults in containers with sugarcane plants and soil. The semi-controlled study was conducted in 2021 and included assessing the mortality levels of *S. levis* adults with field-simulated applications of liquid and solid insecticides. The three field studies were conducted in 2020 with evaluations in 2020 and 2021 and consisted of assessing *S. levis* control, sugarcane injury level and sugarcane yield for liquid and solid applications of insecticides (thiamethoxam, fipronil, thiamethoxam + lambda-cyhalothrin, imidacloprid) and one entomopathogenic fungus (*Metarhizium anisopliae*). *S. levis* adults were not repelled nor attracted to thiamethoxam + lambda-cyhalothrin treated sugarcane stems and *S. levis* adults were confirmed to be nocturnal with the greatest number of exposed and active adults occurring from 18:00 pm until 2:00 am. In the field-simulated experiment, low *S. levis* adult mortality was detected (< 53%) but doubling the solid applied thiamethoxam + lambda-cyhalothrin dosage improved *S. levis* adult mortality to 76.7%. Regarding the field studies, solid insecticide applications showed great potential for *S. levis* control improvement in despite of an overall low *S. levis* control among all treatments. Soil drill applications were seen to promote better efficacy than broadcast and band spraying methods. Insecticide applications at planting and in ratoon cane were not very effective for *S. levis* control and frequent pest monitoring may help determining the best moment for insecticide application after planting and might help recommending a second application in ratoon cane. Therefore, positive effects from solid insecticide applications were observed indicating potential benefits for *S. levis control* and the necessity of further studies with appropriate granular formulations with adequate dosages and new insecticides.

Keywords: granular, billbug, weevil, ratoon, residue, mortality

EFEITO DE APLICAÇÕES LÍQUIDAS E SÓLIDAS DE INSETICIDAS E DO COMPORTAMENTO DO INSETO NO CONTROLE DE *Sphenophorus levis* VAURIE, 1978 (COLEOPTERA: CURCULIONIDAE) EM CANA-DE-AÇÚCAR

RESUMO – O uso de inseticidas e métodos de aplicação atualmente utilizados no controle de *Sphenophorus levis* Vaurie, 1978 (Coleoptera: Curculionidae) não têm sido suficiente para reduzir sua população devido à baixas concentrações do inseticida como resultado de sua localização abaixo da superfície do solo. Poucas informações a respeito do comportamento do inseto estão atualmente disponíveis de modo que novas pesquisas devam ser conduzidas para auxiliar o manejo de *S. levis* em cana-de-açúcar. Além disso, métodos convencionais de controle ainda se limitam em aplicações líquidas de inseticidas apesar de aplicações sólidas terem se mostrado eficazes para diversas pragas de solo e culturas, devido a liberação gradual do inseticida resultando em um efeito prolongado contra as pragas. Desse modo, é extremamente importante o melhor entendimento do comportamento do *S. levis*, além da necessidade de se investigar o potencial de aplicações sólidas de inseticidas no controle de *S. levis* em cana-de-açúcar. O trabalho objetivou avaliar a repelência de adultos de *S. levis* a inseticidas e avaliar a atividade e localização de adultos de *S. levis*. O estudo também objetivou avaliar a eficácia dos métodos de aplicações de diferentes inseticidas por via líquida e sólida em condições semicontroladas e a campo durante o plantio e o tratamento de soqueiras de cana-de-açúcar. O estudo que avaliou o efeito do comportamento do inseto foi conduzido em 2021 e consistiu de avaliações horárias de adultos de *S. levis* em recipientes com plantas de cana-de-açúcar e solo. O estudo em condições semicontroladas foi conduzido em 2021 e incluiu avaliações de mortalidade de adultos de *S. levis* sob aplicações simuladas de campo com inseticidas líquidos e sólidos. Os três estudos de campo foram conduzidos em 2020 com avaliações em 2020 e 2021 avaliando o controle de *S. levis*, os níveis de toco atacado e a produtividade de cana-de-açúcar com aplicações líquidas e sólidas de inseticidas (tiametoxam, fipronil, tiametoxam + lambda-cialotrina, imidacloprido) e de um fungo entomopatogênico (*Metarhizium anisopliae*). Os adultos de *S. levis* não foram repelidos nem atraídos por colmos de cana-de-açúcar tratados com tiametoxam + lambda-cialotrina e confirmou-se o comportamento noturno de adultos de *S. levis* com maior número de adultos expostos e ativos ocorrendo das 18:00 p.m. até as 2:00 a.m. No experimento simulando aplicações a campo, detectou-se baixa mortalidade de adultos de *S. levis* (<53%) mas ao se dobrar a dosagem de tiametoxam + lambda-cialotrina aplicado via sólida, a mortalidade de adultos de *S. levis* aumentou para 76.7%. Em relação aos estudos de campo, as aplicações de inseticidas via sólida demonstraram grande potencial para aumentar o controle de *S. levis* apesar de uma baixa eficácia geral de todos os tratamentos. As aplicações com corte de soqueira demonstraram promover melhor eficácia do que pulverizações em área total ou em faixa. Já as aplicações de inseticidas durante o plantio e na soqueira não foram muito efetivas para o controle de *S. levis* enquanto que o frequente monitoramento de pragas pode ajudar a determinar o melhor momento para aplicações de inseticidas depois do plantio e pode ajudar a recomendar uma segunda aplicação em cana soca. Desse modo, observou-se efeitos positivos das aplicações de inseticidas via sólida mostrando benefícios em potencial no controle de *S. levis*, além da necessidade de novos estudos com formulações sólidas apropriadas, com dosagens adequadas e com novos inseticidas.

Palavras-chave: grânulo, bicudo, gorgulho, soqueira, resíduo, mortalidade

CHAPTER 1 – GENERAL CONSIDERATIONS

INTRODUCTION

Brazil is the largest sugarcane (*Saccharum officinarum* L.) grower in the world and the state of São Paulo is the largest producer with over 51% of the national production (Conab, 2022). Despite its importance for Brazil, sugarcane yield has been declining over recent years. In 2005, mean sugarcane yield in the southeast was 84 t ha^{-1} while in 2022 yield dropped to 72 t ha^{-1} (Conab, 2022). The transition from manual harvesting of burnt sugarcane to mechanical harvesting of green sugarcane was a main factor influencing yield decline. Sugarcane burning provided outstanding control for pests like root spittlebug (*Mahanarva spp*.), sugarcane borer (*Diatraea saccharalis* Fabricius) and sugarcane billbug [*Sphenophorus levis* Vaurie, 1978 (Coleoptera: Curculionidae)] leading to higher yields (Dinardo-Miranda and Fracasso, 2013). Without burning, sugarcane residue on soil surface helps sheltering pests like *S. levis* as it was observed in a study comparing harvesting systems with and without burnt sugarcane (Dinardo-Miranda, 2009).

As one of the most damaging pests in sugarcane, *S. levis* reduces plant stand and longevity with up to 60% of cane ratoon death and yield losses of 40 t ha^{-1} (Terán and Precetti, 1982; Precetti and Arrigoni, 1990; Dinardo-Miranda et al., 2006). Being a soil pest, *S. levis* is mostly located under the soil surface which makes it extremely difficult to properly place insecticides at the right amount near the insect. As the definition of pesticide application states, the adoption of scientific knowledge for correct pesticide placement on target, when and if necessary, at the right amount, with minimal off-target contamination must be considered for optimum *S. levis* control (Matuo, 1990). Unfortunately, current insecticide applications have not been sufficiently effective to reduce *S. levis* populations due to inadequate insecticide concentrations near the insect.

Low insecticide efficacy for *S. levis* control in sugarcane has been reported by several authors, indicating the difficulty of insect management with current application methods (Dinardo-Miranda et al., 2006; Tavares, 2006; Dinardo-Miranda, 2008; Dinardo-Miranda, 2013; Canassa, 2014; Simi, 2014; Alencar, 2016, Evangelista et al., 2017). As most insecticide treatments for *S. levis* control are conducted through liquid applications, alternative treatment methods should be considered, including the application of solid insecticides. Numerous studies have shown the great potential of solid insecticide applications, for different pests and crops (Salvadori, 2001; Barbosa et al., 2004; Possebon, 2011; Gunewardena and Madugalla, 2012; Larsen et al., 2015). Solid applications of imidacloprid in sugarcane, for example, promoted up to 4-year canegrub control (Ward, 2016). Granular insecticides, when solid applied, requires soil moisture to initiate the active ingredient dissipation from the granule to the soil solution (Davis et al., 1996). Depending on its granule coating and thickness, a prolonged rate of release of the active ingredient can be achieved promoting greater insecticide residual (Kimoto et al., 2007; Matthews et al., 2014)

As proven to be highly effective for many insects and crops, *S. levis* control could also potentially be improved by solid application of insecticides. Because no research has ever been conducted, up to date, evaluating solid insecticide application for *S. levis* control, it is extremely necessary to investigate its potential for *S. levis* management in sugarcane.

Therefore, the present study aimed to evaluate the benefit of different methods, including solid applications of insecticides, for *S. levis* control, but also aimed to examine behavior aspects of *S. levis* adults that could potentially improve future management pest recommendations. Thus, the specific objectives of each study were:

1. to assess the repellency of *S. levis* adults from a mixture of two insecticides, lambda-cyhalothrin and thiamethoxam, and to evaluate the activity and location of observable *S. levis* adults.
2. to evaluate the efficacy of two insecticides products (lambda-cyhalothrin + thiamethoxam and imidacloprid) solid and liquid applied and to compare two insecticide rates on *S. levis* adult control through a novel bioassay methodology.
3. to investigate the effect of different methods of insecticide application in field on *S. levis* control, sugarcane injury levels and sugarcane yield.

4. to examine and compare liquid and solid application of insecticides at sugarcane planting for *S. levis* insect control, plant injury
5. to analyze the effect of liquid and solid application of insecticides on *S. levis* control, plant injury level and yield in sugarcane ratoon fields

LITERATURE REVIEW

Sphenophorus levis Vaurie, 1978 (Coleoptera: Curculionidae)

Sphenophorus levis is an important soil pest in sugarcane and it was first described in the Piracicaba region in the state of São Paulo, Brazil (Vaurie, 1978) being currently present in several other states of Brazil, including MT, GO, MG, MS, SP, PR (Pérez, 2008; Moraes and Ávila, 2013; Canassa, 2014).

The insect's total life cycle is approximately 6 months long, with an egg incubation period of 8 days, larvae stage of 35 to 50 days, pupae period of 10 to 12 days and adult phase of 120 days, although adult longevity of up to 250 days has also been reported (Degaspari et al., 1987; Casteliani et al., 2020). In addition, *S. levis* is a gregarious pest with slow spatial distribution, ranging from 5.2 to 6.6 m month^{-1} of field dispersion (Precetti and Arrigoni, 1990; Degaspari, 1978; Simi, 2014; Rosa, 2022). Regarding its gregarious behavior, *S. levis* tends to aggregate in distinct points distributed in the field (Zarbin et al., 2003; Izeppi, 2015; Rosa, 2022). In fact, when studying the spatial distribution of *S. levis*, Izeppi (2015) observed the range of adult incidence in field varied from 28 m to 53 m. Regarding its distribution within the year, several authors have reported greater *S. levis* adult populations between October and November and between February and March while larvae and pupae populations are mostly observed during June and July (Degaspari et al., 1987; Precetti and Arrigoni, 1990; Canassa, 2014; Izeppi et al. 2014). Another described behavior of *S. levis* is the low flight capacity of adults (Precetti and Arrigoni, 1990; Simi, 2014), similar to other Curculionidae and *Sphenophorus* species as related by Kindler and Spomer (1986) for hunting billbugs (*Sphenophorus venatus vestitus*) with limited flight ability. It has also been reported *S. levis* to be a nocturnal insect although further studies detailing its behavior are required (Casteliani et al., 2020).

Despite its low dispersion in field, *S. levis* has been disseminated to several sugarcane regions. During sugarcane harvesting, seed cane billets containing *S. levis* adults are transported to planting areas without insect presence. In addition, the transportation of infested seed cane billets carrying *S. levis* adults may also infect surrounding areas where the transporting route of seed cane is being conducted. I fact, in one study evaluating the infestation of *S. levis* in transferring areas of harvested seed cane, 12% greater infestation levels were observed in areas close to seed cane billet transferring, showing the potential of new infestations not only in newly planted areas but also in areas where seed cane billets were transported and handled (Rosa, 2022). In another study, researchers evaluated *S. levis* infestation in one common cane planting system known as MEIOSI (Method of Inter-Rows Occurring Simultaneously) consisting of seed cane rows planted a few meters apart with one crop cultivated between seed cane rows or without any crop (bare fallow) (Recieri et al., 2018). In that study, authors observed greater *S. levis* infestation, 154% more, in the MEIOSI cane planting with soybean [*Glycine max* (L.) Merr.] cultivated between seed cane rows than in areas without any crop between seed cane rows (Recieri et al., 2018). Once the presence of *S. levis* is detected in a field, insect eradication will not probably occur and will demand constant care to keep its population low with as few plant injury levels as possible.

Plant injuries are caused by female adults depositing their eggs inside the sugarcane crown and tiller where it hatches and the emerged larvae starts feeding and causing the main plant damage. Larvae’s injuries in the cane crown and tiller are characterized by a white and yellow frass formed inside tunnels. Plant symptoms may include leaf yellowing, starting from the outer leaves, and leaf necrosis, followed by plant death characterizing yellow and brown patches distributed across the field. Nearly 90% of formed tunnels are opened bellow the soil surface and most of sugarcane openings are filled with soil or with the white and yellow frass from larvae’s waste (Casteliani et al., 2020).

Sugarcane yield is often directly affected by plant injuries caused by *S. levis*. In fact, it has been reported that for every 1% of plant injury from *S. levis*, 1% of yield is lost (Casteliani et al., 2020). Unfortunately, great yield losses with consequent economic damage to sugarcane farmers are frequent. It has described, for example, significant reduction of plant stand and crop longevity with up to 40 t ha^{-1} yield losses and 60% death of sugarcane ratoon (Terán and Precetti, 1982; Precetti and Arrigoni, 1990).

Control Methods

Because *S. levis* is a complex soil pest, with extensive life-cycle and mostly located underneath the soil, several control methods are recommended to reduce the insect population and mitigate losses. Some examples of different management methods include the desiccation and destruction of volunteer cane using a ratoon eliminator equipment, bare fallow, crop rotation, seed cane billets of high phytosanitary quality, variety selection, insect baiting, biological control and the rotation of insecticide's mode of action.

The adoption of a ratoon eliminator equipment is often indicated during the dry and low rainfall season attempting to physically eliminate the immature pest stages and expose larvae and pupae out of the sugarcane rhizome to predators including some insects and birds. A significant reduction of *S. levis* population using the ratoon eliminator equipment when compared to an untreated area has been reported in a study (Dinardo-Miranda, 2014).

The implementation of bare fallow before sugarcane planting can also help reducing *S. levis* population. Although it may not be recommended due to increasing the potential of soil erosion, bare fallowing was shown to reduce *S. levis* infestation in one study. Researchers evaluated *S. levis* infestation in the MEIOSI cane planting system with soybean cultivated between seed cane rows and without any crop between seed cane rows (bare fallow) and it was observed up to 154% more *S. levis* infestation in areas with soybean than in the areas without any crop (Recieri et al., 2018).

Selecting the right sugarcane variety to be planted can also affect *S. levis* preference and injury levels. However, only a few studies have been conducted evaluating sugarcane variety susceptibility and preference to *S. levis* (Dinardo-Miranda, 2014) showing the potential of some varieties even though no currently available varieties are known to effectively tolerate *S. levis* infestations. Thus, it might be recommended each sugarcane grower and in each sugarcane mill unit, to evaluate, in its own environment and meteorological conditions, which varieties may promote fewer losses from *S. levis* infestation.

Additionally, when using seed cane billets for sugarcane planting, special attention must be taken to avoid using infested planting materials with potential of dispersing *S. levis* to areas without its presence. Intensive and precise pest monitoring should be conducted throughout the season prior to harvesting the sugarcane to be used as seed cane. Moreover, planting with pre-sprouted cane seedlings should significantly reduce the chances of *S. levis* infestation considering it presents greater phytosanitary conditions and process control than conventional planting systems (Landell et al., 2012; Dinardo-Miranda, 2014).

Another control method for *S. levis*, mostly targeting the adult stage, is a type of attract-and-kill behavioral method of pest control using sugarcane stems as baits. In this method, sugarcane stems are treated with insecticides and distributed across the field aiming to attract and control *S. levis* adults. When mixing with another attractant, *S. levis* control is usually improved. Studying the control efficacy of this method, Pérez (2008) observed greater attraction when sugarcane stems were mixed with sugarcane molasse and greater field control levels when treating stems with carbaryl and thiamethoxam insecticides. In another study, assessing the attractiveness of vinasse, a sugarcane byproduct, to *S. levis* adults, Martins et al. (2020) also reported great attraction to sugarcane stalk baits mixed with vinasse. Thus, treating sugarcane stalks with vinasse, molasse and insecticides could improve the attract-and-kill control method.

Biological control methods have also been adopted, although less common, as alternative and supplementary to other methods. Most current biological control options for *S. levis* control are microorganisms, including fungus, nematodes and bacteria. Currently, the most used entomopathogenic fungus is the *Beauveria bassiana* which has shown some potential for *S. levis* control (Canassa, 2014; Simi, 2014; Santos, 2017;

Smaniotto, 2019). Another fungus, the *Metarhizium anisopliae*, has also shown to provide some effect over *S. levis* insects as described by different authors (Delfanti, 2012; Simi, 2014; Santos, 2017). In addition, entomopathogenic nematodes, including the *Steinernema puertoricense*, *S. braziliense* and *Heterorhabditis indica* have been studied showing potential benefits for *S. levis* control. The nematode *Steinernema sp*., for example, has shown to promote effective *S. levis* larvae control in a study of Tavares (2006). *H. indica* has also been indicated to provide some *S. levis* control although higher efficacy was reported when using *S. braziliense* (Leite et al., 2011). Although no significant efficacy has been reported so far, the potential of using bacteria for *S. levis* control, such as *Bacillus thuringiensis*, has also been described in studies assessing different bacteria isolates for larvae control (Polanczyk et al., 2004; Campanini et al., 2012).

Regarding chemical control methods, up to date, eight insecticide active ingredients are registered for *S. levis* control in sugarcane, including thiamethoxam, fipronil, lambda-cyhalothrin, imidacloprid, chlorantraniliprole, alphacypermethrin, bifenthrin and carbosulfan (Agrofit, 2022). Although insecticides can, potentially, promote high mortality rates of *S. levis* insects, low control efficacies have been obtained due to uneven applications and low insecticide residues on target as a consequence of insect behavior and location underneath the soil.

Unsatisfactory *S. levis* control has been reported by several authors (Dinardo-Miranda, 2000; Polanczyk et al., 2004; Dinardo-Miranda et al., 2006; Tavares, 2006; Dinardo-Miranda, 2008; Dinardo-Miranda and Fracasso, 2010; Fracasso et al., 2011; Delfanti, 2012; Leite et al., 2012; Nunes, 2012; Dinardo-Miranda, 2013; Canassa, 2014; Simi, 2014; Alencar, 2016, Evangelista et al., 2017). These vast number of scientific reports clearly indicates the necessity of better insect control solutions. Comparing *S. levis* control in ratoon cane treatment with different insecticides, researchers observed maximum control efficacy of only 60% (Dinardo-Miranda et al., 2006). In cane planting studies, researchers also observed poor control for all products due to low insecticide concentration on target with maximum insecticide efficacy of only 64% (Dinardo-Miranda and Fracasso, 2010; Fracasso et al., 2011).

To overcome current poor and uneven insecticide applications for *S. levis* control, alternative application methods must be considered. At present, liquid applications are still the main method used for chemical and biological *S. levis* control despite its unsatisfactory effectiveness.

Solid Application of Insecticides

Granular insecticide applications have proven to be effective in a number of pests and crops (Apple et al., 1969; Ritcey et al., 1991; Corso and Gazzoni, 1983; Martinelli et al., 1998; Salvadori, 1998; Salvadori, 2001; Barbosa et al., 2004; Possebon, 2011; Gunewardena and Madugalla, 2012; Larsen et al., 2015). In a banana borer (*Cosmopolites sordidus*) control study, authors observed great insect control with granular applications of terbufos and carbofuran (Barbosa et al., 2004). Moreover, when testing insecticides for wireworm (*Melanotus communis*) control in sugarcane, researchers noticed better insect control with granular phorate insecticide treatments. In addition, granular insecticide studies in sugarcane showed up to 3-year control of Childers grub (*Antitrogus parvulus*) using a controlled-release (CR) imidacloprid granule and effective control for Greyback canegrub (*Dermolepida albohirtum*) in Australia (Samson et al., 2010; Ward, 216).

Even water-dispersible granule formulations (WG) applied without water have shown to provide great control against soil pests (Buhler and Gibb, 1993; Rao et al., 2008; Bhawar et al., 2015; Kumar, 2016; Pandey and Kumar, 2020). Studying the efficacy of granular insecticides for early shoot borer (*Chilo infuscatellus*) in sugarcane, Bhawar et al. (2015) observed better control for a WG formulation of imidacloprid + fipronil than granular (GR) formulations of chlorantraniliprole, fipronil, cartap hydrochloride, chlorpyriphos, carbofuran and phorate.

Furthermore, GR formulations of entomopathogenic fungus have also been indicated as a viable pest control tool (Ekesi et al., 2005; Jaronski and Jackson, 2008). In fact, after 668 days of soil inoculation, GR formulation of *M. anisopliae* had greater control of three fruit flies’ larvae species in comparison with oil and liquid formulations (Ekesi et al., 2005).

The key advantage of granular over liquid insecticide application is the increase of release rate controlled by granule coating and thickness properties (Kimoto et al., 2007; Matthews et al., 2014). As a consequence of this prolonged release rate, greater insecticide residual may be achieved. However, despite several benefits of solid insecticide applications, this application method has not been used by sugarcane growers and sugarcane mills for *S. levis* control.

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CHAPTER 2 – *Sphenophorus levis* behavior: repellency to insecticide treatment and nocturnal adult activity and location pattern

ABSTRACT - *Sphenophorus levis* is a difficult to control pest in sugarcane that causes great damage to the subterranean part of the plant, especially the rhizome, significantly reducing sugarcane yield. Most insecticide treatments have not been effective to control this insect in part because of the pesticide application technology adopted but also because of the lack of studies regarding the pest's behavior that may affect insecticide applications efficacy. The present study 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 mixed with thiamethoxam and untreated soil as choice options and both had sugarcane stems as attractant to *S. levis* adults. Insect activity and location behavior were assessed by hourly observations of *S. levis* adults in containers with soil and cultivated sugarcane plant under a 24-hour period. Results indicated that *S. levis* adults were not repelled nor attracted to soil treated with lambda-cyhalothrin + thiamethoxam. In addition, insects presented nocturnal behavior with most activity starting at 18:00 pm until 2:00 am. Activities included walking, digging and mating. 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. Regardless of time, exposed insects were primarily located on soil surface and subsurface. Neither air temperature or humidity were strongly correlated to insect activity. According to these results, nocturnal insecticide applications may improve *S. levis* adult control due to greater insect activity and exposure at night. Further behavior and biology studies should be conducted to better understand and manage *S. levis*.

Keywords: Billbug; sugarcane weevil; attraction; soil; circadian rhythm

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 (Dinardo-Miranda, 2013).

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 (Dinardo-Miranda et al., 2006; Tavares, 2006; Alencar, 2016). 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*) (Dobrin and Hammond, 1985) and repellency of imidacloprid to pollinator beetles (Easton and Gouson, 2013). 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 (Canassa, 2014).

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 (Zarbin et al., 2003; Izeppi, 2015; Rosa, 2022). Studying the spatial distribution of *S. levis*, Izeppi (2015) observed the range of adult incidence in field varied from 28 m to 53 m. Another described behavior of *S. levis* is the low flight capacity of adults (Precetti and Arrigoni, 1990; Simi, 2014), similar to other Curculionidae and *Sphenophorus* species as related by Kindler and Spomer (1986) for hunting billbugs (*Sphenophorus venatus vestitus*) with limited flight ability. Some reports have also indicated that *S. levis* adults are nocturnal (Casteliani et al., 2020) despite no specific studies examining insect activity. 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 (Gold et al., 2001) and *S. venatus vestitus* being most active from 00:00 to 4:00 for (Huang and Buss, 2009), no research has been conducted to evaluate *S. levis* maximum activity hours. 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 (Figure 1c) based on previous studies (Mazzonetto and Vendramin, 2003; Fouad et al., 2012; Viteri Jumbo et al. 2014). 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 (Figure 1a). Insecticide soil treatment was conducted in 50 L pots with ratoon sugarcane plants 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. (2001) methodology 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 cut in half, 35 g and 10 cm long (Figure 1a), was placed on top and with cut surface facing the soil. In the central container E, five *S. levis* adults were released in the center (Figure 1b) and after 24, 48 and 72 h the total number of insects per container was assessed (Figure 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 with previous infestation history and no insecticide application in the year. The percentage of repellency

(PR) was calculated as in Mazzonetto and Vendramin (2003) and in Viteri Jumbo et al. (2014) following Eq. (1):

$$\mathrm{RI} = \frac{(2\times\mathrm{T})}{(\mathrm{T}+\mathrm{C})} \quad (1)$$

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).

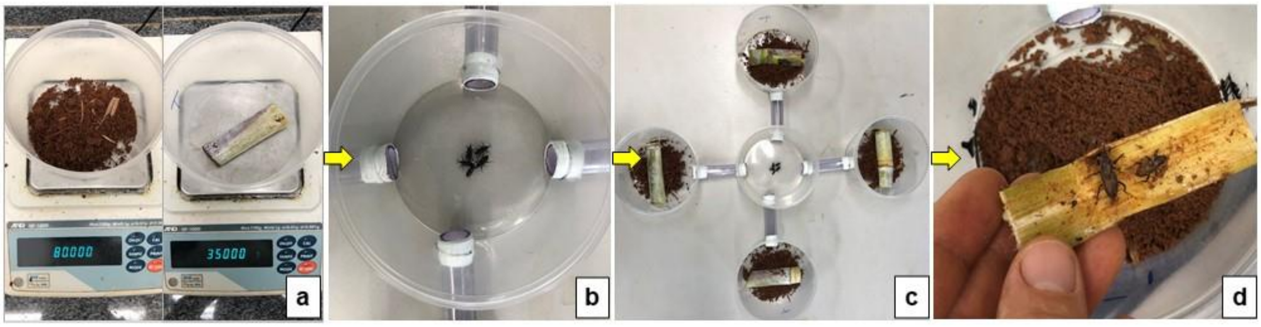

Figure 1. Methodology steps for the insecticide repellency activity including treated soil and sugarcane placement (a) in containers; placement of *S. levis* adults in the central container (b); study apparatus with five containers (c) and repellency evaluations after 24, 48 and 72 h (d).

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. 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 of Pérez (2008). 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.

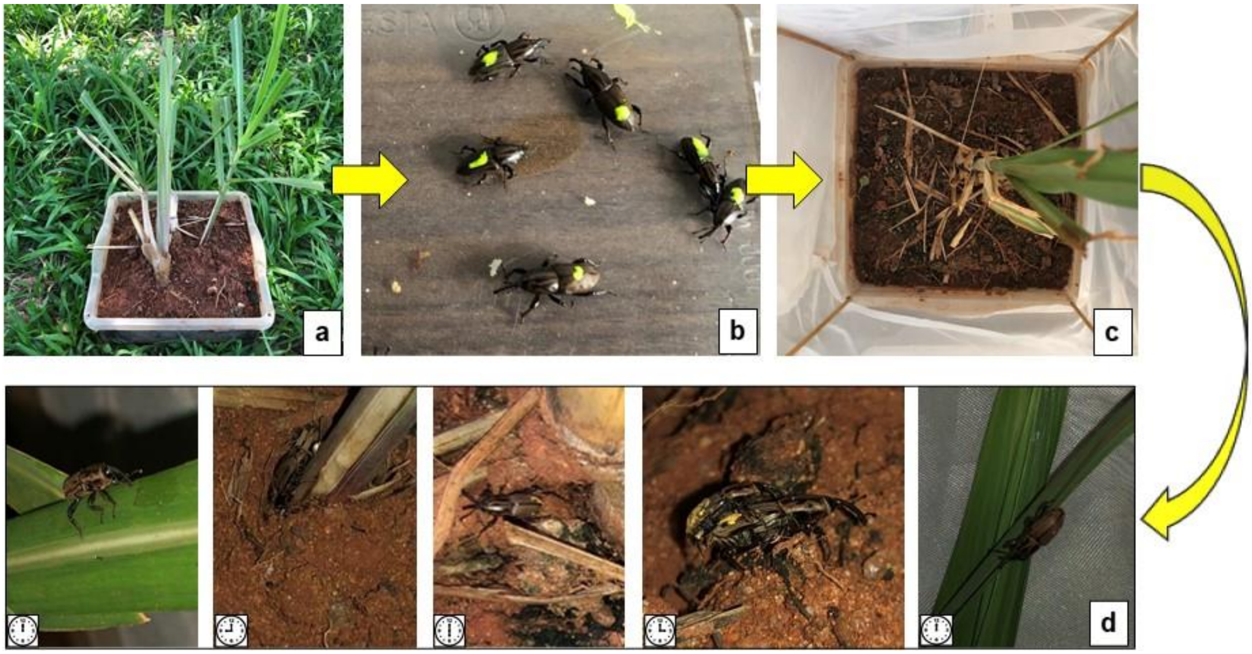

Figure 2. Methodology steps for the *S. levis* adult activity pattern study including ratoon sugarcane in containers (a); placement of 20 marked *S. levis* adults (b) in containers under natural weather conditions (c); and hourly insect activity evaluations recording adult location and behavior (d).

Data analysis

Statistical analysis for both experiments was conducted using the RStudio Version 1.4.1717 software (RStudioTeam, 2021). 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 in R software (Moral et al., 2017) 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 with Sidak's test at $p < 0.05$ (Lenth 2019).

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 (Brooks et al., 2017). Mean number of observed insects were submitted to an analysis of deviance (type II Wald chi-square tests) using the Car package (Fox and Weisberg, 2019) and significant differences between hours of evaluation were analyzed using the emmeans package with Sidak's test at $p < 0.05$ (Lenth 2019). 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).

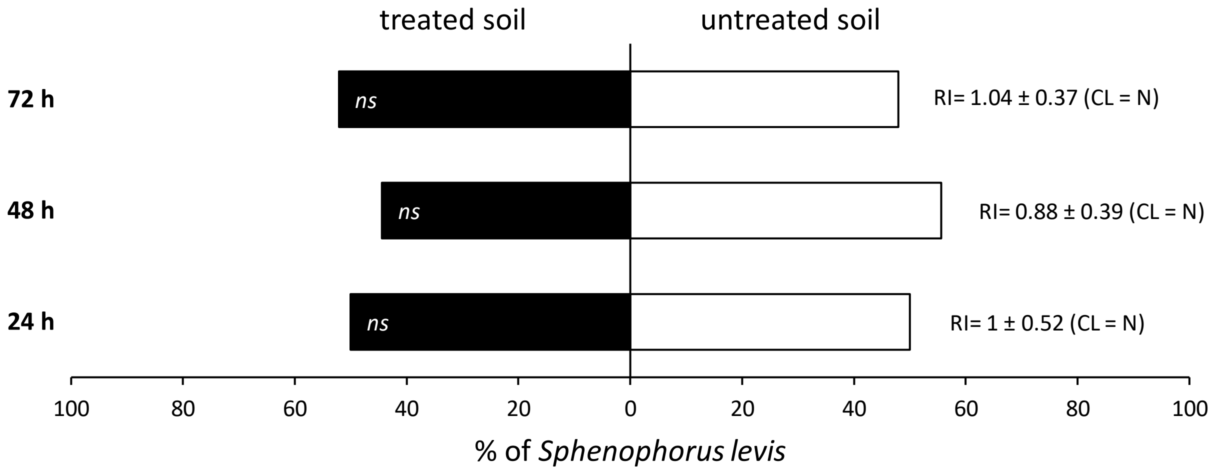

Figure 3. Percentage of *S. levis* adults that moved to soil treated with lambda-cyhalothrin + thiamethoxam and to untreated soil per evaluation period. Each bar represents the mean results of four replicates of two study repetitions. Repellency index (RI) with SD and RI classification (CL) are provided. *ns* – no significant differences were observed at α = 0.05. N – neutral activity.

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, Video 1 and Video 2) and 8% were freely in the soil (Figure 7).

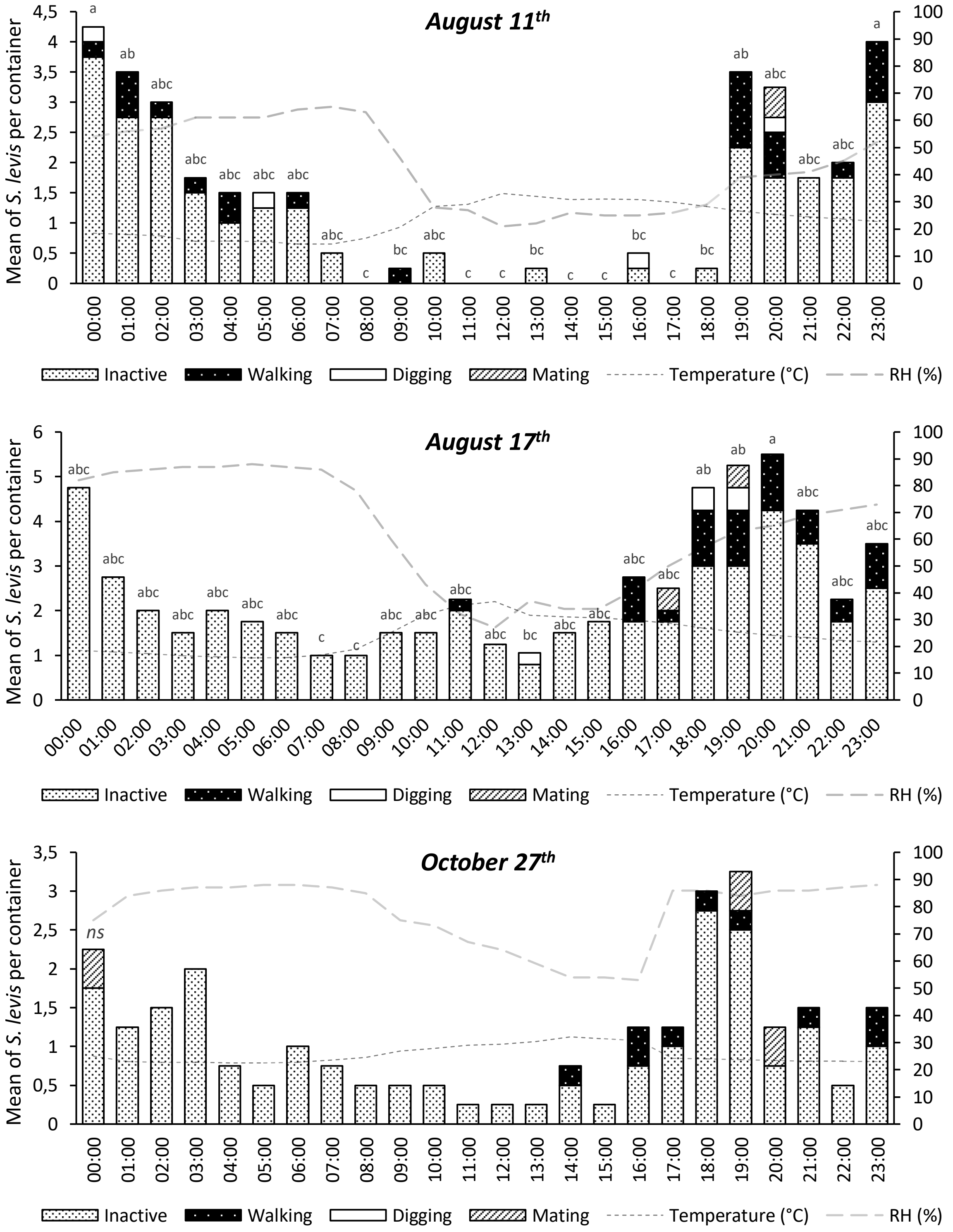

Figure 4. Number of exposed *S. levis* adults per container at every hour in different dates considering insect activity, temperature (temp) and relative humidity (RH%).

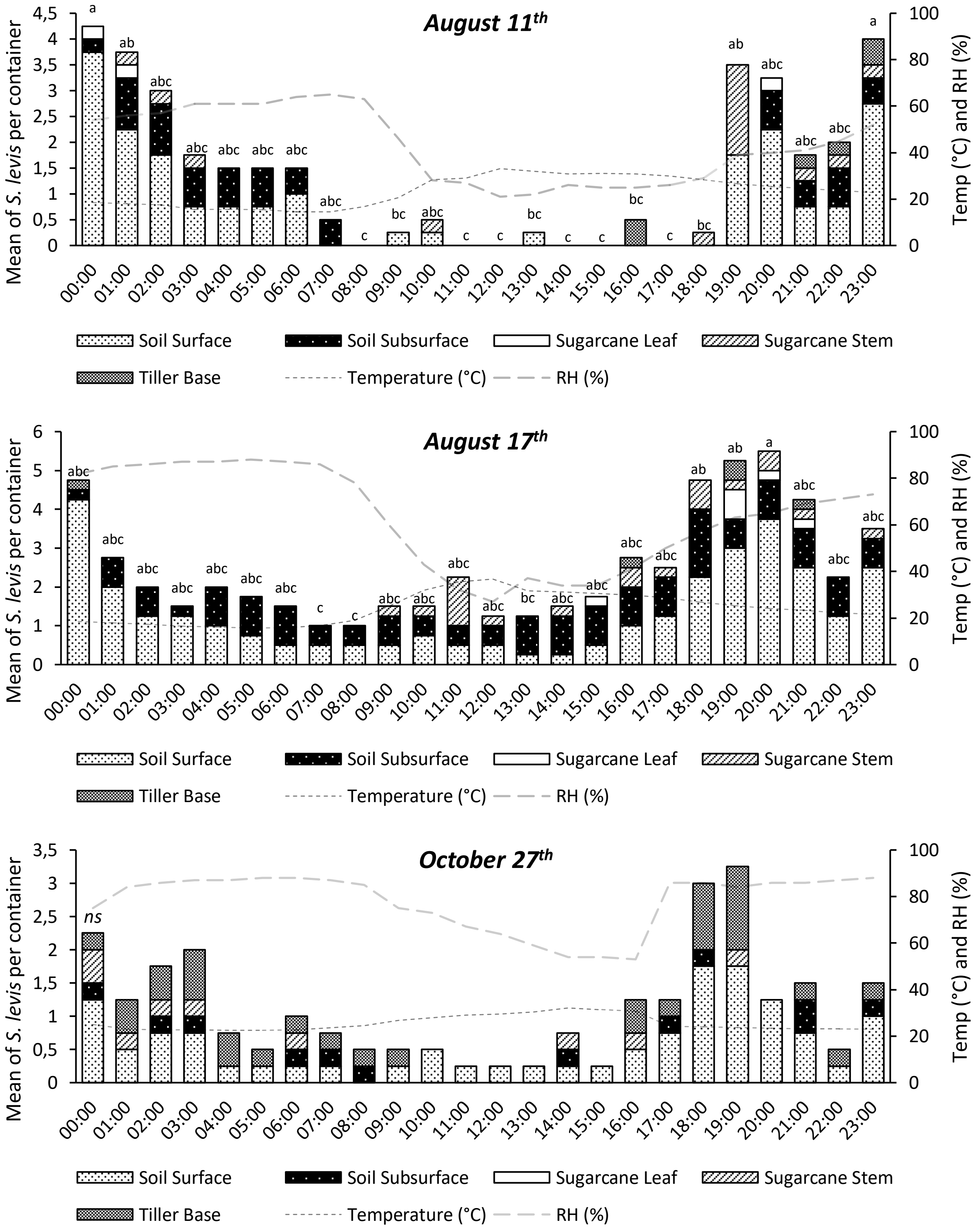

Figure 5. Number of exposed *S. levis* adults per container at every hour in different dates considering insect location, temperature (temp) and relative humidity (RH%).

Figure 6. *Sphenophorus levis* adults attached to sugarcane rhizomes and roots.

Video 1. *Sphenophorus levis* adult inside soil tunnel adjacent to sugarcane rhizome. To access the video, open the camera on your phone, hold it so that the QR code appears in view and tap the notification to open the link associated with the QR code.

Video 2. *Sphenophorus levis* adults partially hidden in soil adjacent to sugarcane rhizome. To access the video, open the camera on your phone, hold it so that the QR code appears in view and tap the notification to open the link associated with the QR code.

Figure 7. *Sphenophorus levis* adults found freely on soil and not attached to sugarcane rhizomes and roots.

DISCUSSION

Experiment 1 – Insecticide Repellent Activity

Sphenophorus levis adults were not attracted nor repelled by treated soil with lambda-cyhalothrin + thiamethoxam which, according to Mazzonetto and Vendramin (2003) classification, 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, as in Dobrin and Hammond (1985) with pyrethroid repellency to *E. varivestis* and as described by Easton and Gouson (2013) showing imidacloprid repellency to different species of pollinator beetles, 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, Viteri Jumbo et al. (2014) observed that *A. obtectus* were only repelled by higher dosages of cinnamon oil, specifically those above the lethal dose of 50% (LD_{50}). 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 (Dinardo-Miranda et al., 2006; Tavares, 2006; Alencar, 2016). 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, Martins et al. (2020) reported great attraction to sugarcane stalk baits mixed with vinasse. 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 (Zarbin et al., 2003) could be identified and synthesized, adding them to sugarcane baits would possibly increase even more its attraction to insects. For instance, a similar attraction response was observed when testing sugarcane baits mixed with aggregation pheromones from *Sphenophorus incurrens* to capture adults in field (Illescas-Riquelme et al., 2016).

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 by Casteliani at al. (2020). Other Curculionidae species are also known for their nocturnal behavior such as *C. sordidus* with maximum activity from 21:00 to 4:00 am (Gold et al., 2001) and other *Sphenophorus* species. Similar results were described for *S. venatus vestitus* in which insects were most active between 00:00 and 4:00 am (Huang and Buss, 2009). In fact, one monitoring option recommended for *S. venatus vestitus* in turfgrass is to scout adults at night (Reynolds, 2014). 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 (Veleri et al., 2007; Klowden, 2013). 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 (Huang and Buss, 2009). *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 by Casteliani at al. (2020).

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 (Degaspari et al., 1987; Precetti and Arrigoni, 1990; Canassa, 2014; Izeppi et al. 2014). 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, Reynolds (2014) observed larvae better development under 20% of total pore space with water, 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 (Durant, 1985).

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* (Dinardo-Miranda et al., 2006; Tavares, 2006; Alencar, 2016). 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 (Polato and

Oliveira, 2011). 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 (Rincão et al., 2020). 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 (Dinardo-Miranda, 2014). According to the author, the application method with two spray nozzles should be recommended during the rainfall periods due to greater adult distribution (Dinardo-Miranda, 2014) 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 (Sondhi et al., 2022).

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, Video 1 and Video 2) and with 8% of adults freely in the soil (Figure 7). Several authors have reported the gregarious behavior of *S. levis* (Zarbin et al., 2003; Izeppi, 2015; Rosa, 2022). Such behavior is induced by aggregation pheromones like the 2-methyl-4-octanol (Zarbin et al., 2003). Additionally, *S. levis* are known to have a slow spatial distribution capacity, ranging from 5.2 to 6.6 m month^{-1} (Degaspari, 1978; Rosa, 2022) in part because of its rare flying behavior but also because of its aggregation activity. 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 by Casteliani et al. (2020) 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

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CHAPTER 3 – Effect of liquid and solid insecticide application and product rate on *Sphenophorus levis* control

ABSTRACT - Application methods of insecticides for *Sphenophorus levis* control in sugarcane have not been effective mostly due to the insect's habitat behavior bellow soil surface suppressing the correct placement of the active ingredient on target. To better understand that, two experiments were conducted using a novel bioassay methodology to evaluate *S. levis* adult mortality. The first study aimed to assess the efficacy of two insecticides liquid and solid applied while the second study aimed to examinate the effect of increasing the dose of lambda-cyhalothrin + thiamethoxam on *S. levis* adult control. Bioassays were conducted through simulating liquid and solid insecticide applications (imidacloprid and lambda-cyhalothrin + thiamethoxam) on ratoon sugarcane plants and exposing *S. levis* adults to residual rhizome and soil after application. In the first experiment, low *S. levis* adult control was detected (< 53%). Both solid and liquid applications of lambda-cyhalothrin + thiamethoxam provided higher efficacy levels than the imidacloprid and control treatments. A short residual activity of insecticides was noticed with maximum control levels at 7 days after application (DAA). In the second experiment, the higher insecticide dose significantly improved *S. levis* adult control (76.7% of mortality) in comparison with the recommended rate (58.8% of mortality).

Keywords: Billbug; sugarcane weevil; granular; residue; mortality

INTRODUCTION

The sugarcane weevil [*Sphenophorus levis* Vaurie, 1978 (Coleoptera: Curculionidae)] is one of the most important pests in sugarcane (*Saccharum officinarum* L.) in Brazil. It was first reported in 1977 (Degaspari et al., 1987) but had its importance significantly increased in the last twenty years following the shift of cane harvesting system, from manual harvesting of burnt cane to mechanical harvesting of green cane. Burning sugarcane as a harvesting tool had a direct impact on pest control. After the new mechanical harvesting system without burning was implemented, an increase of pest pressure in sugarcane has been noticed, especially for sugarcane borer [*Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae)], root spittlebug [*Mahanarva fimbriolatta* Stål (Hemiptera; Cercopidae] and *S. levis* (Dinardo-Miranda and Fracasso, 2013). Since then, *S. levis* has caused great and increasing damage for sugarcane growers with losses up to 30 t ha^{-1} (Precetti and Arrigoni, 1990). Casteliani et al. (2020) reported, for instance, that every 1% of damage caused by *S. levis* resulted in 1% yield loss.

S. levis is a soil-inhabiting pest that damages sugarcane plants by larvae feeding the rhizome. Its larvae stage is, on average, 50 days long, followed by the pupae period with mean duration of 12 days while *S. levis* adults can live up to 250 days (Degaspari et al., 1987; Casteliani et al., 2020). Females deposit their eggs inside the sugarcane base and rhizome where it hatches and the larvae starts to feed causing the main damage. Larvae's damage in the rhizome is characterized by a white/yellow frass formed inside tunnels. Plant symptoms include leaf yellowing, starting from outer leaves, leaf necrosis, followed by plant death forming yellow/brown patches distributed across the field. Nearly 90% of formed tunnels are opened bellow soil surface and most of rhizome's openings are filled with soil or with the white/yellow frass (Casteliani et al., 2020). In addition to pest's immature stages hiding inside buried rhizomes, *S. levis* adults are also mostly hidden underground as observed by Ferreira (2022). Due to the pest's biology and behavior location underneath the soil, it has been extremely difficult to obtain effective control levels of *S. levis*. Specific *S. levis* management alternatives have been developed in sugarcane farming systems, including the desiccation with herbicides and destruction of volunteer cane, insect baiting and biological and chemical insecticide applications during planting and ratoon treatment. However, despite the development of different methods, low efficacy of control has been achieved, especially in insecticide applications (Dinardo-Miranda et al., 2006; Tavares, 2006; Alencar, 2016).

Among the insecticide application methods currently being used for *S. levis* control, the most common in ratoon treatments is conducted with ratoon drill applicators. This equipment physically opens the rhizome with a drilling disc at each sugarcane row and applies insecticides through a full jet spray in the rhizome aiming to deposit the active ingredient as close as possible to the target. Unfortunately, this method has not been fully effective, possibly due to inaccurate applications and low insecticide residual. In one study evaluating different insecticides for *S. levis* control, the mean insecticide efficacy was of only 60% (Dinardo-Miranda et al., 2006). In another study comparing several insecticides, no treatment was effective to reduce *S. levis* infestation or to improve sugarcane yield in comparison with the untreated control (Alencar, 2006). All insecticides currently being used for *S. levis* control are liquid applied through hydraulic nozzles while no solid applications of granular insecticides are used. Meanwhile, several authors have reported pest control benefits when adopting the application of solid insecticides (Buhler and Gibb, 1993; Roy et al., 2014; Ward, 2016; Allsopp, 2020, Pandey and Kumar, 2020). Granular applications of imidacloprid with controlled-release technology, for example, have provided up to 4 years of satisfactory canegrub control (Ward, 2016). Granular insecticides may improve *S. levis* control through the gradual release of the active ingredient concentrated on a granule to the soil profile and for plant uptake. In addition, insecticide concentrations being gradually released underneath the soil could enhance insect control especially due to the gregarious characteristic of this and other Curculionidae species.

Moreover, considering the low efficacy of current insecticides, dose-response studies comparing different product concentrations should be conducted to evaluate if recommended rates are in fact effective or not for *S. levis* control. Studying the lethal concentration of different insecticides for hunting billbug (*Sphenophorus venatus vestitus* Chittenden) control, for example, authors reported it was necessary 6.4 times more insecticide concentration to provide 95% billbug mortality (LC_{95}) compared to the concentration required to control 50% of insects (LC_{50}) (Doskocil et al., 2012).

Due to ineffective *S. levis* control with current insecticide application methods and recommended product rates, the present study aimed to evaluate the efficacy of two insecticides products (lambda-cyhalothrin + thiamethoxam and imidacloprid) applied through solid and liquid application and to compare two insecticide rates (recommended and double dose) on *S. levis* adult control through a novel bioassay methodology.

MATERIALS AND METHODS

Experiment 1 – Liquid and Solid Application of Insecticides

An insecticide efficacy study in ratoon sugarcane for *S. levis* adult control was conducted in 2021 in Jaboticabal, SP, Brazil. The experiment was conducted in a factorial arrangement of treatments (three efficacy evaluation dates by five application treatments) in a completely randomized design with four replications. The experiment was performed in duplicate with insecticide treatment applications on May 27th and June 8th, respectively.

Sugarcane plants

Seedlings of sugarcane variety CTC 4 (Centro de Tecnologia Canavieira S.A., Piracicaba, SP, Brazil) were planted on November 19th, 2020, in 50 L pots of 0.44 m^2 diameter containing a mixture of soil, sand and manure in a proportion mix of 3:1:1, respectively (Figure 1a). Pot mixture was sent for soil analysis at the Soil Fertility Laboratory at UNESP following Raij et al. (2001) methodology for organic matter content (14 g dm^{-3}) cation exchange capacity (73 $mmol_c$ dm^{-3}), base saturation (81%) and soil pH (6.0). Sugarcane pots were continuously watered through drip irrigation (Figure 1b) with 10 mm of water per day. On April 29th, 162 days after planting, sugarcane plants of the first experiment duplicate were manually harvested (Figure 1c, 1d). Harvesting was conducted to induce sugarcane ratoon shoots and tillering. On May 13th, the second experiment duplicate was harvested. All plant residue (dry leaves) from each pot was placed on the soil surface of the corresponding harvested pot. The average amount of residue was of 220 g per sugarcane pot (5,000.0 kg ha^{-1}).

Treatment Application

Insecticide treatment application was conducted on ratoon sugarcane plants 28 days after harvesting. The first and second experiment duplicates had insecticide treatments applied on May 27th and June 8th, respectively. As the experiment aimed to simulate ratoon drill application of insecticides in field, ratoon cane plants in each pot were manually cut using a small size axe. A vertical and uniform drill depth of 10 cm was ensured using a 30 cm rule (Figure 1e). As in field ratoon applications, in which insecticides are applied right after a drilling disc mechanically opens sugarcane tillers and rhizomes, experiment treatments were applied in the same manner after manual drilling. Two insecticides were used with two application methods (solid and liquid application) and one untreated check as in Table 1. Insecticide dosage was calculated based on the average surface area of sugarcane cylindrical pots, 0.44 m^2, and the recommended dosage of each insecticide in grams per hectare (g ha^{-1}) following the Equation 1 (Eq 1) where *CID* is the Calculated Insecticide Dose given in g per treated pot and *RCPD* is the Recommended Commercial Product Dose given in g ha^{-1}:

$$CID = \frac{0.44\ x\ RCPD}{10000} \qquad (1)$$

Liquid treatments were applied adopting an application volume of 200 L ha^{-1} and using a 10 mL syringe size that was constantly pressed towards the entire rhizome cut and pot diameter (Figure 1f). Solid treatments were manually applied inside the whole plant fissure ensuring uniform granular application (Figure 1f).

Figure 1. Methodology stages for the insecticide efficacy study: planting of sugarcane seedlings (a); drip irrigation (b); sugarcane pots before (c) and after (d) harvesting; ratoon drill (e) and application of liquid and solid insecticides in ratoon plants (f) simulating ratoon drill applications.

Table 1. Insecticide application treatments including insecticides, trade names, dosage, and active ingredient application rate.

Application treatments	Trade name	Dosage L or kg ha^{-1}	a.i. Rate g ha^{-1}
T1 - untreated	---------	---------	---------
T2 - liquid - lambda-cyhalothrin + thiamethoxam	[1]Engeo Pleno™ S	2.0 L ha^{-1}	212 + 282
T3 - solid - lambda-cyhalothrin + thiamethoxam	[2]Kaiso Sorbie BR GR + [1]Actara® 250 WG	0.883 + 1.128 kg ha^{-1}	212 + 282
T4 - liquid - imidacloprid	[3]Warrant® 700 WG	1.5 kg ha^{-1}	1200
T5 - solid - imidacloprid	Warrant® 700 WG	1.5 kg ha^{-1}	1200

[1]Syngenta, Basel, Switzerland; [2]Nufarm Limited, Laverton North, VIC, Australia; [3]FMC Química do Brasil Ltda, SP, Brazil.

Air temperature and relative humidity were measured during insecticide treatment applications with a digital thermo hygrometer Jprolab (JProlab, São José dos Pinhais, PR, Brazil). Pot subsurface soil temperature was measured with a digital thermometer TE-400 (Instrutherm, São Paulo, SP, Brazil) and soil humidity was assessed with a ph-2500 pH/soil humidity meter (Instrutherm, São Paulo, SP, Brazil). Daily meteorological data including the entire study period was received from the Processing, Automatization and Instrumentalization Laboratory (LIAP) at the Exact Sciences and Engineering Department at FCAV/UNESP, Jaboticabal, SP, Brazil. The data was obtained from an automatic weather station (Davis Instruments, Hayward, CA, USA) closely installed to the study area in the LIAP premises. Average meteorological and soil conditions during application are available in Table 2 and mean rainfall and temperature during the study period is available in supplementary material section.

Table 2. Harvest date, meteorological and soil conditions of each duplicate in experiment 1.

	Duplicate 1	Duplicate 2
Harvest date	29/04/2021	13/05/2021
Treatment application date	27/05/2021	08/06/2021
Temperature (°C)	28.9	25.9
Relative humidity (%)	43.0	67.5
Soil temperature (°C)	24.2	25.0
Soil humidity (%)	53.6	54.0
First rain after application / rain amount	14 DAA / 24.8 mm	2 DAA / 24.8 mm

Plant and Soil Removal

Treated sugarcane rhizome, tiller and soil were removed for insect exposure and residue evaluation in three different dates including 1 day after application (DAA), 7 and 14 DAA. In each insect exposure period, sugarcane rhizomes were manually extracted from all pots with a grubbing hoe (Figure 2a). Extracted plants were manually chopped in small pieces (6 cm) using a machete and were placed in individual plastic bags. A uniform amount of treated soil (265 g) was collected from the 5 cm surface of each pot and was placed in identified individual plastic bags. To avoid any source of contamination during plant and soil removal, both grubbing hoe and machete were washed with water and soap between each pot extraction procedure.

Insects

Sphenophorus levis adults used in the mortality evaluations of the experiment were collected between March and May of 2021 in sugarcane fields with previous infestation history and no insecticide application in the year. Sugarcane stalks cut in half (30 cm) were used as *S. levis* baits. Following an adapted methodology of Pérez (2008), cane stalks were immersed in 50 L water containers with 10% of melted sugar solution for 24 h. The following day, cane baits were distributed in sugarcane fields with the stalk cut in half section facing the soil and were covered with sugarcane residue. Five days after bait distribution, *S. levis* adults found in baits were collected and placed in containers with cane stalks in it. Collected insects were maintained in rectangular plastic containers (15 cm x 11 cm x 6 cm) sealed with small punctured lids for air exchange. In each container 30 adults of *S. levis* were placed with 3 cane stalks cut in half (14 cm). Containers were cleaned with soap and 70% ethyl alcohol and sugarcane stalks were replaced every four days. Insects were maintained under 12 h photoperiod, at room temperature (23.2°C ± 1.5) and relative humidity (65% ± 12) until were used in the insecticide efficacy study. Room temperature and relative humidity were measured with a digital thermo hygrometer Jprolab (JProlab, São José dos Pinhais, PR, Brazil).

Insecticide Efficacy for *S. levis* control

Cane rhizomes, tillers and soil that were removed from each treated pot and placed in individual bags were weighed with a GF-1000 precision scale (A&D Company, Limited, Tokyo, Japan). 50 g of treated soil and 85 g of chopped rhizomes, with average length of 6 cm, from each pot were placed in 1 L round containers. Four *S. levis* adults were then placed in each round container for treatment exposure. One treated sugarcane pot was used to fill two containers with soil, rhizome and insects. The containers were maintained in a laboratory room with 12 h of photoperiod, room temperature (22.0°C ± 1.3) and relative humidity (64% ± 7).

After 96 hours of *S. levis* insect’s initial exposure to treated soil and rhizomes, insects were replaced to new 1 L containers with one half sliced cane stalk inside it, with average weight of 40 g and 10 cm long. These new containers were now filled with eight insects from two containers with soil and rhizome of the same treated pot. Thus, each new container had 8 insects that were in contact with treated soil and rhizome of the same individual sugarcane pot. Insects were kept inside the new containers with cane stalks for 96 h until the first mortality evaluation.

The mortality evaluation consisted of counting the number of live, moribund and dead *S. levis* adults in each container. As *S. levis* exhibits the behavior of thanatosis, insects were considered dead when no movement was noticed during one minute and when no movement was observed after an involuntary reflex was induced (slight grasp in the abdomen with entomology forceps). Insects were considered live when vigorous and expected movement (insect walking; movement of legs, antenna and head) were observed. The insects assessed as moribund were either lying on their back, even after flipping the insects over, or had an atypical slow and uncoordinated body-part movement. There was a total of three insect control evaluations dates including at 8 days, at 12 days and at 14 days after insect exposure (DAIE) to treated soil and rhizomes. At every mortality evaluation, live and moribund insects were replaced to new containers with half sliced cane stalk. Dead *S. levis* adults were discarded.

Figure 2. Laboratory methodology stages for the insecticide efficacy study: sugarcane plants (a) and soil (b) were removed at each evaluation date; treated plants and soil were placed in containers (c) with *S. levis* adults placed right after it (d); insects were exposed to treated soil and rhizome (e) for 96 h and were replaced to new containers with sugarcane stalks (f) at every mortality evaluation (g).

Experiment 2 – Insecticide Rate Comparison in Solid Application

A second insecticide efficacy experiment was conducted. The experiment aimed to evaluate solid application efficacy of one insecticide mixture at two rates. The insecticide mixture consisted of lambda-cyhalothrin (Kaiso Sorbie BR GR, Nufarm Limited, Laverton North, VIC, Australia) with thiamethoxam (Actara® 250 WG, Syngenta, Basel, Switzerland). Treatments included granular lambda-cyhalothrin + thiamethoxam at two rates: 212 + 282 and 424 + 564 g a.i. ha^{-1}, respectively. All experiment methodology steps (planting, harvesting, treatment application, plant/soil removal and laboratory work) were conducted as previously described in Experiment 1. Harvesting was conducted on May 13th and treatment applications on ratoon cane of first and second duplicates were made on August 13th and August 26th, respectively. The only differences between methodologies were that in Experiment 2 only one period of soil/rhizome removal and insect exposure (14 DAA) was adopted, a total of eight round containers with twelve *S. levis* adults were used for each insecticide rate and four evaluation periods were conducted including 4, 8, 12 and 14 days after insect exposure (DAIE) to treated soil and rhizomes. Average meteorological and soil conditions during application are available in Table 3 and mean rainfall and temperature during the study period is available in supplementary material section.

Table 3. Harvest date, meteorological and soil conditions of each duplicate in experiment 2.

	Duplicate 1	Duplicate 2
Harvest date	13/05/2021	13/05/2021
Treatment application date	13/08/2021	26/08/2021
Temperature (°C)	27.4	29.0
Relative humidity (%)	32.5	35.0
Soil temperature (°C)	28.1	30.7
Soil humidity (%)	56.2	57.0
First rain after application / rain amount	3 DAA / 4.2 mm	0 mm

Data analysis

Descriptive analysis and model fitness were conducted in RStudio Version 1.4.1717 software (RStudioTeam, 2021) for the dependent variables of dead and moribund *S. levis* adults. Before selection of the best model, different model error distributions and link functions were tested and adjusted to correct for overdispersion and assess goodness of fit. To select the best model, model's performances were compared by half-normal plots with simulation envelopes using the hnp package in R software (Moral et al., 2017). *S. levis* mortality and moribund percentage data of each period after application and each evaluation date was treated as dependent variable in a quasibinomial generalized linear model with application treatment as the independent variable in experiment 1 and with insecticide rate as the independent variable in experiment 2. After model selection, results of *S. levis* mortality and moribund percentage were submitted to an analysis of deviance (type II Wald chi-square tests) for main effects. Significant effects were analyzed using the emmeans package with Sidak's test at $p < 0.05$ (Lenth, 2019) to determine significant differences between treatments. Treatment efficacy was calculated using Schneider-Orelli's correction formula (Schneider-Orelli, 1947) in which it considers both mortality of treated and untreated mean values in its equation.

RESULTS

Experiment 1 - Liquid and Solid Application of Insecticides

Adults of *S. levis* exposed to treated sugarcane 1 day after application (1 DAA) had significant results of control for each evaluation period. At 8 days after insect exposure (8 DAIE) to treated rhizome and soil, application treatments were significant to affect percentage of dead and moribund insects ($p < 0.0001$) as in Figure 3. Despite the liquid application of lambda-cyhalothrin + thiamethoxam (T2) had higher mortality level, 18,7% of *S. levis* control, it was only significantly higher than T4. Additionally, T2 also presented the greatest level of moribund insects, 23.4%. At 12 days after insect exposure (12 DAIE), *S. levis* dead and moribund percentage was significantly affected by application treatment ($p = 0.0018$ and $p = 0.0067$, respectively) as in Figure 3. The liquid application of lambda-cyhalothrin + thiamethoxam (T2) had higher *S. levis* control, 34.4%, than the untreated check, but it was not significant different than the solid application of lambda-cyhalothrin + thiamethoxam (T3) and both imidacloprid treatments (T4 and T5). At 12 DAIE, the percentage of moribund insects decreased while the level of dead insects increased for all treatments in comparison with the previous evaluation at 8 DAIE, especially for the lambda-cyhalothrin + thiamethoxam treatments (T2 and T3). At 14 days after insect exposure (14 DAIE), *S. levis* control was also significantly affected by application treatment ($p = 0.0028$ and $p = 0.0152$) as in Figure 3. Such as at 12 DAIE, liquid application of lambda-cyhalothrin + thiamethoxam also had greater insect control than T1 and T4 but was not significantly different from the solid application of the same insecticide (T3) and solid application of imidacloprid (T5).

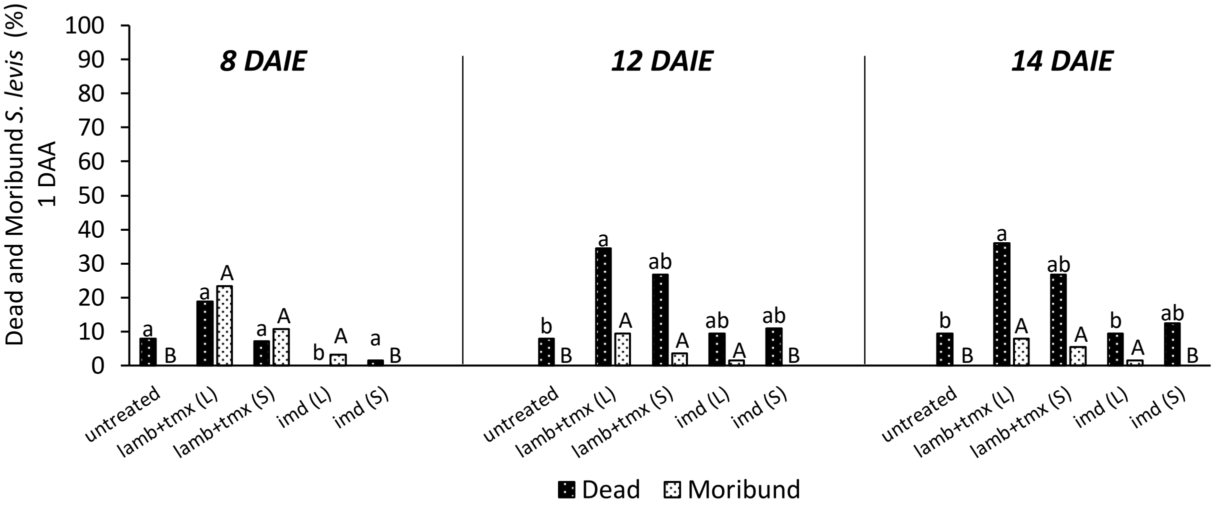

Figure 3. Percentage of dead and moribund *S. levis* adults exposed to different treatments on sugarcane one day after application (1 DAA) at different evaluation periods. DAIE – Days After Insect Exposure; lamb+tmx – lambda-cyhalothrin + thiamethoxam; imd – imidacloprid; L – liquid applied; S – solid applied; Bars with mean values in each period followed by same lowercase letter indicate no significant difference for dead insects and bars in each period followed by same uppercase letter indicate no significant difference for moribund insects at α=0.05.

Insects exposed to treated sugarcane and soil 7 days after application (7 DAA) were significantly affected by treatment application. At 8 DAIE, application treatment was not significant to affect *S. levis* control ($p = 0.2583$) but it was significant to affect the percentage of moribund insects ($p < 0.0001$) as in Figure 4. Both liquid and solid applications of lambda-cyhalothrin + thiamethoxam (T2 and T3) presented greater concentration of moribund *S. levis* adults than the untreated and the imidacloprid treatments. The T2 treatment had 15.6% while T3 had 17.5% of moribund insects at 8 DAIE.

At 12 DAIE, treatments were significant to affect insect control ($p < 0.0001$) as in Figure 4. The T3 treatments was more effective than the other treatments, except T2, with

41.3% of *S. levis* control. Despite the percentage of moribund insects at 12 DAIE being reduced in comparison with 8 DAIE, both T2 and T3 had greater levels of moribund insects. For the T3 treatment, for example, the level of moribund insects decreased from 17.5% to 12.7% while the percentage of dead insects increased from 11.1% to 41.3% in four days. At 14 DAIE, treatments also impacted significantly the levels of dead insects ($p < 0.0001$) but not the concentration of moribund adults ($p = 0.0731$). Both T2 and T3 were better effective for *S. levis* control (40.6 and 52.4%) than the untreated check (T1) and the imidacloprid treatments (T4 and T5).

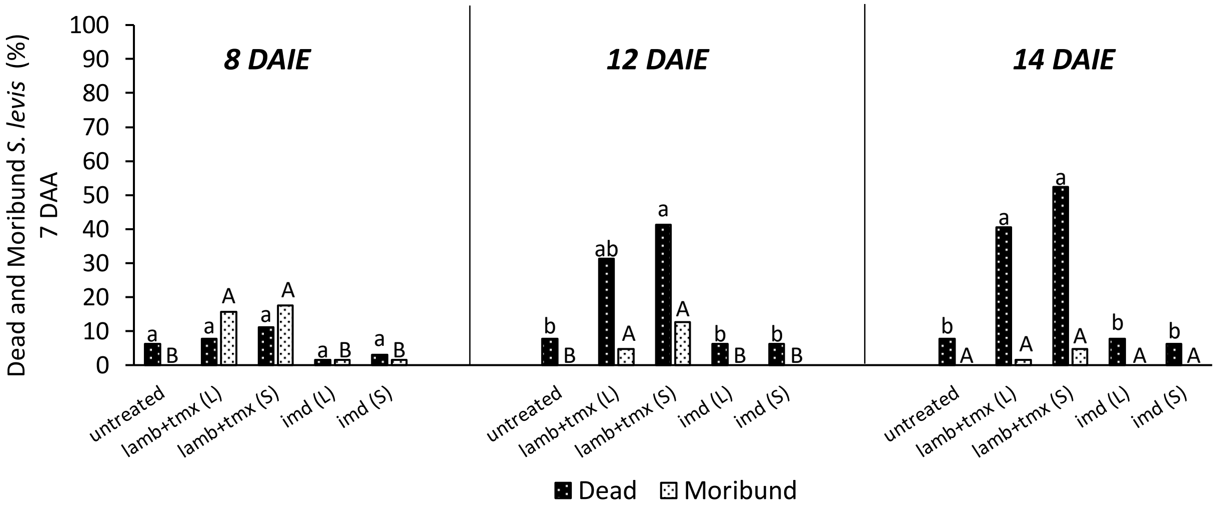

Figure 4. Percentage of dead and moribund *S. levis* adults exposed to different treatments on sugarcane seven days after application (7 DAA) at different evaluation periods. DAIE – Days After Insect Exposure; lamb+tmx – lambda-cyhalothrin + thiamethoxam; imd – imidacloprid; L – liquid applied; S – solid applied; Bars with mean values in each period followed by same lowercase letter indicate no significant difference for dead insects and bars in each period followed by same uppercase letter indicate no significant difference for moribund insects at α=0.05.

Application treatments on sugarcane 14 days after application were also significant to affect *S. levis* control. At the evaluation period of 8 DAIE, significant differences across treatments were observed for dead and moribund insects ($p = 0.0004$ and $p < 0.0001$, respectively) (Figure 5). Regarding insect control, the T4 treatment was significantly lower than the remaining treatments while no other differences were observed. The moribund percentage results, however, showed the T3 treatment with the highest concentration, 26.6%, followed by T2 treatment with 15.6%. At 12 DAIE, application treatments were significant to impact insect control and moribund concentration (*p* < 0.0001) as in Figure 5. The percentage of dead insects increased considerably from 8 to 12 DAIE. In the T3 treatment, it increased from 14% to 36%. Treatments in the last evaluation period at 14 DAIE also affected significantly (*p* < 0.0001) insect control and moribund levels with T3 having greater control values than the remaining treatments, except T2 (Figure 5). Thus, the solid application (T3) had 42.2% of dead adults followed by the liquid application of the same insecticide (T2) with 25%. In addition, both liquid and solid applications of imidacloprid and the untreated check presented less than 10% of *S. levis* control.

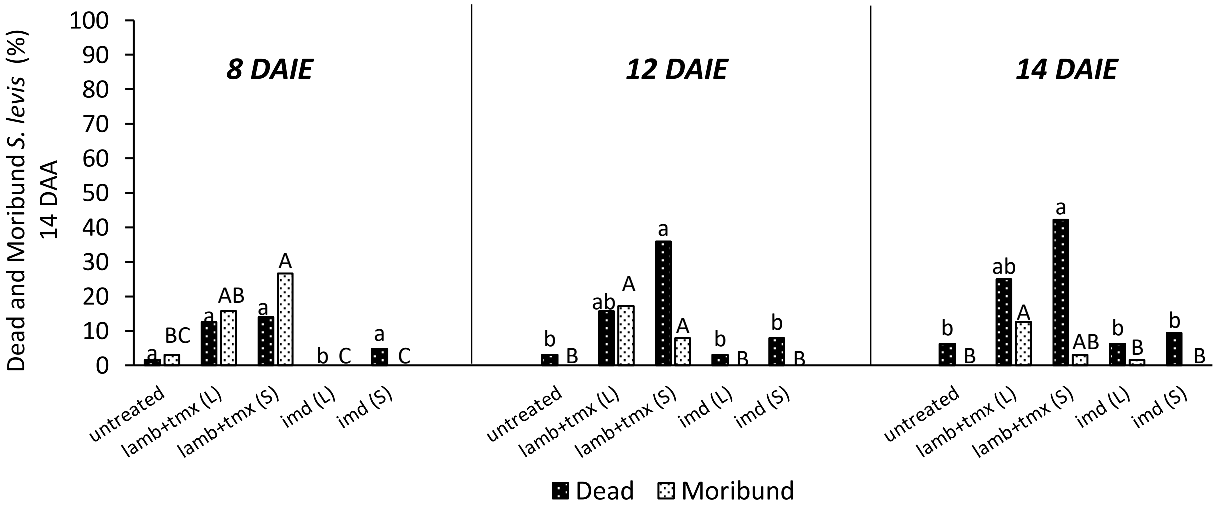

Figure 5. Percentage of dead and moribund *S. levis* adults exposed to different treatments on sugarcane fourteen days after application (14 DAA) at different evaluation periods. DAIE – Days After Insect Exposure; lamb+tmx – lambda-cyhalothrin + thiamethoxam; imd – imidacloprid; L – liquid applied; S – solid applied; Bars with mean values in each period followed by same lowercase letter indicate no significant difference for dead insects and bars in each period followed by same uppercase letter indicate no significant difference for moribund insects at α=0.05.

To evaluate *S. levis* control over time after application, results of evaluations at 14 DAIE were analyzed for each period after application (1, 7 and 14 DAA) as in Figure 6. Thus, no significant differences of insect control among periods after application were observed for each treatment (*p* = 0.5061). However, despite not statically significant, it was possible to notice the liquid application of lambda-cyhalothrin + thiamethoxam (T2) presenting greater insect control at 1 DAA followed by a slight increase on control at 7 DAA and then a substantial decrease at 14 DAA (Figure 6). The T3 treatment, on the other hand, had lower control levels at 1 DAA, followed by a peak on insect control at 7 DAA followed by a percentage decrease at 14 DAA (Figure 6).

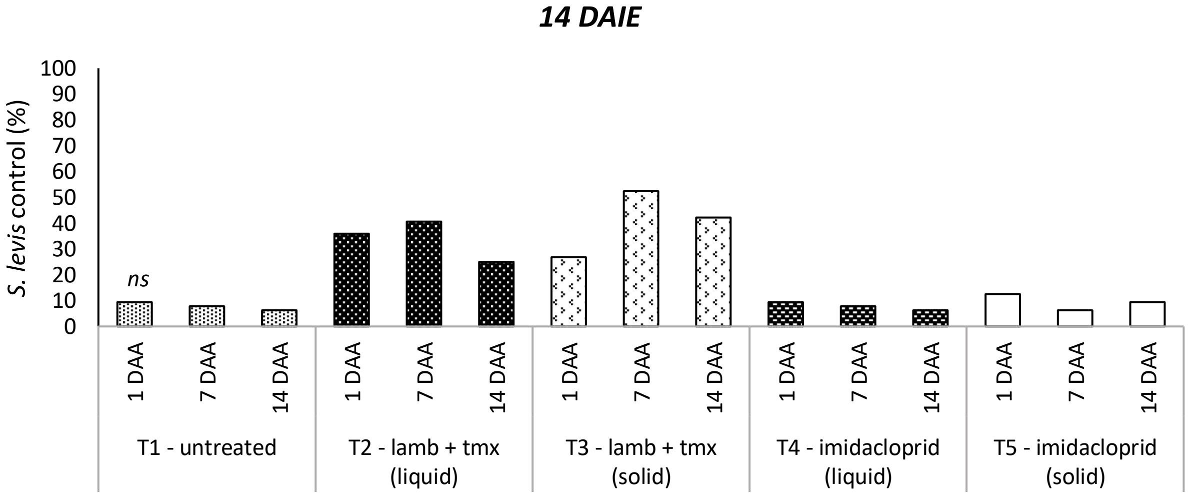

Figure 6. Control of *S. levis* adults, considering dead insects only, exposed to different treatments on sugarcane at 1, 7 and 14 DAA for the evaluation period of 14 DAIE. DAA – Days After Application; DAIE – Days After Insect Exposure; lamb + tmx –lambda-cyhalothrin + thiamethoxam; *ns* – not significant at α=0.05.

Analyzing the treatment efficacy in relation to the untreated check (Schneider-Orelli correction formula) for each period after application and evaluation period as in Table 4, it was possible to observe that for insects exposed to sugarcane and soil at 1 DAA, the highest insecticide efficacy was obtained by the liquid application of lambda-cyhalothrin + thiamethoxam (T2), with maximum efficacy at 14 DAIE (Table 4). *S. levis* adults that were exposed to treated soil and sugarcane at 7 DAA were better controlled by the solid application of lambda-cyhalothrin + thiamethoxam (T3) (Table 4). Similarly, insects that were exposed to sugarcane and soil treated at 14 DAA were also better controlled by the T3 treatments showing greater treatment efficacy than the other application treatments. As it was also noticed in the percentage of dead insects, the imidacloprid treatments had also extremely low efficacy values. And as observed in Figure 6, both T2 and T3 treatment's efficacy were better achieved on insects exposed to plant/soil at 7 DAA.

Table 4. Insecticide application efficacy (Schneider-Orelli, 1947) in relation to the untreated check for each date of insect exposure to treatments (1, 7 and 14 DAA) and at each evaluation period (8, 12 and 14 DAIE).

Insecticide Exposure Period	Evaluation Period	Treatment Efficacy (%)*			
		T2	T3	T4	T5
1 DAA	8 DAIE	11.9	0	0	0
	12 DAIE	28.8	20.6	1.7	3.4
	14 DAIE	29.3	19.2	0	3.4
7 DAA	8 DAIE	1.7	5.2	0	0
	12 DAIE	25.4	36.3	0	0
	14 DAIE	35.6	48.4	0	0
14 DAA	8 DAIE	11.1	12.7	0	3.2
	12 DAIE	12.9	33.9	0	4.8
	14 DAIE	20.0	38.3	0	3.3

*T2 – lambda-cyhalothrin + thiamethoxam liquid applied; T3 - lambda-cyhalothrin + thiamethoxam solid applied; T4 – imidacloprid liquid applied; T5 – imidacloprid solid applied.

Experiment 2 - Insecticide Rate Comparison in Solid Application

The second experiment comparing two rates of solid applied lambda-cyhalothrin + thiamethoxam were conducted with *S. levis* adults exposed to insecticide residue on sugarcane and soil at 14 DAA during four evaluation periods. At the first evaluation period, 4 DAIE, treatments were significantly different to affect the concentration of moribund insects (*p* = 0.0110) but not to affect the mortality levels (*p* = 0.3334) as shown in Figure 7. Doubling the insecticide rate significantly increased *S. levis* control. The higher insecticide dose (double dose) resulted in 67.4% of moribund *S. levis* adults while the lower dose (recommended dose) provided 40.7% of moribund insects. Less than 6% of insect were dead at 4 DAIE for both doses. At 8 DAIE, treatments were significantly different regarding insect mortality (*p* = 0.0287) but were not for moribund insects (*p* = 0.2182). The higher insecticide dose provided better *S. levis* control than the lower dose (Figure 7). Additionally, the percentage of moribund insects decreased substantially while the number of dead insects increased. At 4 DAIE for the double dose treatment, for example, the level of moribund insects was of 67.4% and four days later, at 8 DAIE, it dropped to 21.2%. *S. levis* control was also significantly affected by treatments at 12 DAIE (*p* = 0.0086) while the level of moribund insects was not (*p* = 0.9032). The solid application of lambda-cyhalothrin + thiamethoxam with higher insecticide rate had better *S. levis* control (65.3%) than the lower dose treatment (44%). The percentage of moribund insects decreased even more when compared to the previous evaluation period at 8 DAIE while the level of dead insects increased. Finally, at the last evaluation period of 14 DAIE, significant differences on *S. levis* control between insecticide doses were detected (*p* = 0.0426) with no differences on moribund concentration (*p* = 0.2069). The higher dose treatment had better control than the lower dose treatment, 76.7% and 58.8%, respectively (Figure 7).

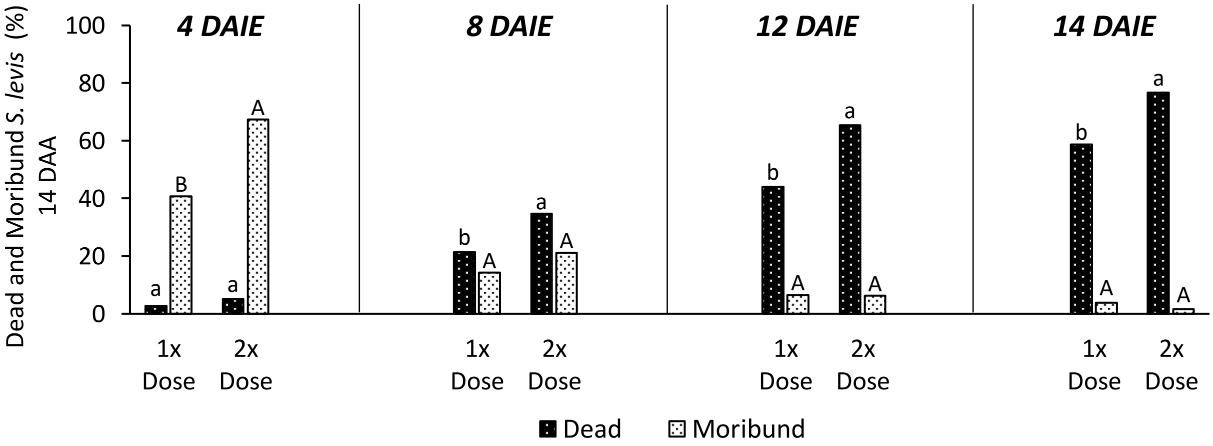

Figure 7. Percentage of dead and moribund *S. levis* adults exposed to two insecticide rates on sugarcane fourteen days after application (14 DAA) at different evaluation periods. DAIE – Days After Insect Exposure; Bars with mean values in each period followed by same lowercase letter indicate no significant difference for dead insects and bars in each period followed by same uppercase letter indicate no significant difference for moribund insects at α=0.05.

DISCUSSION

Experiment 1 - Liquid and Solid Application of Insecticides

Results of *S. levis* adult control indicated low treatment efficacy across all insecticides and application methods, including liquid and solid applications. The maximum percentage for insect control was of only 52.38% with lambda-cyhalothrin + thiamethoxam solid applied at 7 DAA at the last evaluation period (Figure 4) while the maximum treatment efficacy using the correction formula was of 48.4% for the same treatment and period (Table 4). According to Health and Safe Executive (HSE) British regulator agency, pest control levels between 40 and 60% are considered to provide some control or to reduce pest damage (HSE, 2020) although some regulation agencies may require pest control levels above 80% (Embrapa, 2011). Similar results have also been reported by different authors regarding low efficacy of insecticides on *S. levis* control. In one field study evaluating different insecticides for *S. levis* control, average efficacy was of only 60% (Dinardo-Miranda et al., 2006). No success of control was also observed in another field study testing different insecticides (Alencar, 2016). In one laboratory experiment, however, Tavares (2006) reported high *S. levis* adult control efficacy with entomopathogenic nematodes associated with insecticides, but the adopted methodology in that study consisted of direct treatment applications on sugarcane stalks carrying *S. levis* adults in it and buried in sand, probably facilitating insecticide translocation and improving control levels. The current experimental methodology, on the other hand, simulated ratoon applications by applying treatments on actual ratoon plants and exposing insects to treated ratoon plants and soil. Even though the current experiment was conducted in a more realistic scenario in comparison to other methodologies, laboratory results are expected to present higher efficacy levels than field trials. Even when highly effective treatments from laboratory results were tested in field, low *S. levis* controls were reported as in Tavares (2006). Laboratory experiment conditions are often controlled and restricted to some standards while field conditions are susceptible to several variables. These field variables can, therefore, impact directly the active ingredient’s degradation, absorption and efficacy. The soil half-life (DT_{50}) of thiamethoxam in laboratory conditions, for example, is of 121 days, while in field conditions it drops to 39 days (Lewis et al., 2016). Thus, if recommended insecticide rates were not effective against *S. levis* adults in laboratory, as observed in the present study, field applications may present even worst efficacy levels. In addition, both liquid and solid applications of imidacloprid had practically zero *S. levis* adult control such as the untreated control. Similar results were also reported in a study comparing insecticides and entomopathogenic nematodes for *S. levis* adult control. In this study, the liquid application of imidacloprid provided less than 10% of *S. levis* adult mortality (Tavares, 2006). Hence, based on present results and previous research (Tavares, 2006; Alencar, 2016) both solid and liquid application of imidacloprid should not be recommended for *S. levis* adult control while applications of lambda-cyhalothrin + thiamethoxam showed some potential for *S. levis* adult control requiring further studies with higher insecticide rates.

Present results showed treatments efficacy considering residual exposure of adults to insecticides as an attempt to better simulate field reality. However, if treatments were topically tested on *S. levis* adults, efficacy levels would probably be greater than the ones reported here. But as *S. levis* is a soil-inhabiting pest, the biology cycle and adult activity takes place predominantly in the soil subsurface (Ferreira, 2022) while the minority of *S. levis* larvae, pupae and adults in field would be directly exposed to insecticide applications. In addition, treatment efficacy values reported here are only valid for adult *S. levis* control. If treatments were to be tested for larvae/pupae mortality, greater control levels would also be expected. Some granular insecticide labels have, for example, specified at which pest's life stage the product is recommended to be applied (Anonymous, 2022).

During the evaluation periods of insect control assessment, it was also possible to observe the progression of insecticide poisoning symptomology on *S. levis* adults, especially for those exposed to lambda-cyhalothrin + thiamethoxam. During the first evaluations, at eight and twelve days after insects were exposed to the insecticide, the concentration of moribund insects was higher than in the last evaluation period, at 14 DAIE. It was clear that *S. levis* adults exposed to lambda-cyhalothrin + thiamethoxam were initially affected showing poisoning symptoms like uncoordinated, slow and atypical movement (Video 1). Most insects that showed initial poisoning symptoms were dead by the last evaluation period. The usual symptoms of pyrethroid insecticides, like lambda-cyhalothrin, include convulsive activity, vigorous tremors, incorporated movements, rapid paralysis and death (Nishimura et al., 1987; Tomlin, 1997). Similar uncoordinated movements as those observed on *S. levis* adults in the experiment were also reported in a study by Maccuaig (1980) evaluating pyrethroid symptoms on desert locusts (*Schistocerca gregaria* Forskål). Within few minutes after application, symptoms were evident on desert locusts but were less apparent after two days (Maccuaig, 1980). However, due to lambda-cyhalothrin's low solubility (0.005 mg L^{-1}) and extremely high adsorption to organic matter (Koc = 283707) (Lewis et al., 2016), the insecticide was probably not available through soil and plant rhizome when *S. levis* adults were exposed due to organic matter binding of the insecticide. The strong sorption and hydrophobic characteristics of lambda-cyhalothrin can affect its bioavailability causing pest control reduction (Oudou and Hansen, 2002). Therefore, based on physicochemical properties and literature, it is hypothesized that most residual poisoning symptoms on *S. levis* adults were caused by thiamethoxam toxicity. Thiamethoxam and other neonicotinoid insecticides are also known to induce continuous nervous excitation, loss of coordination and orientation, paralysis, decrease in plant feeding and death, as similarly reported in the present study (Martinou et al., 2014; Goulson, 2013; Yao et al., 2015). In an experiment investigating the reduction in feeding and locomotion pattern of carabids (*Platynus assimilis*), it was noticed a significant reduction of food consumption even for insects treated with very low thiamethoxam rates (Tooming et al., 2017). In the same study, beetle adults showed similar symptoms of locomotor hypoactivity state after thiamethoxam treatment (Tooming et al., 2017). According to thiamethoxam's physicochemical properties, such as its solubility (4100 mg L^{-1}) and adsorption (Koc = 56.2) (Lewis et al., 2016), it is possible to suggest that thiamethoxam was probably more available in soil and for plant uptake (rhizome) with consequent higher exposure on *S. levis* adults than lambda-cyhalothrin was. This characteristic high mobility of thiamethoxam in soil has been reported by Mörtl et al. (2016). In addition, when associating both the insecticide's degradation path and insecticide's efficacies as observed in the present study, it is important to state that low *S. levis* control can also be related to the adsorption of lambda-cyhalothrin on organic matter or the high mobility of thiamethoxam causing the active ingredient to leach and dilute its concentration within the soil profile.

A B C

Video 1. QR codes for videos showing poisoning symptoms of moribund *S. levis* adults exposed to thiamethoxam + lambda-cyhalothrin (A, B and C). To access the video, open the camera on your phone, hold it so that the QR code appears in view and tap the notification to open the link associated with the QR code.

Despite no great levels of insect control, both liquid and solid applications of lambda-cyhalothrin + thiamethoxam were better alternatives to control *S. levis* adults than the remaining treatments. The results indicated similar *S. levis* adult control for both liquid and solid application of lambda-cyhalothrin + thiamethoxam, however, it was evident the liquid application (T2) provided higher control levels and efficacy than T3 (Figure 3) when insects were exposed to treatments shortly after application (1 DAA). Pesticides when liquid applied are usually readily available for plant/insect uptake once the active ingredient reaches the soil solution, depending only on the kinetics of dissolution of the active ingredient (Davis et al., 1996). Insecticide granules, however, are applied dry and need to be wetted first so the active ingredient is dissipated from the granule into the soil solution for later target absorption (Davis et al., 1996). Thus, the liquid treatment (T2), in association with the drip irrigation used on sugarcane pots, had more lambda-cyhalothrin + thiamethoxam initially available for insect exposure than the solid treatment. It was also possible to notice the liquid application efficacy of lambda-cyhalothrin + thiamethoxam decreasing faster over time than the solid treatment, which indicates low potential for long-term crop protection (Figure 6 and Table 4). As previously stated, liquid pesticides can quickly dissolve into the soil solution accelerating its availability but also its losses in the environment through biological and chemical degradation, photolysis, evaporation, runoff and leaching (Davis et al., 1996; Fernández-Pérez, 2007).

On the other hand, despite not significant different, the solid application of lambda-cyhalothrin + thiamethoxam (T3) had greater *S. levis* adult control percentage than T2 when insects were exposed to treatments at 7 and 14 DAA demonstrating some potential for long-term insect control. Considering the solid treatment, T3, was not composed by a specific formulation for solid application but by a mixture of two insecticides with different formulations (GR of lambda-cyhalothrin and WG of thiamethoxam), the observed behavior of maximum efficacy at 7 DAA could be highly improved if a proper and adequate GR formulation was used for both active ingredients. Proper granular formulations may include those with controlled release (CR) technologies that can maintain effective control levels during longer and controlled periods (Roy et al., 2014). Since the early 1990s, the Australian sugar industry has been working with CR formulations of insecticides aiming the control of canegrubs species in sugarcane (Allsopp, 2020). More recently, with a newer imidacloprid CR formulation product, trial results have indicated control of different canegrub species from 2 to 4 years using the granular formulated insecticide (Ward, 2016). In a study developing a CR formulation for imidacloprid, Kimoto et al. (2007) observed that both the amount and concentration of the coating membrane of granules had direct effects on release time of the active ingredient. The authors also observed the effect of temperature on the insecticide release profile, in which lower temperatures extended the release period (Kimoto et al., 2007). Even without an adequate formulation of lambda-cyhalothrin + thiamethoxam, the potential of insecticide solid applications on sugarcane for *S. levis* adult control was observed in the present study. As no granular and controlled-release insecticides are currently available for *S. levis* control in sugarcane, the development of new insecticide formulations should be encouraged by the sugarcane farming community and by the pesticide industry. Moreover, based on the results, it is clear the recommended insecticide rates were not effective to control *S. levis* adults, thus, new experiments comparing different insecticide rates should be conducted.

Experiment 2 - Insecticide Rate Comparison in Solid Application

As observed in Experiment 1, current insecticide rates were not effective to successfully control *S. levis* adults. The second experiment considered the treatment with greatest control efficacy from experiment 1, the lambda-cyhalothrin + thiamethoxam solid applied (T3) and compared two insecticide rates regarding *S. levis* adult control. The higher dose clearly provided better adult control than the recommended rate at 14 DAIE, giving a maximum control percentage of 76.7% in comparison to 58.8%. Despite potential insecticide losses from different degradation pathways such as leaching, adsorption, microbiological and photodegradation, it is hypothesized the greater dosage treatment maintained greater concentrations in soil and rhizome and, as consequence, provided greater insect control. According to the HSE British regulator agency, insect control levels between 60 and 80% can be classified as useful for pest control while levels between 40 and 60% are considered to provide some control or to reduce pest damage (HSE, 2020). Thus, doubling the recommended lambda-cyhalothrin + thiamethoxam rate improved *S. levis* control's classification as useful for control and improved the mortality levels by 1.3 times. In an experiment evaluating the susceptibility of pepper weevil (*Anthonomus eugenii* Cano) to thiamethoxam, for example, the insecticide concentration to provide 50% weevil mortality (LC_{50}) was of 0.53 mg ai L^{-1} while the concentration to control 95% of insects (LC_{95}) was 3.6 times higher, 1.91 mg ai L^{-1} (Caballero et al., 2015). Similarly, studying the toxicity of lambda-cyhalothrin to Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky), authors reported a dose increment of 5.7 required to change the lethal dose of 50% of insects (LD_{50}) to a 90% (LD_{90}) (Wu et al., 2015).

As the experiment was conducted in controlled conditions, including sugarcane plants grown in pots and a confined area (plastic container) for insect exposure to insecticide residue on soil and cane rhizome, observed control results are probably higher than under field conditions. Especially due to intrinsic field variability. Therefore, it is expected that *S. levis* adult control in field applications may present lower efficacy in comparison with current laboratory results. Further studies including additional insecticide rates should be conducted to better asses the most effective active ingredient concentration for *S. levis* adult control. Several authors have evaluated the toxicity profile of different insecticides to a range of agricultural pests but no study has assessed the dose response of insecticides on *S. levis* control to present date. Doskocil et al. (2012) evaluated the lethal dose and concentration of some insecticides on *S. venatus vestitus* in laboratory bioassays. In that study, authors evaluated bifenthrin, imidacloprid and clothianidin insecticide dose responses by topical application on the ventral side of the insect's thorax (Doskocil et al., 2012). Future *S. levis* laboratory bioassays, however, should consider the insect's subterranean behavior and should consider applying insecticide treatments directly on soil/plant instead of topical applications as a more realistic scenario. The present study's bioassay, for example, provides a better representation of field reality by considering the insect behavior and the most probable path of exposure to applied insecticides, the soil and plant rhizome.

If the double dose treatment in the present study provided control levels up to 76.7%, higher doses may provide control results close to 90 or 100%. As previously stated, several researchers have conducted similar studies for different insects and products (Doskocil et al., 2012; Caballero et al., 2015; Wu et al., 2015) and new experiments should consider dose-response insecticide effects for *S. levis* control. However, a cost-effectiveness analysis of higher insecticide rates should also be conducted to evaluate the economic viability of the operation. Although increasing insecticide application rate increases the application cost, potential yield increment through *S. levis* control may reveal to be economically valid. Evaluating the cost-effectiveness of different granular insecticides for pink stem borer (*Sesamia inferens* Walker) control, Sidar et al. (2017) reported the economic viability of two applications of solid thiamethoxam (WG) in corn (*Zea mays* L.). Moreover, if precision agriculture tools for insect symptomology mapping is proper used, such as satellite multispectral/hyperspectral imagery and unmanned aerial vehicles (UAVs) photogrammetry, site-specific maps for insecticide applications can be used for spot spraying. By only spraying where *S. levis* symptoms are detected, the total amount of insecticide to be applied can be drastically reduced in comparison with conventional broadcast/band applications and, therefore, could possibly justify insecticide dose increment. In addition, improved pest control as a result of greater insecticide doses may require less applications and may reduce operational costs in despite of pesticide inputs.

The higher insecticide rate results also indicate a great potential for solid applications on *S. levis* control. As a number of researchers have reported low field efficacies with current insecticides liquid applied for *S. levis* control (Dinardo-Miranda et al., 2006; Tavares, 2006; Alencar, 2016), current results are an indication that insecticides solid applied can improve pest control, especially if proper granular formulation is adopted (Roy et al., 2014; Ward, 2016; Allsopp, 2020). In addition to new studies including more insecticides and different active ingredient concentrations, sequential insecticide applications and other integrated pest management (IPM) alternatives should also be tested for *S. levis* control potential. Examples of IPM actions should include desiccation and destruction of volunteer cane, bare fallow, crop rotation, seed cane billets of high phytosanitary quality, variety selection, insect baiting, biological control and insecticide's mode of action rotation.

Similar to Experiment 1 results, earlier evaluations in the dose experiment also showed greater concentration of moribund adults than later ones (Figure 7) for both treatments. The first evaluation (4 DAIE), especially, had a great number of *S. levis* adults showing symptoms of slow, uncoordinated and uncommon movement (Video 1). By the last evaluation, most insects that were initially showing poisoning symptoms were diagnosed dead (Figure 7).

CONCLUSION

The proposed bioassay methodology simulating *S. levis* adult control though insecticide exposure after ratoon sugarcane treatments has proven to be effective for *S. levis* adult control evaluation. Based on the results, low *S. levis* adult control (< 53%) and short residual activity was achieved with both liquid and solid application of insecticides in the first experiment. Solid and liquid application of lambda-cyhalothrin + thiamethoxam had maximum pest control when insect adults were exposed to insecticide residue collected after seven days from the application. Doubling lambda-cyhalothrin + thiamethoxam dose resulted in greater *S. levis* adult control (76.7%) in comparison with the recommended labelled dose (58.8%). The application of solid lambda-cyhalothrin + thiamethoxam demonstrated great potential for *S. levis* adult control in sugarcane but the development of proper granular formulations including higher active ingredient concentration formulations should optimize insecticide performance for *S. levis* control.

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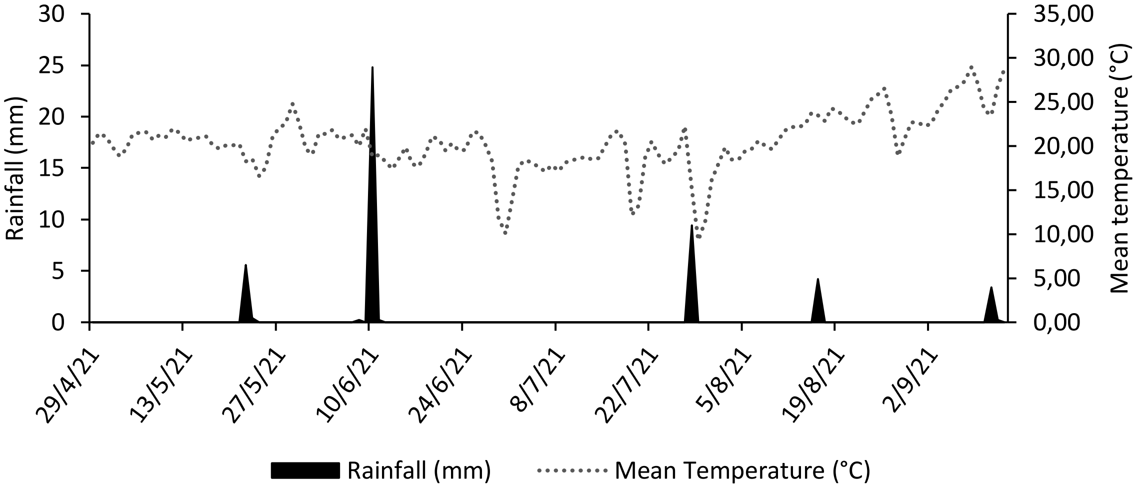
SUPPLEMENTARY MATERIAL



Mean rainfall and temperature data during the conduction of both experiments 1 and 2.

CHAPTER 4 - Application methods for *Sphenophorus levis* control in sugarcane

ABSTRACT - Current application methods used to control *Sphenophorus levis*, an important sugarcane pest, have shown low efficacy due to the difficulty of reaching the insect underneath the soil with adequate insecticide concentrations. Different application methods could possibly improve insecticide placement with consequent enhanced insect control. Thus, the present study aimed to investigate the efficacy of different application methods on *S. levis* control, plant injury and sugarcane yield. Six treatments using the same insecticides rate (lambda-cyhalothrin at 212 g a.i. ha^{-1} + thiamethoxan at 282 g a.i. ha^{-1}) and no treatment (as check) were tested in three different ratoon sugarcane fields. Treatments included insecticide application with a ratoon drill applicator with solid application and with liquid application; broadcast application; band sprayings with one solid stream nozzle, with three-solid stream nozzles and with an even-spray nozzle. Analysis parameters consisted of *S. levis* insect counts and sugarcane rhizome injury evaluations at 0, 60, 120, 200 days after application (DAA) and 30 days after harvest (DAH). Yield assessment was conducted at 200 DAA. In general, just a few differences on the number of *S. levis* per soil sample, sugarcane injury levels and yield were seen among treatments. However, soil applications with solid and liquid insecticides demonstrated positive results with high efficacies for insect control and low number of *S. levis* insects for longer periods, especially with the solid insecticide application providing low insect counts even at 30 DAH. Developing proper granular formulations with effective active ingredient concentrations should improve *S. levis* control in solid applications. Additionally, an increase of *S. levis* population was observed between June and July during the evaluation period at 200 DAA.

Keywords: Granular, weevil, ratoon, insecticide

INTRODUCTION

Sphenophorus levis Vaurie, 1978 (Coleoptera: Curculionidae) is considerate one of the most important pests in sugarcane (*Saccharum officinarum* L.) in Brazil. Great yield loss and economic damage to farmers are frequent as *S. levis* injuries to sugarcane reduces plant stand and crop longevity with up to 40 t ha^{-1} yield losses and 60% death of sugarcane ratoon (Terán and Precetti, 1982; Precetti and Arrigoni, 1990). *S. levis* is a weevil that attacks the root system of sugarcane, especially the plant rhizome. Among the plant symptoms, the most common one is the formation of rhizome tunnels by *S. levis* larvae filled with white-yellow frass (Casteliani et al., 2020) with consequent drying of tillers, leaf yellowing and plant death. One of the factors that has influenced *S. levis* control is the timing to detect the pest in the field. Because *S. levis* lives underneath the soil, when plant symptoms become visible it may be too late to effectively control it.

Most of the attacks are caused by the insect larvae hiding underneath the soil surface making it difficult to properly place insecticides and to obtain adequate pest control. The total life cycle of *S. levis* is of approximately 6 months, with egg incubation period of 8 days, larvae stage period of 35 days, pupae period of 10 days and adult stage period of 120 days (Degaspari et al., 1987). Aiming to improve the control efficacy of an inaccessible insect with such a long life cycle, different types of application equipment have been developed to apply liquid insecticides inside the ratoon cane targeting the insect immature stages. These ratoon cane applicators that are currently adopted in sugarcane fields are composed by a sprayer with drilling discs that physically opens the soil and cane ratoon's root system, while a high-pressure nozzle, attached behind the drilling discs, sprays a full jet inside the exposed soil and rhizome. Although most sugarcane farmers have been using this application equipment for *S. levis* treatment on ratoon cane, proper pest control has not been accomplished (Dinardo-Miranda et al., 2006). When sugarcane tillers are too tall and the ratoon applicator equipment cannot be used, some farmers have used broadcast applications with standard spray nozzles while some have used band spray applications with jet stream or even-spray nozzles. Although some methods have been used, only a few studies have evaluated application methods for *S. levis* control. In one study, for example, insecticide applications with drilling discs and nozzles placed in the sugarcane row and sideways to the sugarcane row were compared (Dinardo-Miranda et al., 2006). In other studies, band sprayings with 70% of the application volume directed to the sugarcane plant base and with 30% of the volume directed to the soil were compared with band sprayings with jet stream nozzles and were also compared with drill disc ratoon applications directed to the soil (Dinardo-Miranda, 2014). Despite some studies of application methods effect on *S. levis* control, no research has evaluated and compared a wide range of methods, including solid insecticide applications and different band spraying methods.

In addition to chemical control methods and as part of an integrated pest management program, farmers should also be encouraged to adopt different management techniques including crop desiccation and destruction of volunteer cane, crop alternation, bare fallow, high phytosanitary quality seed cane billets, insect baiting and biological control based on pest monitoring. Thus, sugarcane farmers should use all available agronomic methods to improve plant tolerance and yield against pests (Goebel and Nikpay, 2018)

However, even with most adopted *S. levis* management techniques, poor control and yield loses are still a reality in the majority of fields with *S. levis* occurrence. As no research, up to date, has been conducted evaluating and comparing different application methods for *S. levis* control considering current and proposed methods, the present study objective was to evaluate the effect of different insecticide application methods on *S. levis* control, sugarcane injury levels and sugarcane yield.

MATERIALS AND METHODS

The study consisted of testing different application methods in three sites of ratoon cane fields, with insecticide applications in 2020 and evaluations in 2020 and 2021. All field experiments were conducted in three sugarcane mil units including one field located in Santa Ernestina, SP, Brazil (Field 1), one located in Pradópolis, SP, Brazil (Field 2) and another in Jaboticabal, SP, Brazil (Field 3). Field sites were located in areas with *S. levis* infestation history. Previous crop management operations in past ratoon cane and before sugarcane planting included conventional soil tillage in field 1, mechanical elimination of ratoon cane and conventional soil tillage in field 2 and conventional soil tillage in field 3. Information of each field site is available in Table 1.

Table 1. Field sites information including sugarcane variety, harvest date, ratoon number, soil specification, soil analysis and crop residue amount.

	Field Sites		
	Field 1	Field 2	Field 3
Coordinates	21° 27.7475' S 48° 20.1158' W	21° 18.539' S 48° 05.2005' W	21° 11.5522' S 48° 20.1363' W
Sugarcane variety	CV6654	CTC 4	CTC 4
Harvest date	15/10/2020	04/09/2020	30/10/2020
Ratoon number	3° ratoon	3° ratoon	3° ratoon
Soil type	Oxisol	Oxisol	Oxisol
Soil texture	Medium Clay	Clay	Medium
Organic matter (g dm^{-3})	20	42	18
CEC ($mmol_c$ dm^{-3})	74	117	77
Base saturation (%)	69	66	59
pH ($CaCl_2$)	5.4	5.4	5.0
Residue (kg ha^{-1})	18,268.0	14,978.0	13,391.0

The experiment was conducted in a randomized complete block design with seven treatments, four blocks and two replicates per block. Each row was considered as one replicate and each treatment was composed of two rows with length of 50 m and row spacing of 1.5 m. Six treatments of different application methods were applied with the same insecticide active ingredients, lambda-cyhalothrin and thiamethoxam (Table 2) and one untreated check (T1) was included. The solid application treatment (T2) was applied with lambda-cyhalothrin at 212 g a.i ha^{-1} (Kaiso Sorbie BR GR, Nufarm Limited, Laverton North, VIC, Australia) mixed with thiamethoxam at 282 g a.i. ha^{-1} (Actara® 250 WG, Syngenta, Basel, Switzerland). Treatment T2 was applied using two solid applicator equipment (FRS Equipamentos, Limeira, SP, Brazil) with a fluted rotor, attached at the end of a ratoon drill applicator, one at each sugarcane row (Figure 1) distanced at 0.05 m from the soil and plant rhizome. Feed hoses linking the granular hopper to ratoon rows were placed right behind the drilling discs in which insecticide was delivered through gravity once the equipment's electric motor was activated by an on/off switch control. The electric motor of the solid applicator equipment was powered by the tractor battery.

Table 2. Application method treatments with technologies, spray pressure and operation speed used.

Treatments		Pressure kPa	Speed m s^{-1}
Application type	Nozzle/Delivery mechanism		
T1-untreated	---	---	---
T2-granular soil application	feed hose (⌀ 5 cm) - Fluted rotor mechanism	---	2.77
T3-liquid soil application	[1]TP0006 – Solid stream jet	214	2.77
T4-broadcast spraying	[1]TTJ60-11002 – Dual orifice with 110° each	173	1.00
T5-band spraying	[2]MJS5 - Solid stream jet	210	0.83
T6-band spraying	[1]SJ3-015 – Three solid stream jets	320	0.83
T7-band spraying	[1]AI95015EVS – Air induction even flat spray	310	0.83

[1]Spraying Systems Inc., Wheaton, IL, US; [2]Magno Jet Ind Ltda, Ibaiti, PR, Brazil

Figure 1. Application method treatments including an untreated check (T1), ratoon drill with solid applicators (T2), ratoon drill with liquid application (T3), broadcast application

(T4), band application with one jet stream nozzle (T5), with three jets (T6) and one even flat fan nozzle (T7).

Treatments with liquid application were also sprayed with lambda-cyhalothrin + thiamethoxam, 212 + 282 g a.i. ha^{-1} (Engeo Pleno™ S, Syngenta, Basel, Switzerland). All liquid application treatments (T3, T4, T5, T6 and T7) were calibrated to deliver 200 L ha^{-1}. The spray pressure, operation speed and nozzle type of different application methods are provided in Table 2. The T3 treatment was applied with a ratoon drill applicator with one solid stream jet nozzle directed towards the rhizome distanced at 0.05 m and placed behind the drilling disc in each sugarcane row (Figure 1). The T4, T5, T6 and T7 treatments were sprayed with a CO_2 pressurized backpack sprayer. The broadcast treatment (T4) used a four-nozzle boom with nozzle spacing and height of 0.5 m (Figure 1) while liquid band application treatments (T5, T6 and T7) used a two-nozzle boom with nozzle spacing of 1.5 m (Figure 1) and nozzle height of 0.5 m. All band application treatments (T2, T3, T5, T6, T7) were calibrated for a spray zone width of 0.6 m and nozzle spacing of 1.5 m corresponding to distance between sugarcane rows.

Meteorological and soil conditions were measured and are available in Table 3. Temperature and relative humidity were measured at the time of application using a digital thermo hygrometer Jprolab (JProlab, São José dos Pinhais, PR, Brazil), wind speed with an anemometer Kestrel 3000 (Kestrel Instruments, Boothwyn, PA, USA), soil temperature with a digital thermometer TE-400 (Instrutherm, São Paulo, SP, Brazil) and soil humidity with ph-2500 pH/soil humidity meter (Instrutherm, São Paulo, SP, Brazil). Four soil samples were collected at each field and were analyzed by the Soil Fertility Laboratory at UNESP following methodology of Raij et al. (2001) for organic matter (OM) content, cation exchange capacity (CEC), base saturation and soil pH (Table 1). Crop residue quantification was conducted in each field site prior to treatment application using a 1 m^2 frame, randomly selecting four points in field and collecting all residue in each point. Residue collection was conducted after a minimum of 7 days without rain to ensure dry residue weigh using a portable electronic scale BM-A06 (Guangzhou Wei Heng Electronics Co., Guangzhou, China).

Table 3. Information of treatment application including date, time, meteorological and soil conditions and first rainfall event after ratoon application.

	Field Sites		
	Field 1	Field 2	Field 3
Application date	06/11/2020	04/12/2020	20/11/2020
Application time	09:00 am	10:00 am	15:00 pm
Temperature (°C)	31.5	35.5	31.9
Relative humidity (%)	29.6	55.5	38.0
Wind speed (m s^{-1})	3.97	0.88	2.62
Soil humidity (%)	43	48	52
Soil temperature (°C)	23.0	25.6	25.1
First rain after application/rain amount	5 DAA[1] / 4.9 mm	2 DAA / 20 mm	7 DAA / 5.1 mm

[1] DAA - days after application.

Sphenophorus levis insect count and rhizome injury evaluations were conducted one day before application, at 60, 120, 200 days after application (DAA) and at 30 days after harvest (DAH) in each replication. Insect count evaluation considered all *S. levis* development stages (larvae + pupae + adult) found in each soil sample. Cane injury evaluations were conducted by counting the total number of rhizomes and the number of rhizomes injured by *S. levis* per replication. Both insect count and plant damage evaluations consisted of digging a 0.50 x 0.50 m x 0.30 m trench and pulling out cane rhizomes, cutting subterranean shoots and steams and assessing insect presence in soil/plant and assessing cane injury symptoms in the center of each row (replication). Sugarcane yield was estimated at 200 DAA by counting plants in 10 m of each replication, manually harvesting 10 cane stalks of each row and weighting it with a portable electronic scale BM-A06 (Adapted from Arizono et al., 1998; Landell et al., 1999). Weather data including rainfall, humidity and temperature during the study period was obtained for each field and is available in supplementary material section.

Data analysis

Treatment and evaluation period effects were analyzed for individual and pooled field site data aiming to observe specific and overall effects across field sites. Descriptive analysis and model fitness were conducted for insect count (all insect development stages), sugarcane injury and yield data in R version 1.4.1717 software (RStudioTeam, 2022). Before selection of the best model, different model error distributions and link functions were tested and adjusted to correct for overdispersion and assess goodness of fit. To select the best model, model's performances were compared by half-normal plots with simulation envelopes using the hnp package in R software (Moral et al., 2017) and based on Akaike information criterion (AIC) and residual deviance values.

Insect count data was treated as dependent variable in a negative binomial generalized linear model of a two-way interaction of independent variables (treatment x evaluation period) using the MASS package (Venables and Ripley, 2002). Cane injury data was treated as dependent variable in zero-inflated negative binomial models and in quasibinomial models of a two-way interaction of independent variables (treatment x evaluation period). The zero-inflated model was used to compensate for excessive zeros and overdispersion using the pscl package (Jackman, 2020) and zeroinfl function (Zeileis et al., 2008). Sugarcane yield data was treated as dependent variable in a gaussian generalized linear model with treatment as the independent variable.

After model selection, results of *S. levis* count, cane injury and yield were submitted to an analysis of deviance (type II Wald chi-square tests) considering two-factor interaction and main effect, respectively. Significant interactions and main effects were analyzed using the emmeans package with Sidak's test at $p < 0.05$ (Lenth, 2019) to determine significant differences between treatments. When the interaction between treatment and evaluation period was significant, the corrected treatment efficacy was calculated using the Henderson and Tilton correction formula in Eq. (1) (Henderson and Tilton, 1955):

$$CTE = (1 - \frac{UTR\ before\ treatment\ x\ T\ after\ treatment}{UTR\ after\ treatment\ x\ T\ before\ treatment})\ \mathrm{x}\ 100 \tag{1}$$

where *CTE* is the corrected treatment efficacy, *UTR* is the mean number of insects per soil sample in the untreated check, *T* is the mean number of insects in one determined treatment, in which both *UTR* and *T* values before treatment refers to values from the evaluation period at 0 DAA and values after treatment refers to those from 60, 120, 200 DAA or 30 DAH.

RESULTS

Treatment and Evaluation Period Effect on *S. levis* Count

The interaction between treatments and evaluation period was not significant to affect the number of *S. levis* insects found in field 1 ($p = 0.5785$). Therefore, no significant insect number differences were observed between treatments during the same period or during different periods in field 1. However, the evaluation period, independently of treatments, was significant to affect the number of *S. levis* ($p < 0.0001$). Thus, the number of *S. levis* was significantly higher at 200 DAA, with 1.6 insect trench^{-1}, in comparison with other evaluation periods in field 1 (Figure 2).

Regarding the field 2 results, both treatment and evaluation period effects did not interact to affect the *S. levis* count ($p = 0.9975$). Moreover, each effect alone was not significant to influence the number of insects, including the treatment effect ($p = 0.3330$) and the evaluation period effect ($p = 0.2026$). Hence, regardless of application treatment or evaluation period, the number of insects was not affected by any of these variables in field 2.

The number of *S. levis* larvae, pupae and adults found in field 3 was also not affected by the interaction between treatment and evaluation period variables ($p = 0.05768$) meaning that there were no significant differences in the number of *S. levis* in field 3 between treatments from the same period and from different periods. However, both treatment and evaluation period variables in field 3, when analyzed independently, affected the mean number of *S. levis* insects found. The evaluation period variable ($p < 0.0001$), for instance, showed significant higher count values at 200 DAA, with 1.1 insect trench^{-1}, in comparison with other periods (Figure 2). The treatment variable alone (p = 0.0347) also significantly affected the mean number of insects in field 3. The broadcast application treatment with 0.53 insect trench^{-1}, the one nozzle band application with 0.36 insect trench^{-1} and the three-nozzle band application with 0.25 insect trench^{-1}, all had significant higher counts than the remaining treatments (Figure 3).

In addition, when considering the results of *S. levis* insects found in all fields, a significant effect of treatment and evaluation period interaction (p = 0.0474) was detected, meaning that there were significant differences of insect count among treatments for the same evaluation period and among different periods. Regarding differences at the same evaluation period, the only ones observed were between the liquid soil application (T3) and the band application with one nozzle (T5) at 120 DAA in which T3 presented 10 times less insects than T5 (Figure 4). In relation to significant differences between evaluation periods, all treatments had an increase on the number of insects at 200 DAA in comparison with previous evaluations (Figure 4). Despite some significant differences, the number of *S. levis* insects in the untreated check was not significant different than other treatments (Figure 4). However, it is possible to notice an efficacy tendency among application treatments when considering *S. levis* count change over time with the Henderson and Tilton efficacy correction formula in Eq. (1). For example, the granular soil application (T2) presented high efficacy values with a gradual decrease over time (Table 3). Additionally, the liquid soil application (T3) presented high efficacy values but had a faster reduction in efficacy over time than T2 (Table 3). At 60 DAA, for example, the liquid soil application (T3) had zero insect trench^{-1} and at 30 DAH the number of insects was greater than the untreated control (Figure 4). Meanwhile, for the granular soil application (T2), the number of insects per trench was lower than the untreated control during all periods after application (Figure 4).

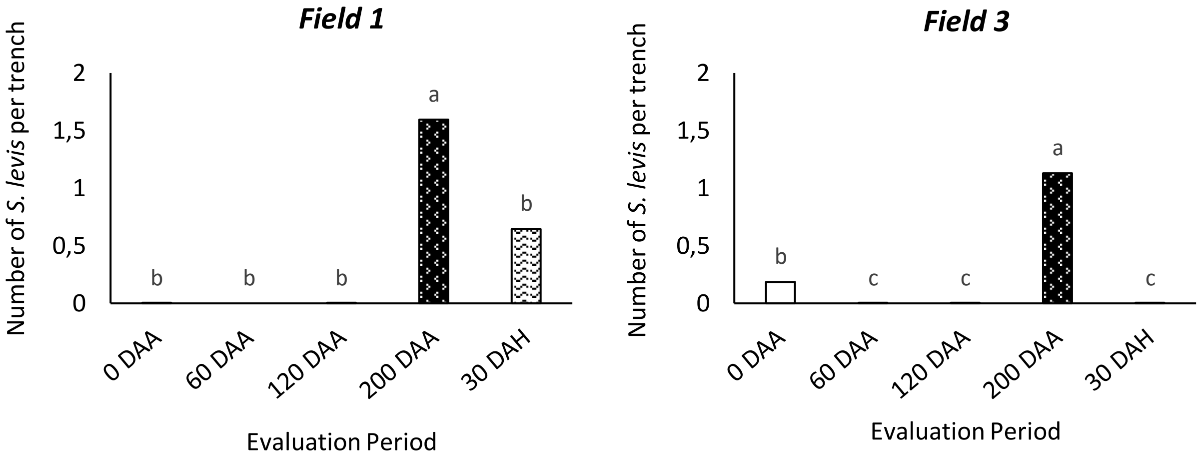


Figure 2. Number of *S. levis* insects per evaluation period regardless of treatment, considering field 1 (left) and field 3 (right). Bars with same letter are not significantly different at $p \leq 0.05$.

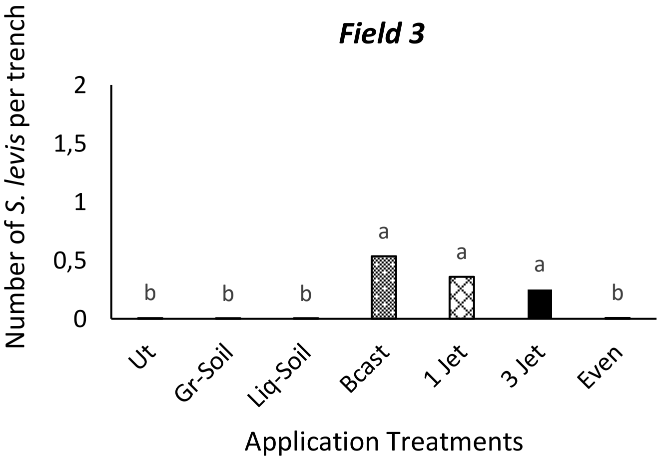


Figure 3. Number of *S. levis* insects in field 3 per application treatment regardless of evaluation period. Bars with same letter are not significantly different at $p \leq 0.05$. Ut-Untreated; Gr-Soil - Granular soil application; Liq-Soil - Liquid soil application; Bcast-Broadcast application; 1 Jet-One jet band spraying; 3 Jet-Three jet band spraying; Even-Even flat fan band spraying.

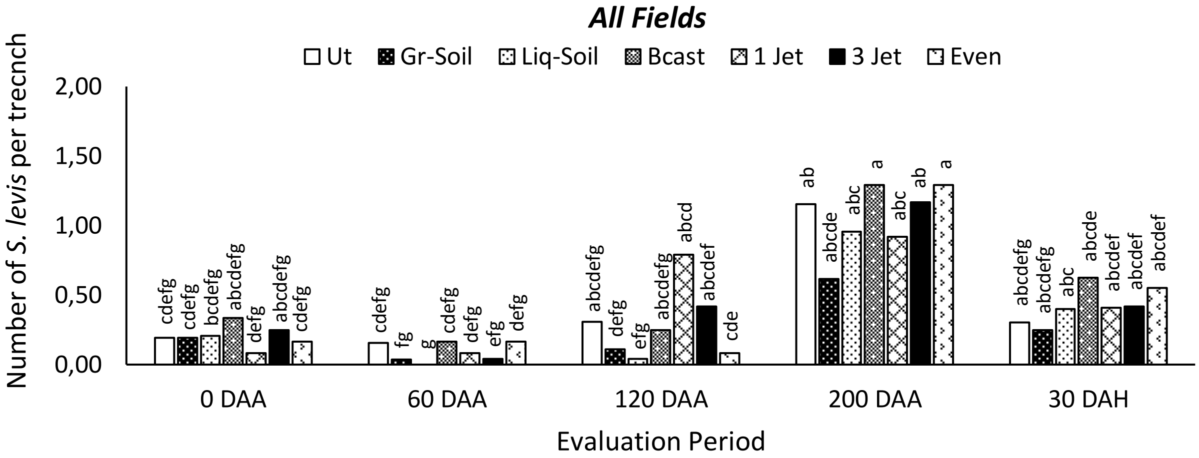


Figure 4. Number of *S. levis* insects per treatment for each evaluation period considering pooled data of all fields. Bars with same letter are not significantly different at $p \leq 0.05$. Ut- Untreated; Gr-Soil - Granular soil application; Liq-Soil - Liquid soil application; Bcast- Broadcast application; 1 Jet-One jet band spraying; 3 Jet-Three jet band spraying; Even- Even flat fan band spraying.

Table 3. Treatment efficacy (%) of *S. levis* insect control, based on the number of insects per soil sample for each evaluation time (days after application) and the untreated control, obtained by the Henderson and Tilton correction formula.

Application Treatments	Treatment Efficacy (%)			
	60 DAA	120 DAA	200 DAA	30 DAH
T1 – untreated	-	-	-	-
T2 – granular - soil application	75.0	63.9	46.7	17.8
T3 – liquid - soil application	100.0	87.5	23.4	0
T4 – broadcast spraying	37.4	53.1	35.4	0
T5 – band spraying - 1 jet nozzle	0	0	0	0
T6 – band spraying - 3 jets nozzle	79.2	0	22.2	0
T7 – band spraying - even flat fan nozzle	0	68.8	0	0

Treatment and Evaluation Period Effect on Sugarcane Injury

Both treatment and evaluation period did not interact to significantly affect the percentage of sugarcane injury caused by *S. levis* in field 1 ($p = 0.0526$). This result represents no differences between application treatments for the same period or between different periods. The effect of treatments, however, regardless of the evaluation period, was significant to affect sugarcane injury percentage in field 1 ($p = 0.0368$) in which the band application with an even nozzle (T7) had lower injury levels than the untreated check T1 (Figure 5). In addition, the evaluation period itself significantly affected the level of cane injury in field 1 ($p < 0.0001$). High injury levels were detected at 200 DAA, followed by 30 DAH and 0 DAA (Figure 6).

In field 2, the level of sugarcane injury from *S. levis* was also not affected when treatments and evaluation periods interacted ($p = 0.0899$). Thus, no differences of injury levels were seen across treatments and evaluation dates. Treatment by itself was also not significant to affect sugarcane injury level in field 2 ($p = 0.4636$) meaning that in despite of evaluation periods, no treatment was more or less effective to affect sugarcane injury values. In contrast, the period of evaluation, regardless of application treatments, had a significant effect on injury level in field 2 ($p < 0.0001$) as in Figure 6. At 60 DAA, low injury levels were observed for all treatments while at 200 DAA a significant injury increment was noticed for all treatments (Figure 6).

Results from field 3 also showed the level of sugarcane injury not being affected by the interaction of application treatment and evaluation period ($p = 0.3090$) nor by the treatment effect alone ($p = 0.1135$) and, therefore, injury percentage was not influenced by treatments during the same period or different periods neither was influenced by treatments when the evaluation period was not considered. On the other hand, the evaluation period itself was significant to affect sugarcane injury in field 3 ($p < 0.0001$) regardless of application treatments. At both 200 DAA and 30 DAH periods, the mean cane injury levels were significantly greater than previous evaluation periods (Figure 6).

Moreover, when all field data of sugarcane injury was pooled to detect the overall effect of application treatment and evaluation period interaction, no significance was observed ($p = 0.0920$). The period of evaluation, however, was significant to affect the level of sugarcane injury when considering all fields ($p < 0.0001$). At 60 DAA, injury percentage was the lowest across evaluation periods while at 200 DAA the injury percentage was the highest (Figure 7). In addition, a significant decrease of injury level was noticed after sugarcane harvesting, from 28.5% to 15.2% (Figure 7).

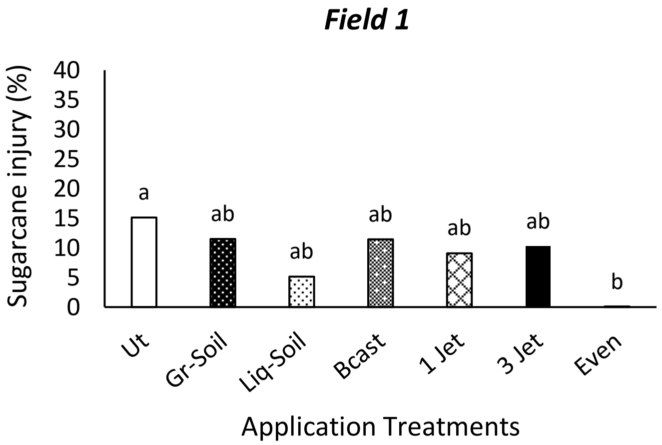


Figure 5. Sugarcane injury (%) in field 1 per application treatment regardless of evaluation period. Bars with same letter are not significantly different at $p \leq 0.05$. Ut-Untreated; Gr-Soil - Granular soil application; Liq-Soil - Liquid soil application; Bcast-Broadcast application; 1 Jet-One jet band spraying; 3 Jet-Three jet band spraying; Even-Even flat fan band spraying.

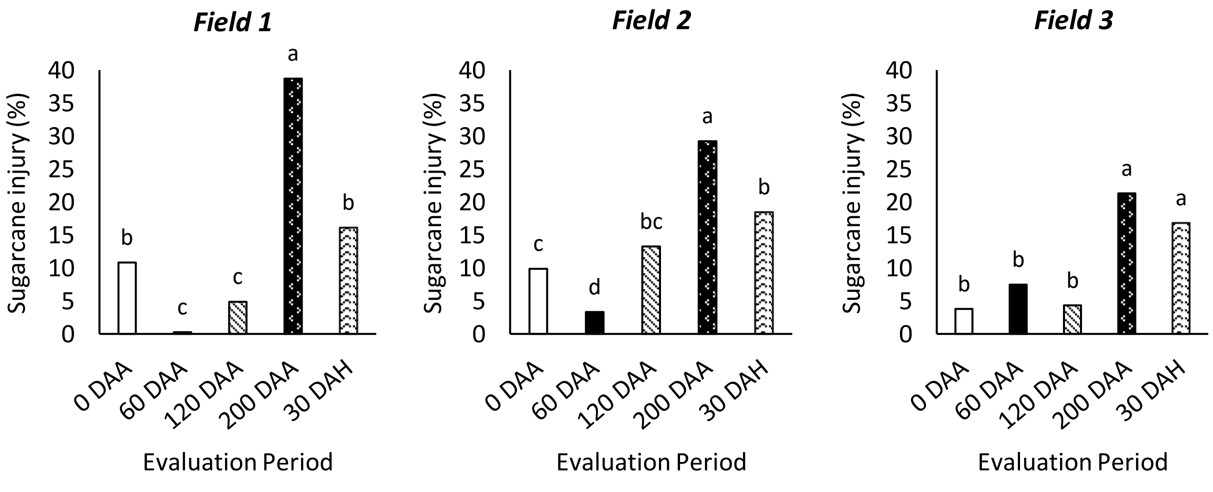


Figure 6. Sugarcane injury (%) per evaluation period regardless of treatment, considering field 1 (left), field 2 (center) and field 3 (right). Bars with same letter are not significantly different at $p \leq 0.05$.

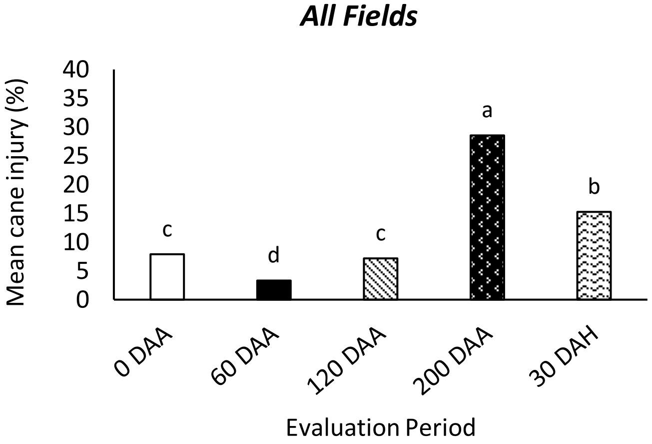


Figure 7. Sugarcane injury (%) per evaluation period regardless of treatment, considering pooled data of all fields. Bars with same letter are not significantly different at $p \leq 0.05$.

Treatment Effect on Sugarcane Yield

Treatments were significant to affect sugarcane yield in field 1 ($p = 0.0020$) as seen in Figure 8. Both band applications with one and three nozzles (T5 and T6) provided higher sugarcane yield (98.6 and 106.5 t ha^{-1}, respectively) in comparison with the band application with one even nozzle (T7) with 76.7 t ha^{-1}. All remaining treatments had no significant differences on sugarcane yield. In field 2, no significant differences were observed across treatments in relation to sugarcane yield ($p = 0.2412$) with sugarcane yield values ranging from 110 t ha^{-1} to 134 t ha^{-1} (Figure 8). In field 3, sugarcane yield was also not influenced by application treatment ($p = 0.1892$) and yield values ranged from 57.4 to 84.5 t ha^{-1} as in Figure 8. When considering the overall effect of treatment on yield for all field, no significant differences among application treatments for sugarcane yield values were seen ($p = 0.8247$) and sugarcane yield values varied from 90.8 to 102.2 t ha^{-1} (Figure 9).

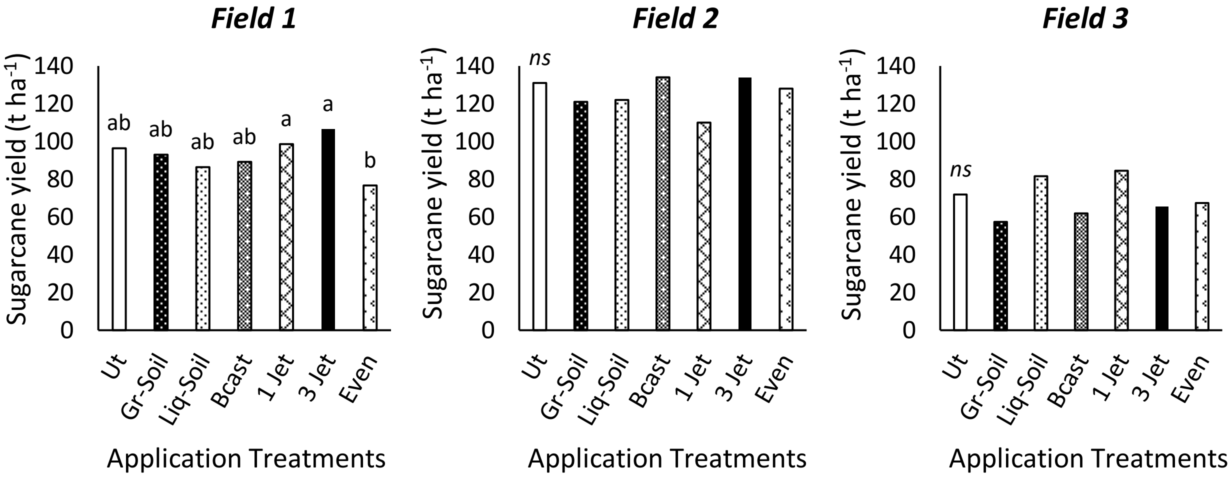


Figure 8. Sugarcane yield (t ha^{-1}) per treatment, considering field 1 (left), field 2 (center) and field 3 (right). Bars with same letter are not significantly different at $p \leq 0.05$. *ns* – not significant; Ut-Untreated; Gr-Soil - Granular soil application; Liq-Soil - Liquid soil application; Bcast-Broadcast application; 1 Jet-One jet band spraying; 3 Jet-Three jet band spraying; Even-Even flat fan band spraying.

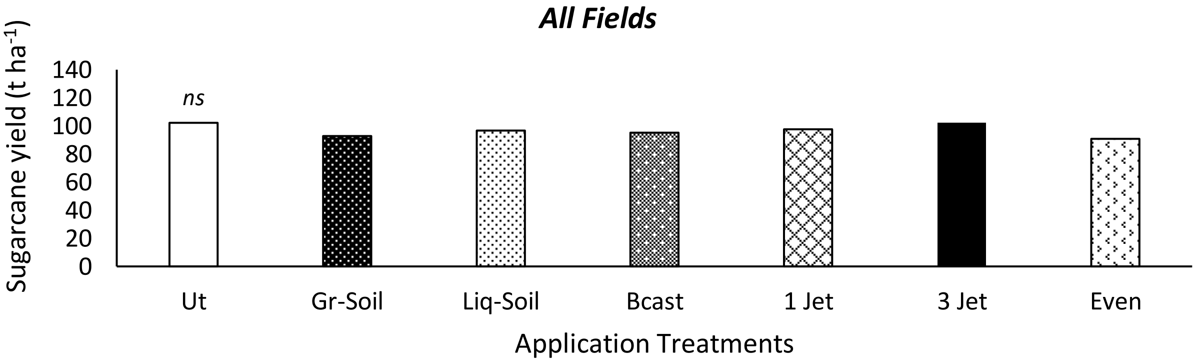


Figure 9. Sugarcane yield (t ha^{-1}) per treatment, considering pooled data of all fields. Bars with same letter are not significantly different at $p \leq 0.05$. *ns* – not significant; Ut-Untreated; Gr-Soil - Granular soil application; Liq-Soil - Liquid soil application; Bcast-Broadcast application; 1 Jet-One jet band spraying; 3 Jet-Three jet band spraying; Even-Even flat fan band spraying.

DISCUSSION

Treatment and Evaluation Period Effect on *S. levis* Count

Low mean values of insect count were observed across treatments and evaluation periods with values ranging from 0 to 1.6 *S. levis* insects trench^{-1}. Similar results of insect count values were also observed in other *S. levis* control studies. In one study, for example, insect values ranged from 0 to 2.2 insect trench^{-1} (Dinardo-Miranda et al., 2006) whereas in another study, the number of *S. levis* per trench varied from 0.1 to 1.8 insect trench^{-1} (Alencar, 2016). These low count values are consequence of a high proportion of zero values commonly observed in ecological count datasets (Sileshi, 2006), especially when studying soil pests. Excessive zeros and overdispersion can be a result of pest distribution variation, insect aggregation and habitat heterogeneity (Sileshi, 2006). However, even low insect numbers detected during soil sampling can represent a potential risk for yield losses. When converting the number of insects per trench to number of insects per hectare, for example, one single *S. levis* insect per trench indicates 13333 insects per hectare, considering only insects found underneath the soil and inside cane rhizomes in trenches dug over canes row (0.5 x 0.5 x 0.3 m) with row spacing of 1.5 m. In addition, because of *S. levis* potential damage, the control threshold adopted for this pest is the detection of one single insect per field (Dinardo-Miranda et al., 2008).

At 200 DAA, the number of *S. levis* found, especially larvae and pupae, was greater than at other evaluation periods. Evaluations at 200 DAA were conducted between June and July, which, according to several studies, is the period with most occurrence of *S. levis* larvae and pupae (Degaspari et al., 1987; Precetti and Arrigoni, 1990; Canassa, 2014; Izeppi et al. 2014). Thus, *S. levis* larvae-pupae management alternatives should be studied during this period of high infestation, including studies with sequential pesticide applications, higher product rates, different insecticides and other IPM alternatives. Ferreira (2022a) observed, for example, significant *S. levis* adult control by doubling the recommended insecticide dosage.

Despite low *S. levis* insect counts, no treatment was effective to significantly reduce the number of *S. levis* in comparison to the untreated check and no treatments significantly reduced *S. levis* initial infestation of 0 DAA. In field 3, however, the broadcast application (T4), the one-jet (T5) and the three-jet band spraying (T6) presented overall higher insect counts than the other treatments, possibly because of insect distribution and field variability with greater insect pressure in those treated plots. In addition, low insecticide efficacy has been reported by several authors (Dinardo-Miranda et al., 2006; Tavares, 2006; Alencar, 2016). Among some of the variables affecting *S. levis* control, the insect's behavior and habitat underneath the soil surface (Precetti and Arrigoni, 1990; Ferreira, 2022b) are one of the main reasons insecticides are not properly deposited and absorbed by insects. Although not significant, both solid and liquid soil applications (T2 and T3) had consistently lower insect count values than the untreated check until 200 DAA while the solid application provided lower *S. levis* counts at all periods including thirty days after sugarcane harvesting (Figure 4). When considering the corrected efficacy formula by Henderson and Tilton (1955) in Eq. (1), it was also possible to notice the potential of both soil applications with the liquid application presenting higher efficacy for a shorter period while the solid application promoted longer efficacy over time. In one study evaluating the effect of liquid and solid insecticide applications on *S. levis* control with the same insecticide used in the present study (lambda-cyhalothrin + thiamethoxam), the author also observed the potential of solid applications providing 52.4% *S. levis* control in relation to the liquid application with 40.6% control (Ferreira, 2022a). In the same study, it was also noticed a longer residual effect of solid applications on *S. levis* adult control than liquid applications (Ferreira, 2022a). Considering that no adequate insecticide GR formulation for soil application and *S. levis* control is currently available, field results from the T2 treatment could possibly be improved if a proper and adequate granular formulation was used. Adequate granular formulations may include those with controlled release (CR) technologies providing satisfactory pest control levels with longer and controlled insecticide residual (Roy et al., 2014).

Based on the corrected efficacy values and lower *S. levis* counts over time, the treatments with soil applications using drilling discs for insecticide deposit in the soil and the sugarcane rhizome (T2 and T3) were, therefore, the best options to reduce some of *S. levis* infestation. Although only a few studies have been conducted to compare and evaluate different application methods on *S. levis* control, one experiment comparing liquid soil applications with drilling discs placed over the sugarcane row and aside the row showed greater control efficacy at 120 DAA with the drilling discs positioned over the cane row as in both T2 and T3 treatments from the present study (Dinardo-Miranda, 2006).

Treatment and Evaluation Period Effect on Sugarcane Injury

Sugarcane injury results demonstrated that, in general, no treatment was effective to reduce injury levels. In field 1, however, one exception was noticed with the one even nozzle band application (T7) providing lower injury levels than the untreated check. This result was possibly due to field variability and insect distribution considering that no similar effect was observed with this same treatment for insect count, no similar response was seen on injury level for fields 2 and 3 and this same treatment provided low yield also in field 1. Despite most of sugarcane mill units adopting the soil application of liquid insecticides (T3) and some adopting band spraying with one nozzle for drench applications (T5), the overall injury level was not influenced by any application treatment.

This general low efficacy of treatments to control sugarcane injury cause by *S. levis* has been also observed by different authors. In one study comparing *S. levis* control with fipronil and with thiamethoxam at two dosages, Evangelista et al. (2017) saw no differences among insecticides and the untreated control regarding sugarcane injury levels. In another study, assessing entomopathogenic nematodes mixed with thiamethoxam on *S. levis* control, authors also observed no differences of sugarcane injury among treatments (Leite et al., 2012). Even when mixing different insecticides such as a tank mixture of fipronil + thiodicarb + lambda-cyhalothrin and another mixture of thiodicarb + imidacloprid + lambda-cyhalothrin, no significant *S. levis* injury reductions were observed (Alencar, 2016). As previously discussed, it is hypothesized the *S. levis* poor control being primarily caused by the lack of insecticide deposit and absorption by insect as a result of its location underneath the soil surface (Precetti and Arrigoni, 1990;

Ferreira, 2022b). However, an increment on *S. levis* control has been noticed when increasing insecticide dosage in one study as the author stated that with greater product concentrations, more insecticide would be left available in soil, despite of pesticide degradation pathways, such as leaching, soil adsorption, microbiological and photodegradation (Ferreira, 2022a). Therefore, increasing the insecticide dosage may promote higher concentration in soil/rhizome with greater chances of targeting *S. levis*. Further field studies should be conducted to evaluate the dosage effect among other control alternatives for *S. levis* control.

Similar to most of *S. levis* insects being found between June and July (200 DAA) in all experimental fields (Figures 2 and 4), especially larvae and pupae, the injury levels were also mostly observed during the same evaluation period at 200 DAA (Figures 6 and 7). However, considerable injury levels were also seen during different evaluation periods other than at 200 DAA. At 30 DAH, for example, sugarcane injury levels were also significantly higher than other periods (Figure 7). These high injury levels at 200 DAA in June-July and at 30 DAH in September-October are mostly a result of *S. levis* larvae and pupae seasonal distribution as reported by several authors (Degaspari et al., 1987; Precetti and Arrigoni, 1990; Canassa, 2014; Izeppi et al. 2014). At 60 DAA, for example, insect and injury evaluations were conducted in January and February and was the period with less insects and injuries found in soil/plant samples because during these months most *S. levis* insects found are in the adult stage (Precetti and Arrigoni, 1990; Canassa, 2014).

Treatment Effect on Sugarcane Yield

As described before, no direct effect of application treatment over sugarcane yield was observed similar to the results of *S. levis* control and injury with no treatments effect as well. Although, in field 1, the band application with one even nozzle (T7) providing lower yield than the band applications with one and three jet nozzles (T5 and T6), the overall effect of application method was still not significant. As results of field variability, a greater pressure of other sugarcane pests that were possibly better controlled by the even nozzle treatment (T7) might explain greater yields in field 1 for this treatment although no specific evaluations of different cane pests were conducted. Nevertheless, overall results indicated low influence over yield are possibly result of no *S. levis* control as also observed in several studies evaluating the insect control and treatments effect on yield. It has been reported in one study, for instance, no differences of sugarcane yield and total sugar content among several insecticide treatments for *S. levis* control (Alencar, 2016). In another study, however, significant yield differences were seen when treating sugarcane with two specific treatments, one consisting of a tank mixture of carbofuran and bifenthrin and the other a mixture of carbofuran with fipronil (Dinardo-Miranda et al., 2006). The active ingredient carbofuran, however, has been banned in Brazil since 2017 (Friedrich et al., 2021) and current registered insecticides have not been fully studied and compared regarding its efficacy on *S. levis* and yield. New studies should consider different application approaches as an attempt to evaluate treatment effects on yield, including higher insecticide dosages as proposed by Ferreira (2022a), nocturnal applications targeting *S. levis* adults (Ferreira, 2022b), applications with entomopathogenic fungus and nematodes (Tavares, 2006; Canassa, 2014) among other IPM methods.

CONCLUSION

In general, just a few differences on the number of *S. levis* per soil sample, sugarcane injury levels and yield were seen among treatments. However, positive results were observed for soil treatments with solid and liquid application of insecticides using the ratoon drill applicator. The liquid application in soil presented high efficacy of *S. levis* insect control at shorter periods, up to 120 DAA, whereas solid insecticide applications in soil promoted some efficacy even 30 days after sugarcane harvesting. The adoption of specific granular formulations and adequate active ingredient concentration may improve the efficacy of solid applied insecticides for *S. levis* control. Moreover, the period with most incidence of *S. levis* insects in all fields was between June-July at 200 DAA.

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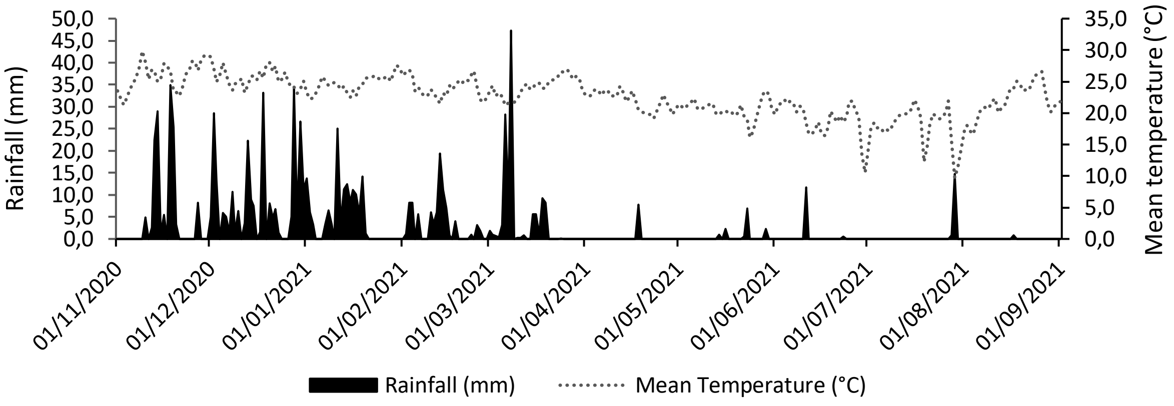
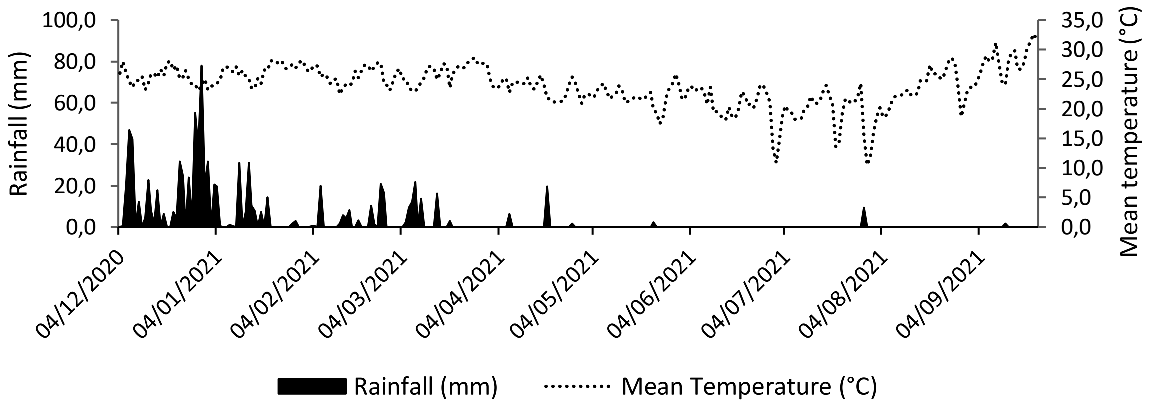
SUPPLEMENTARY MATERIAL

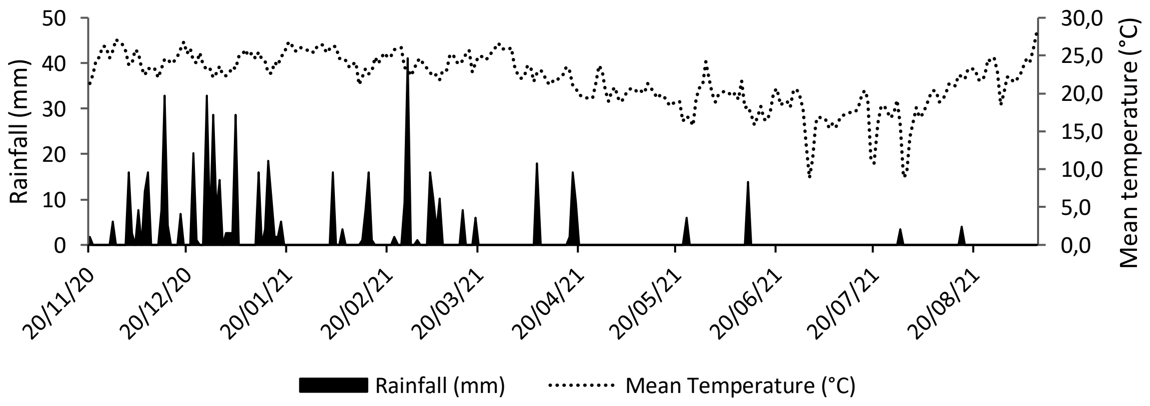
Field 1



Field 2



Field 3



Mean daily rainfall and temperature data during the experiment conduction in field 1, 2 and 3.

CHAPTER 5 - Solid and liquid insecticide applications in sugarcane planting for *Sphenophorus levis* control

ABSTRACT - The sugarcane billbug (*Sphenophorus levis*) is an important soil pest in sugarcane that causes significant yield loss. During sugarcane planting, infestation to new areas begins with infested seed cane billets carrying the insect and with the dispersion of insects from nearby infested areas. Although different insecticides are commonly applied at planting to prevent and control *S. levis*, through liquid application, satisfactory control levels have not been achieved. Solid application of insecticides, however, have proven to be effective for different pest and crops. Thus, the objective of the present study was to evaluate and compare *S. levis* control, cane injury level and plant yield with liquid and solid applications of insecticides at sugarcane planting. The experiment was conducted in 2020 and 2021 in a randomized complete block design with eleven treatments, four blocks and two replicates per block in three sugarcane fields. Treatments included an untreated control, solid and liquid applications of thiamethoxam, fipronil, thiamethoxam + lambda-cyhalothrin, imidacloprid and *Metarhizium anisopliae*. Insect count and sugarcane injury evaluations were conducted at 120 DAA, 360 DAA and 30 DAH and yield was assessed at 360 DAA. Both liquid and solid applications of insecticides presented low efficacy to control *S. levis*, to reduce sugarcane injury and to affect plant yield. However, a common trend across solid applied insecticides with fewer *S. levis* insects and greater yield than in liquid applied treatments was perceived. Additionally, *S. levis* insects and high injury levels were only detected at 360 DAA and 30 DAH. A positive correlation indicated that one *S. levis* insect per soil sample in the sugarcane row represented 12.4% sugarcane injury at 360 DAA. Based on these results, insecticide applications at planting were not very effective for *S. levis* control and frequent monitoring of insect and cane injury after planting can help determining the ideal application timing.

Keywords: Weevil, seed cane billets, granular, furrow, residual

INTRODUCTION

The sugarcane billbug [*Sphenophorus levis* Vaurie, 1978 (Coleoptera: Curculionidae)] is a soil insect that causes significant injuries in sugarcane (*Saccharum officinarum* L.) being considered one of the most important pests for this crop. Subterranean injuries are caused mostly by *S. levis* larvae developing and feeding inside of sugarcane rhizomes. Damaged plant crowns and rhizomes are characterized by tunnels with white/yellow frass with consequent poor root system development, leaf necrosis and plant death. Approximately, every 1% of injured plants represents yield loss of 1 t ha^{-1} (Casteliani et al., 2020). In fact, sugarcane yield losses from up to 30 t ha^{-1} have been previously reported (Precetti and Arrigoni, 1990).

Sphenophorus levis infestations were first described in 1977 in one specific sugarcane region, near Piracicaba, SP, Brazil, but nowadays infestations have been found in several regions including different sugarcane growing states (GO, MG, MS, MT, PR, SP) (Degaspari et al., 1987; Canassa, 2014). Despite the low dispersion of *S. levis* adults, up to 6.6 m per month and its limited flight capacity (Degaspari et al., 1987; Simi, 2014). *S. levis* insects are primarily dispersed during sugarcane planting in which seed cane billets are collected from an infested area, carrying the insect to areas without prior infestation. *S. levis* are attracted to seed cane billets through emitted plant volatiles from sugarcane fermentation including ethyl acetate and ethanol compounds (Girón-Pérez et al., 2009). In one study evaluating the infestation of *S. levis* in transferring areas of harvested seed cane, 12% greater infestation levels were observed in areas close to seed cane billet transferring, showing the potential of new infestations not only in newly planted areas but also in areas where seed cane billets were transported and handled (Rosa, 2022). In another study, researchers evaluated *S. levis* infestation in one common cane planting system known as MEIOSI (Method of Inter-Rows Occurring Simultaneously) consisting of seed cane rows planted a few meters apart with one crop cultivated between seed cane rows or without any crop (bare fallow) (Recieri et al., 2018). In that study, authors observed greater *S. levis* infestation, 154% more, in the MEIOSI cane planting with soybean [*Glycine max* (L.) Merr.] cultivated between seed cane rows than the one without any crop between seed cane rows (Recieri et al., 2018). In addition to infested seed cane billets used for planting, nearby fields with insect presence may also contribute to populate areas without *S. levis*.

Because of its great damage potential, sugarcane farmers have been using insecticides at planting aiming to protect seed cane from *S. levis* and other soil-pests. Currently, nine insecticide active ingredients are commercially available for application at sugarcane planting (Agrofit, 2022). Low insecticide efficacy, however, has been reported by several authors, especially in ratoon sugarcane. For example, when comparing insect control and injury level on cane-ratoon applications of imidacloprid, thiodicarb, fipronil and lambda-cyhalothrin alone and mixed with up to two insecticides, no treatment was effective to control *S. levis* or to reduce plant damage (Alencar, 2016). Similarly, in one study assessing insecticide efficacy at planting for *S. levis* control, authors noticed no significant differences between treatments, including planting-furrow applications of carbofuran, fipronil, imidacloprid, thiamethoxam and bifenthrin (Dinardo-Miranda and Fracasso, 2010).

Although all registered insecticides for *S. levis* control in sugarcane planting are liquid applied, using some water volume, the potential of solid insecticide application without water has been reported for different soil pests and crops. Studying the efficacy of granular insecticides for early shoot borer (*Chilo infuscatellus*) in sugarcane, Bhawar et al. (2015) observed better control for water dispersible granules (WG) formulation of imidacloprid + fipronil than granular (GR) formulations of chlorantraniliprole, fipronil, cartap hydrochloride, chlorpyriphos, carbofuran and phorate. In addition, solid applications of imidacloprid with controlled release (CR) formulation technology provided control of different canegrub species for up to 4 years (Ward, 2016). Solid application of entomopathogenic fungus have also proven to be effective. When assessing the effectiveness of *Metarhizium anisopliae* applied with different formulations, including granular, oil and liquid ones, greater control of fruit fly larvae after 668 of soil inoculation was observed when using the granular formulation (Ekesi et al., 2005).

Despite several reports and studies showing the poor control levels of *S. levis* with liquid applied insecticides at sugarcane planting, no research has been conducted, up to date, to assess the potential of solid insecticide applications for *S. levis* control. Therefore, the objective of the present study was to evaluate and compare liquid and solid application of insecticides at sugarcane planting for *S. levis* insect control, plant injury, to examine the relationship between insect number and injury levels, and assess treatment effects on sugarcane yield.

MATERIALS AND METHODS

The study was conducted in three cane-planting field sites with previous *S. levis* infestation history and potential for new infestation. Insecticide applications at planting were conducted in 2020 and evaluations in 2020 and 2021. Field experiments were conducted in three sugarcane mill units located in Matão, SP, Brazil (Field 1), in Pradópolis, SP, Brazil (Field 2) and Jaboticabal, SP, Brazil (Field 3). Information of each field site is available in Table 1. Previous crop management operations in past ratoon cane and before sugarcane planting included conventional soil tillage in field 1, mechanical elimination of ratoon cane and conventional soil tillage in field 2 and conventional soil tillage followed by *Crotalaria spectabilis* Roth cultivation in field 3. Information of each field site is available in Table 1.

The experiment was conducted in a randomized complete block design with eleven treatments, four blocks and two replicates per block. Each row was considered as one replicate and each treatment was composed of two rows with length of 50 m and row spacing of 1.5 m. Five active ingredients and one entomopathogenic fungus were used in sugarcane planting operations for *S. levis* control with liquid and solid applications.

Treatments were applied in planting furrows directly over seed cane billets (Figure 1a) except the untreated check (T1). Commercially available insecticides with granular formulations (WG and GR) were selected to investigate solid application efficacy and compare it with the same active ingredients applied through liquid application. Each insecticide physicochemical property is available in Table 2.

Table 1. Field sites information including sugarcane variety, planting date, soil specification and soil analysis.

	Field Sites		
	Field 1	Field 2	Field 3
Coordinates	21° 35.1396' S 48° 19.5282' W	21° 28.5568' S 48° 10.5458' W	21° 12.9794' S 48° 21.1087' W
Sugarcane variety	CV7870	CTC 9001	RB975242
Planting date	14/03/2020	07/03/2020	20/04/2020
Soil type	Ultisol	Oxisol	Oxisol
Texture	Sandy-loam	Clayey	Clayey
Organic matter (g dm^{-3})	13	18	23
CEC ($mmol_c$ dm^{-3})	59	85	80
Base saturation (%)	51	66	63
pH ($CaCl_2$)	4.8	5.3	5.3

Table 2. Physicochemical properties of the active ingredient and entomopathogenic fungus used.

	Insecticide				
Physicochemical Property	thiamethoxam	fipronil	lambda-cyhalothrin	imidacloprid	*M. anisopliae*
solubility (mg L^{-1})	4100[a]	3.78[a]	0.005[a]	610[a]	NA[3]
adsorption (Koc)	56.2[a]	825 [b]	283707[a]	249 [c]	NA
volatility (mm Hg)	4.9×10^{-11} [b]	2.8×10^{-9} [b]	1.5×10^{-9} [c]	3×10^{-12} [c]	NA
photolysis[1] (days)	2.7[a]	0.33[a]	40[a]	0.2[a]	0.04 [d]
soil DT50-lab[2] (days)	50[a]	142[a]	175[a]	191[a]	50 [e]
soil DT50-field[2] (days)	39[a]	65[a]	26.9[a]	174[a]	NA

[1]aqueous photolysis; [2]degration time for 50% of a compound; [3]not available.
[a]Lewis et al. (2016); [b]Kim et al. (2019); [c]National Pesticide Informational Center (2019); [d]Zimmermann (1982); [e]Li and Holdom (1993).

A soil covering equipment was used to apply liquid and solid insecticide treatments and to cover seed cane billets with soil (Figure 1b). Solid application treatments (T2, T3, T4, T5 and T6) were applied with two solid applicator equipment (FRS Equipamentos, Limeira, SP, Brazil) with a fluted rotor, attached at the end of a soil covering equipment, one at each sugarcane row (Figure 1b). Feed hoses with 5 cm diameter linking the granular hopper to planting furrows were placed in front of soil covering discs in which insecticide was delivered through gravity once the equipment's electric motor was activated by an on/off switch control. The electric engine of the solid applicator equipment was powered by the tractor battery. The application speed for solid treatments was 2.77 m s^{-1}. Liquid application treatments (T7, T8, T9, T10 and T11) were sprayed with the AI9505EVS nozzle, calibrated at 309 kPa pressure, with application speed of 2.77 m s^{-1} and spray volume of 200 L ha^{-1}. Solid application equipment was calibrated for each insecticide dosage using a precision balance (Kkmoon, Shenzhen TOMTOP Technology Co, Shenzhen, China). Each treatment with the insecticide used, trade name, dose and active ingredient rate is available on Table 3.

Figure 1. Sugarcane planting rows with seed cane billets exposed for insecticide treatments (a); soil covering equipment used for both liquid and solid applications (b); field evaluations (c); *S. levis* larvae and cane injury symptoms (d).

Table 3. Application method treatments including insecticides and trade names, dose, and active ingredient application rate.

Application	Treatment	Trade name	Dose kg ha^{-1}	a.i. Rate g ha^{-1}
-------	T1 – untreated	---------------------	--------------	----------------
solid	T2 – thiamethoxam	[1]Actara® 250 WG	1.41	352.5
	T3 – fipronil	[2]Regent® 20 GR	22.5	450
	T4 – lambda-cyhalothrin + thiamethoxam	[3]Kaiso Sorbie BR GR + Actara® 250 WG	1.104 + 1.41	265 + 352.5
	T5 – imidacloprid	[4]Warrant® 700 WG	1.5	1200
	T6 – *M. anisopliae* IBCB 425	[5]Metarriz® GR	10	1000
liquid	T7 – thiamethoxam	Actara® 250 WG	1.41	352.5
	T8 – fipronil	[2]Regent® 800 WG	0.562	450
	T9 – lambda-cyhalothrin + thiamethoxam	[1]Engeo Pleno™ S	2.5 L ha^{-1}	265 + 352.5
	T10 – imidacloprid	Warrant® 700 WG	1.5	1200
	T11 – *M. anisopliae* IBCB 425	[5]Metarriz® Plus WP	1.11	1000

[1]Syngenta, Basel, Switzerland; [2]BASF SE., Ludwigshafen, Germany; [3]Nufarm Limited, Laverton North, VIC, Australia., [4]FMC Química do Brasil Ltda, SP, Brazil; [5]Biocontrol Sistema de Controle Biológico Ltda, SP, Brazil;

Soil samples were collected with four replications per field site and were analyzed by the Soil Fertility Laboratory at UNESP following methodology of Raij et al. (2001) for organic matter (OM) content, cation exchange capacity (CEC), base saturation and soil pH (Table 1). Weather data of each field was obtained for the experiment duration from sugarcane mill units including rainfall, relative humidity and average temperature data. Temperature and relative humidity were measured throughout treatment application using a digital thermo hygrometer Jprolab (Jprolab, São José dos Pinhais, PR, Brazil). Wind speed was measured with an anemometer Kestrel 3000 (Kestrel Instruments, Boothwyn, PA, USA). Soil temperature was measured with a digital thermometer TE-400 (Instrutherm, São Paulo, SP, Brazil) and soil humidity was measured with ph-2500 pH/soil humidity meter (Instrutherm, São Paulo, SP, Brazil). Meteorological and soil conditions are available in Table 4.

Table 4. Information of treatment application including date, average meteorological and soil conditions and first rainfall event after planting application.

	Field Sites		
	Field 1	Field 2	Field 3
Application/planting date	14/03/2020	07/03/2020	20/04/2020
Average temperature (°C)	27.84	24.2	31.6
Average relative humidity (%)	55.3	57.9	44.0
Average wind speed (m s^{-1})	5.0	4.3	4.3
Average soil humidity (%)	54	55	47
Average soil temperature (°C)	22.6	19.0	27.0
First rain after application/rain amount	3 DAA[1] / 3 mm	9 DAA / 10.6 mm	33 DAA / 20 mm

[1]DAA – days after application.

Evaluations of *S. levis* insect count and rhizome injury were conducted at 120 and 360 days after application (DAA) and 30 days after harvest (DAH). Insect count assessment consisted of counting the number of *S. levis* larvae, pupae, and adult discovered in each replication. Cane injury evaluations were performed by counting the total number of rhizomes and the number of rhizomes injured by *S. levis* per replication. Both insect count and plant damage evaluations consisted of digging a 0.50 x 0.50 m x 0.30 m trench and pulling out cane rhizomes, cutting subterranean shoots and steams and assessing insect presence in soil/plant and cane injury symptoms (Figure 1c and 1d) in the center of each row (replication). Sugarcane stand evaluations were conducted by counting plants in 10 m of each experimental unit at 30, 60, 120 and 360 DAA (Figure 1c).

Yield was estimated at 360 DAA by counting plants in 10 m of each replication, manually harvesting 10 cane stalks and weighting harvested stalks with a portable electronic scale BM-A06 (Guangzhou Wei Heng Electronics Co., Guangzhou, China) (Adapted from Arizono et al., 1998; Landell et al., 1999). Weather data including rainfall, humidity and temperature during the study period was obtained for each field and is available in supplementary material section.

Data analysis

Treatment and evaluation period effects were analyzed for individual and pooled field site data to observe specific and general effects across field sites. Descriptive analysis and model fitness were conducted for insect count (all insect development stages), sugarcane injury and yield data in R version 1.4.1717 software (RstudioTeam, 2022). Prior to model selection, different model error distributions and link functions were tested and adjusted to correct for overdispersion and assess goodness of fit. Model's performances were compared by half-normal plots with simulation envelopes using the hnp package in R software (Moral et al., 2017) and based on Akaike information criterion (AIC) and residual deviance values to select the best model.

Insect count data was treated as dependent variable in a negative binomial generalized linear model of a two-way interaction of independent variables (treatment x evaluation period) using the MASS package (Venables and Ripley, 2002). Cane injury data was treated as dependent variable in zero-inflated negative binomial models and in general linear models with binomial distribution of a two-way interaction of independent variables (treatment x evaluation period). The zero-inflated model was used to compensate for excessive zeros and overdispersion using the pscl package (Jackman, 2020) and zeroinfl function (Zeileis et al., 2008). Sugarcane yield data was treated as dependent variable in a gaussian generalized linear model with treatment as the independent variable.

After model selection, results of *S. levis* count, cane injury and yield were submitted to an analysis of deviance (type II Wald chi-square tests) considering two-factor interaction and main effect, respectively. Significant interactions and main effects were analyzed using the emmeans package with Sidak's test at $p < 0.05$ (Lenth, 2019) to determine significant differences between treatments. Mean values of insect count (insect per trench) were converted to number of insects per hectare considering a trench square area of 0.5 m^2 and row spacing between plants of 1.5 m.

To assess the relationship between sugarcane injury levels and number of insects at each evaluation period, correlations were conducted in R software with the Spearman's rank correlation method for nonparametric data. Field results were pooled to observe the overall relationship across field sites. If treatment effect was not significant, results were also pooled to observe the overall relation regardless of treatment effect. When moderate to strong correlations ($r > 0.6$) were detected, a zero-inflated negative binomial model, considering cane injury as dependent variable and insect count as the independent variable, was adopted to account for overdispersion and excessive number of zeros using the pscl package (Jackman, 2020) and zeroinfl function (Zeileis et al., 2008). Predicted cane injury levels were estimated using the predict function in R considering the adopted zero-inflated negative binomial model.

RESULTS AND DISCUSSION

Treatment and Evaluation Period Effect on *S. levis* Population

The interaction between insecticide treatments and evaluation period was not significant to affect the number of *S. levis* insects found in field 1 ($p = 0.9715$), field 2 ($p = 0.9128$), field 3 ($p = 0.5172$) and neither when data of all fields was pooled ($p = 0.4599$). Therefore, no significant differences on the number of *S. levis* insects per soil sample were observed between treatments during the same period or during different periods. No differences between insecticide treatments at planting have also been reported in one study comparing *S. levis* control with carbofuran, fipronil, imidacloprid and thiamethoxam (Dinardo-Miranda and Fracasso, 2010). In another study, applications of thiamethoxam and two entomopathogenic nematodes, *Steinernema* sp. And *Heterorhabditis indica*, were also not effective for *S. levis* control (Tavares, 2006). The low efficacy of applied insecticides during sugarcane planting may be a result of short insecticide residual and inadequate product dose as Ferreira (2022a) observed in one experiment evaluating *S. levis* adult control with liquid and solid applied insecticides. In addition, during the first few months after planting, sugarcane plants do not have a robust and developed root system/rhizome not being as attractive and suitable to *S. levis* insects as older and more developed plants can be.

However, when variables were analyzed independently, both treatment and evaluation period affected the mean number of *S. levis* insects found in at least one field. In field 2, for example, treatments were significant to affect the population of *S. levis* (p = 0.0492) where both liquid and solid applied imidacloprid treatments had fewer insects per trench than the liquid application of thiamethoxam (Figure 2) despite not being different from the untreated check. Thiamethoxam is a very soluble insecticide (4100 mg L^{-1}) with low adsorption potential to organic matter (Koc of 56.2) and soil dissipation half-life in field (DT_{50}) of 39 days while imidacloprid has lower solubility (610 mg L^{-1}), higher adsorption potential (Koc of 249) and field DT_{50} of 174 days (Table 2). In addition, field 2 received a considerable amount of rain (62 mm) within 30 days after application (supplementary material section). Therefore, considering both physicochemical characteristics and rainfall data, it is expected the thiamethoxam liquid applied was less available for insect uptake due to more insecticide leaching and dissolution in comparison to imidacloprid treatments. For instance, the high mobility of thiamethoxam in soil has also been reported by Mörtl et al. (2016).

When considering the pooled data of all fields, despite not significant (p = 0.0956), it is possible to notice a similar trend for all insecticides with solid applications presenting fewer insects than liquid applications, except the *M. anisopliae* treatment (Figure 2). For example, the solid applied thiamethoxam had, on average, 0.055 insect $trench^{-1}$, or 733.3 insect ha^{-1} considering only insects found underneath the soil and inside cane rhizomes in trenches (0.5 x 0.5 x 0.3 m) dug over canes row with row spacing of 1.5 m, while the liquid applied thiamethoxam presented an average of 0.087 insect $trench^{-1}$ (1160 insect ha^{-1}). Solid applications of insecticides may extend the active ingredient dissolution and release in soil, improving pest control over time. Ferreira (2022b) observed similar effects when comparing application methods for *S. levis* control in ratoon sugarcane treatments, where the solid insecticide application provided low insect counts even one year after application. Moreover, some of the adopted insecticides were not composed of proper solid formulations for direct soil application. Thiamethoxam and imidacloprid treatments, for example, were solid applied with water dispersible granule (WG) formulations.

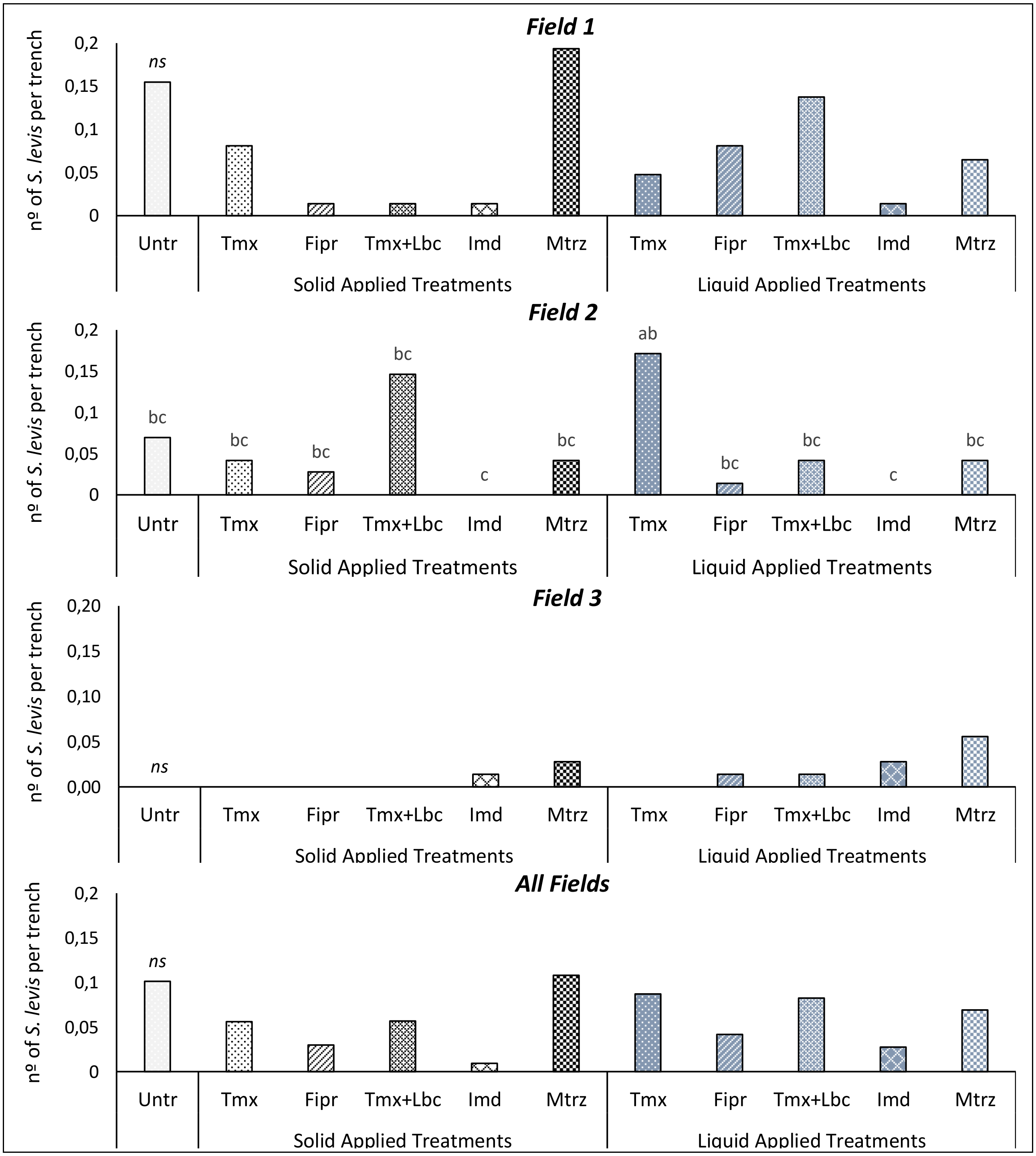


Figure 2. Number of *S. levis* insects in each field and in all fields per treatment regardless of evaluation period. Bars with same letter are not significantly different at $p \leq 0.05$. *ns* – not significant; Untr-untreated; Tmx-thiamethoxam; Fipr-fipronil; Tmx+Lbc-thiamethoxam+lambda-cyhalothrin; Imd-imidacloprid; Mtrz-*Metharizium anisopliae*.

If specific solid soil formulations were used, like formulations with controlled release (CR) technology, better and prolonged *S. levis* control could possibly be achieved as Ward (2016) observed for different canegrub species in sugarcane, including 3-year control of greyback (*Dermolepida albohirtum*), negatoria (*Lepidiota negatoria*), consobrina (*Lepidiota consobrina*) and Bundaberg canegrubs (*Alepida crinita*) and 4-year control of Childers (*Antitrogus parvulus*) and southern (*Antitrogus consanguineus*) canegrubs.

The period of evaluation, when analyzed individually, was also significant to affect the number of *S. levis* found in field 1, field 2 and in the pooled data of all fields ($p < 0.0001$). At 120 DAA no *S. levis* was found (0 insect trench^{-1}) in any field (Figure 3) indicating that during this period (July-August) insects were either not present, not attracted to young developing plants or that meteorological conditions were not favorable for insect dispersion and infestation.

In field 1, however, a few insects were found during the second evaluation period, at 360 DAA (February, 2021), but it was at 30 DAH (August, 2021) that a significant increment of *S. levis* population was noticed (Figure 3). On the other hand, in field 2, the evaluation period with most insects found per soil sample was at 360 DAA (March, 2021) followed by a significant decrease on the number of insects at 30 DAH (September, 2021) as in Figure 3. The earlier increase on *S. levis* population in field 2, compared to field 1, can be accounted to the sugarcane planting system adopted in field 2. The system known as MEIOSI (Method of Inter-Rows Occurring Simultaneously) consisted of seed cane rows planted 13.5 m apart. Ten months later, these seed cane rows were harvested and used to plant the middle-rows of the same area for the actual planting experiment.

Thus, in this planting system, recent planted rows and ratoon cane rows are present in the same area simultaneously, and the ratoon cane rows are, therefore, more prone to *S. levis* presence but also can shelter insects that can damage adjacent planted sugarcane. In fact, evaluating the incidence of *S. levis* under MEIOSI planting systems, some authors observed greater *S. levis* population when soybean plants were cultivated between seed cane rows in comparison with bare fallow between seed cane rows (Recieri et al., 2018).

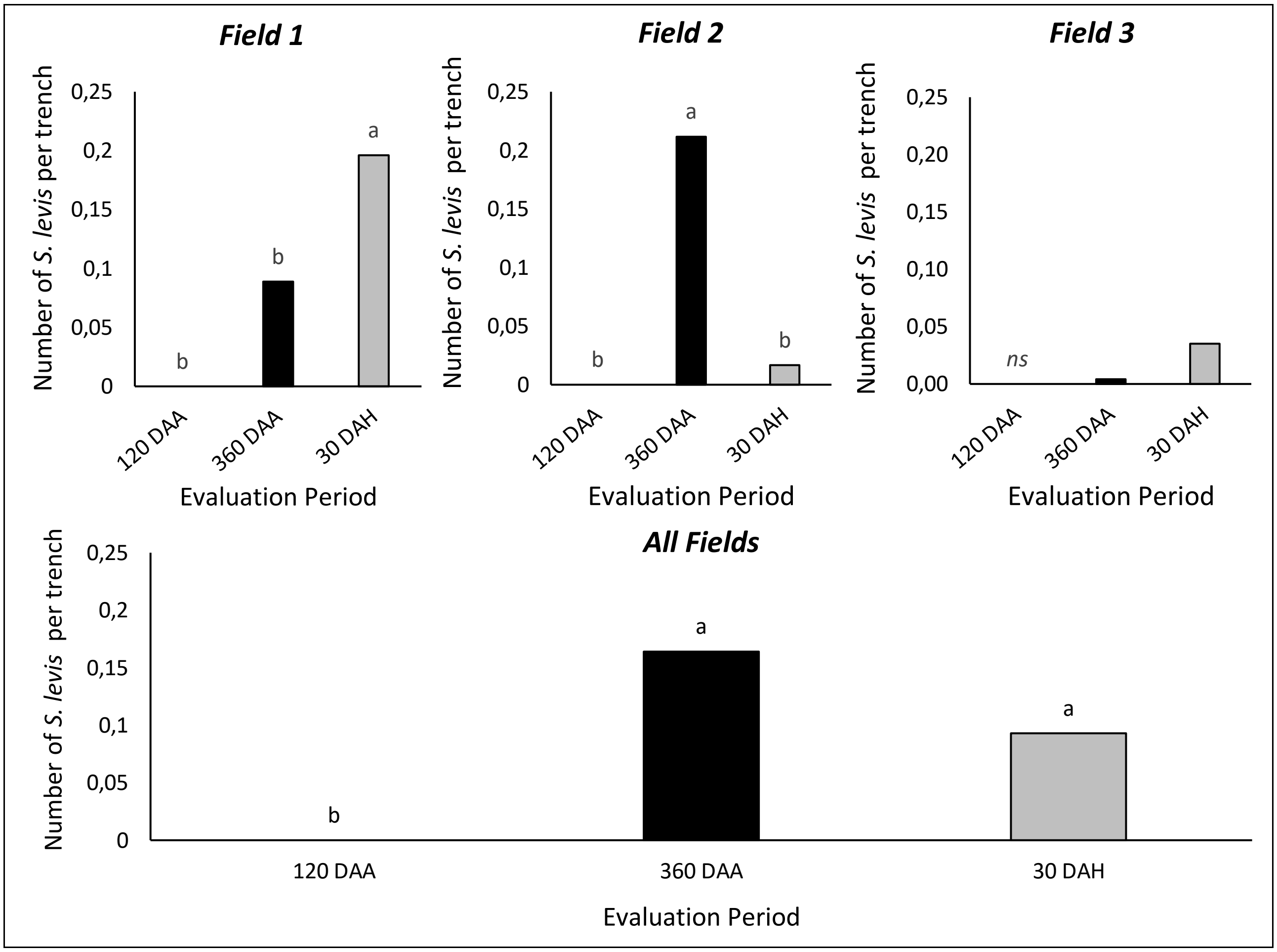


Figure 3. Number of *S. levis* insects per evaluation period regardless of treatment, considering field 1 (left), field 2 (center), field 3 (right) and all fields (bottom). Bars with same letter are not significantly different at $p \leq 0.05$; *ns* – not significant.

According to present results, it is possible to conclude that plants with 120 days and younger were less prone to *S. levis* presence while in older plants it is more likely to find the insect. Lower tillering and root system development at young sugarcane stages may not attract *S. levis* insects as much as developed plants. At 6 months after planting, for example, researchers found up to 1.8 t ha^{-1} of sugarcane roots in a soil profile of 70 cm while at 12 months after planting, up to 8 t ha^{-1} was observed (Inforzato and Alvarez, 1957). In addition, soil tillage and bare fallow prior to planting contributed to *S. levis* reduction as also reported by Pizzano et al. (1987) and Dinardo-Miranda and Fracasso (2010). *S. levis* is known to present a slow dispersion, up to 6.6 m month^{-1}, mostly because of its gregarious behavior and low flight capacity (Degaspari et al., 1987; Rosa, 2022). Thus, it is hypothesized that after sugarcane planting, the remaining *S. levis* population in field, especially adults, despite its slow dispersion, are relocated to nearby areas offering temporarily sheltering, and once optimum host conditions are reestablished in the planted field (developed cane roots and plants) *S. levis* insects may return to reestablish its population. In addition, studies evaluating the low dispersion of *S. levis* were conducted under established ratoon sugarcane while its potential of dispersion during soil preparation and planting has not been investigated yet. Therefore, results indicated that areas with previous *S. levis* infestation may require some time to increase again the insect's population after soil tillering and planting, as it has been observed at 360 DAA in the present study.

Treatment and Evaluation Period Effect on Sugarcane Injury

Treatment and evaluation period did not interact to significantly affect the level of sugarcane injury caused by *S. levis* insects in field 1 (p = 0.4079), field 2 (p = 0.6356), field 3 (p = 0.9529) and when data of all fields was pooled (p = 0.1351). Thus, no differences on sugarcane injury were detected between treatments during the same period or during different periods. Similar results have been observed in one study evaluating the effect of different liquid applied insecticides at sugarcane planting on plant injury in which no differences among treatments and between the untreated control were noticed (Dinardo-Miranda and Fracasso, 2010). As previously discussed for the *S. levis* population results, the low efficacy of control and low reduction of injury levels might be a consequence of several factors, including low insecticide residual as reported by Ferreira (2022a) assessing thiamethoxam and imidacloprid liquid and solid applications. In addition, the slow development of sugarcane after planting, with reduced root and aerial biomass at earlier stages may have delayed the attraction of *S. levis* in comparison to older sugarcane plants. Besides, the physical effects of soil tillage and bare fallow can significantly reduce *S. levis* populations, especially shortly after planting as previously discussed (Pizzano et al., 1987; Dinardo-Miranda and Fracasso, 2010).

The independent effect of treatment, however, was significant to influence the level of sugarcane injury in field 2 (*p* = 0.0069). Despite significant differences of injury level, the only differences observed were between the liquid applied imidacloprid, with 3.3% injury, and *M. anisopliae*, with 11.9% injury (Figure 4), although no differences of insect number per trench were seen for these same treatments (Figure 2). As also stated in another planting study of insecticide efficacy for *S. levis* control, the sampling method of either insect population or injury level may not have been precise enough to demonstrate the real effect on injury level (Dinardo-Mirada and Fracasso, 2010). Great variability of *S. levis* insect count results have been reported by several authors with insect count results ranging from 0 to 2.2 insect trench^{-1} (Dinardo-Miranda, 2006; Alencar, 2016; Ferreira, 2022b) as a consequence of insect distribution variation, aggregation and habitat heterogeneity (Sileshi, 2006). However, in despite of potential field variability, the overall low efficacy of treatments was in accordance with previous literature results (Tavares, 2006; Dinardo-Miranda and Fracasso, 2010; Alencar, 2016; Ferreira, 2022b, Ferreira, 2022c). In fact, the observed low efficacy of *M. anisopliae* for *S. levis* control and consequent low injury reduction has also been demonstrated by different authors (Delfanti, 2012; Simi, 2014, Santos et al., 2017).

When considering the pooled results of all fields, no differences were detected (*p* = 0.2706). Differently than the results of *S. levis* count number for all fields (Figure 2), with an overall lower insect population in solid applied insecticide treatments, the results of sugarcane injury levels for all fields (Figure 4) did not show the same trend regarding solid and liquid applications. The solid application of thiamethoxam, for example, provided a mean injury level of 7.1% while the liquid application of the same insecticide provided 11.4% of injury level (Figure 4). The imidacloprid solid applied, however, had an injury level of 16.8% whereas its liquid application had an injury level of 7% (Figure 4). Further studies to better examine the benefits of solid and liquid insecticide applications should include more active ingredients with adequate formulations for solid soil application. For example, when a granular CR formulation of imidacloprid was used in sugarcane trials, different canegrub species were controlled from 2 to 4 years (Ward, 2016).

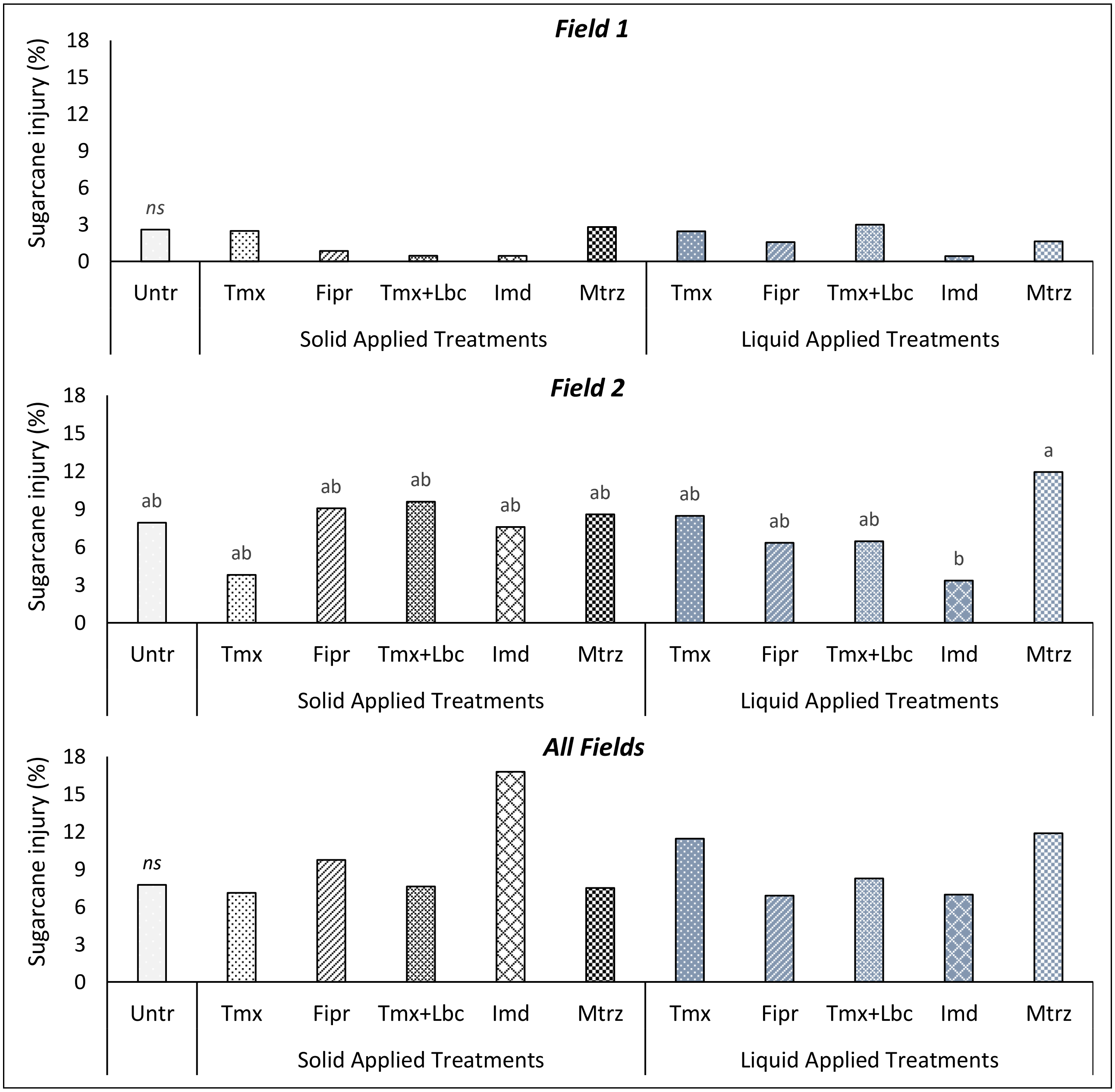


Figure 4. Sugarcane injury level (%) in field 1, field 2 and in all fields per treatment regardless of evaluation period. Bars with same letter are not significantly different at $p \leq 0.05$. *ns* – not significant; Untr-untreated; Tmx-thiamethoxam; Fipr-fipronil; Tmx+Lbc-thiamethoxam+lambda-cyhalothrin; Imd-imidacloprid; Mtrz-*Metharizium anisopliae*.

Furthermore, future research should also examine different insecticide dosages. *S. levis* adult mortality, for example, was enhanced from 58.8% to 76.7% when doubling the dosage of thiamethoxam and lambda-cyhalothrin (Ferreira, 2022a).

In addition to the treatment effect, the evaluation period also had an influence on sugarcane injury level. In field 1, for example, the level of injured sugarcane at 30 DAH (August, 2021) was significantly higher than at previous periods ($p < 0.0001$) as in Figure 5. Although injury levels in field 1 were not remarkably high, up to 3.2%, it is important to understand and detect at initial stages when *S. levis* starts to cause significant damage for proper pest management. In field 2, injury level was also affected by the evaluation period ($p = 0.002601$) in which a significant infestation increase was noticed from 120 DAA (July, 2020) with 0.5% injury level, to 360 DAA (March, 2021) with 7% injury level to an even higher increment at 30 DAH (September, 2021) with 15% injury level (Figure 5).

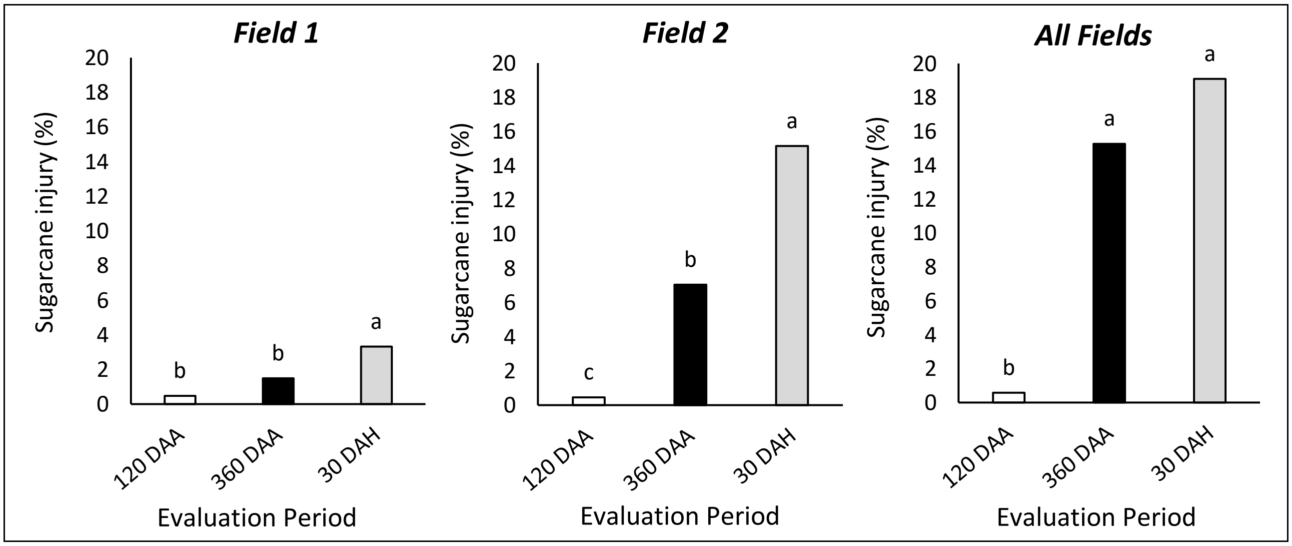


Figure 5. Sugarcane injury level (%) per evaluation period regardless of treatment, considering field 1 (left), field 2 (center) and all fields (bottom). Bars with same letter are not significantly different at $p \leq 0.05$.

Although few insects were found at 30 DAH in field 2 (Figure 3), sugarcane injury level increment could have been resulted from insect presence not evaluated between 360 DAA and 30 DAH (March and September, 2021). Several authors have described frequent occurrence of *S. levis* larvae and pupae during this interval between evaluations, especially during the months of June, July and August (Degaspari et al., 1987; Precetti and Arrigoni, 1990; Canassa, 2014; Izeppi et al. 2014). Finally, when considering the pooled data of all fields, an overall increment of injury levels (*p* < 0.0001) at both 360 DAA and 30 DAH was noticed (Figure 5) as previously discussed for the other experimental fields.

As observed in both *S. levis* population and cane injury results, insects and plant damage were significantly detected only at 360 DAA and 30 DAH. Based on these results and considering that no insecticide control was achieved at planting, it is suggested not to use insecticides aiming *S. levis* control at planting, unless insects are detected early prior to planting or during planting in seed cane billets. Instead, it is recommended frequent *S. levis* monitoring after planting, especially after 3 months, and once *S. levis* insects and plant injury are detected, it is recommended insecticide applications. In one study evaluating the application timing of different insecticides on sugarcane borer [*Diatraea saccharalis* (Lepidoptera: Crambidae)], authors observed reduced levels of bored internodes and adult emergence holes per stalk when applications were conducted shortly after insects were present in comparison with earlier applications without insects (Wilson et al., 2022). However, if proper solid formulations of insecticides are developed, potential prolonged insecticide activity may be achieved as observed by Roy et al., (2014) and Ward (2016) and could extend *S. levis* control even with insecticide applications at planting.

Prediction of *S. levis* sugarcane injury based on insect population

Because no *S. levis* insects (larvae, pupae, adults) were observed at 120 DAA in any field, no relationship between insect population and injury level could be determined. On the other hand, at 360 DAA, a positive moderate correlation (r = 0.64) was observed between the variables of sugarcane injury and number of *S. levis* and a significant influence of insect number (*p* < 0.0001) on the injury level of the same period was detected. An average number 0.5 of *S. levis* insects per trench, or 6,666 insects ha^{-1} considering only insects found underneath the soil and inside cane rhizomes in trenches (0.5 x 0.5 x 0.3 m) dug over canes row with spacing between rows of 1.5 m, was estimated to represent 5.7% of sugarcane injury at 360 days after planting (Figure 6) while a mean number of 1 insect per trench (13,333 insects ha^{-1}) represented 12.4% injury level (Figure 6). The greater the number of insects, the greater the injury level. It has been reported, for instance, that 1% injury level accounted for 1 t ha^{-1} yield loss (Casteliani et al. 2020). Therefore, considering this relation, an average of 0.5 insect trench^{-1} found, which represented an injury level of 5.7%, may cause up to 5.7 t ha^{-1} yield loss. Because of its great damage potential, the control threshold for *S. levis* in sugarcane has been the detection of 1 insect regardless of the number of soil sample or the detection of 5% injury level (Dinardo-Miranda et al., 2008; Dinardo-Miranda, 2014). Similar results were also noticed showing this relationship of *S. levis* insects and injury level in a 4-month ratoon sugarcane field with a mean number of 1 insect trench^{-1} representing up to 17.9% of injury level (Ferreira, 2022c). This greater effect in ratoon cane fields (1 insect trench^{-1} ≈ 17.9% cane injury) in comparison with current seed cane results (1 insect trench^{-1} ≈ 12.4% cane injury) is also in accordance with another study where authors observed greater infestation and injury levels in ratoon cane than in seed cane fields (Dinardo-Miranda and Fracasso, 2010). During the evaluation period at 30 DAH, on the other hand, a week positive correlation (r = 0.40) was detected between sugarcane injury and *S. levis* population, and, therefore, no relationship was considered for further analysis. Similarly, in another study, no strong correlations between the number of insects found and injury levels were noticed in ratoon sugarcane during some periods, indicating the injury level of sugarcane plants may not, necessarily, be directly associated to the number of insects found during pest monitoring (Ferreira, 2022c). Depending on the evaluation period sugarcane injury may not be linked to insect count because some *S. levis* might not be detected at the time of pest monitoring.

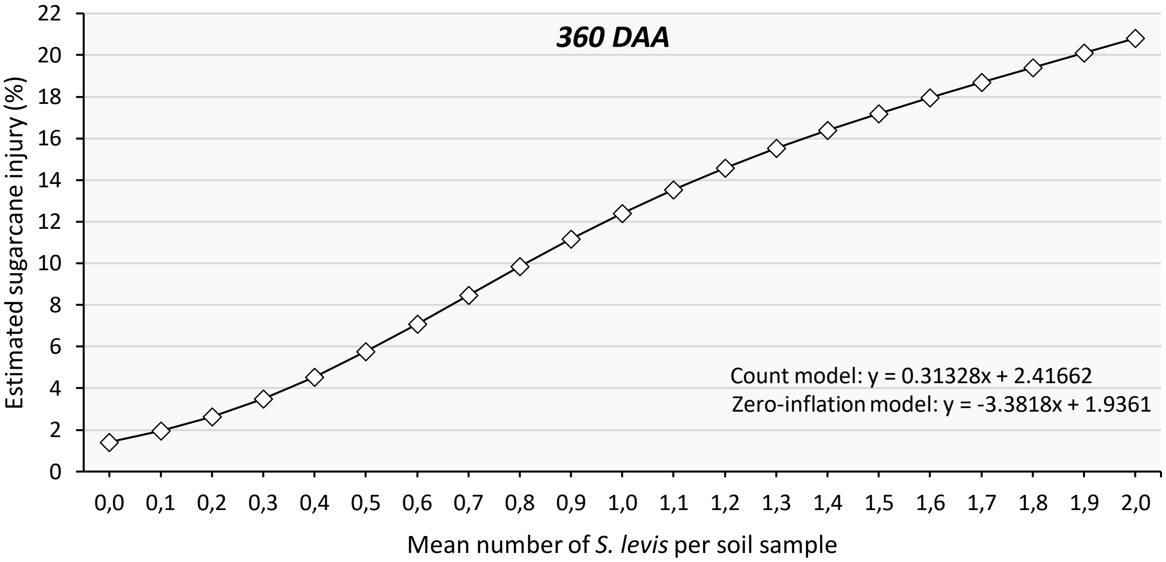


Figure 6. Estimated sugarcane injury prediction based on the mean number of *S. levis* per soil sample from evaluations conducted at 360 days after application/planting. Predicted plant injury values were calculated using a zero-inflated negative binomial model with count and zero-inflated coefficients, and with insect number effect significant at $p \leq 0.05$.

Treatment Effect on Sugarcane Yield

In general, treatments had no influence on sugarcane yield, especially for field 1 ($p = 0.7118$), field 3 ($p = 0.7908$) and for the pooled data of all fields ($p = 0.7796$) as seen in Figure 7. No yield effect was also reported in another study comparing different insecticides for *S. levis* control (Alencar, 2016). In field 2, however, significant differences were detected ($p = 0.0471$) where the liquid application of thiamethoxam provided greater yield than the untreated control (Figure 7) but no other differences were seen. Dinardo-Miranda and Fracasso (2010) also observed yield increments when using thiamethoxam for *S. levis* control. Although thiamethoxam did not reduced significantly *S. levis* population and plant damage, the yield increment caused by its active ingredient has been previously reported. Thiamethoxam was shown to promote plant yield by early root development, alteration of photoassimilates distribution, total soluble protein concentration increment, reduction of nitrate reductase activity and by phenylalanine ammonia-lyase activity increase (Macedo et al., 2011).

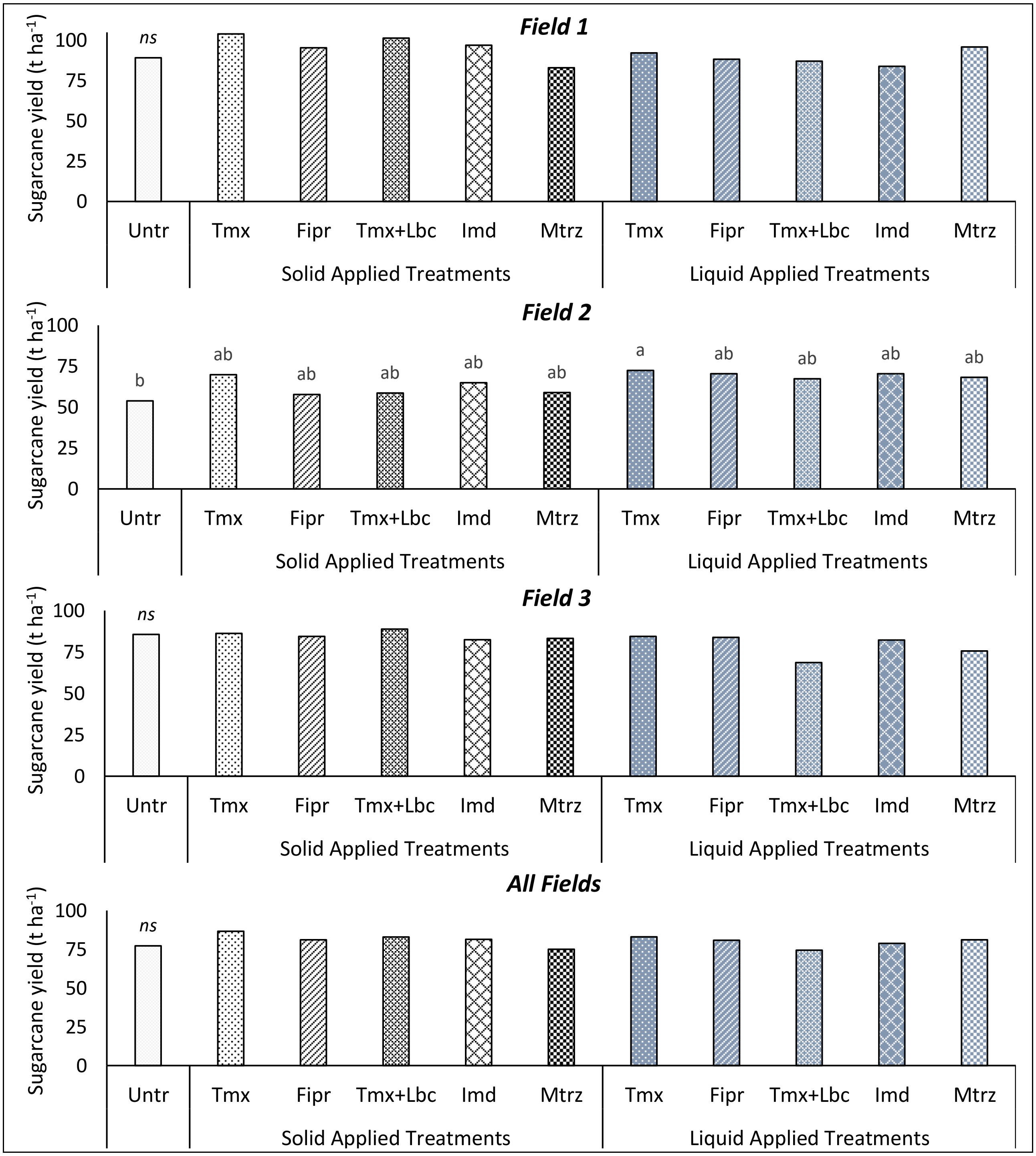


Figure 7. Sugarcane yield (t ha^{-1}) per treatment, in each field and considering pooled data of all fields. Bars with same letter are not significantly different at $p \leq 0.05$. *ns* – not significant; Untr-untreated; Tmx-thiamethoxam; Fipr-fipronil; Tmx+Lbc-thiamethoxam+lambda-cyhalothrin; Imd-imidacloprid; Mtrz-*Metharizium anisopliae*.

In addition, in field 2, an overall tendency of higher yield across liquid applied insecticides than solid applied ones, despite not significant, was observed (Figure 7). Liquid applied insecticides are typically readily available for plant/insect uptake once the active ingredient reaches the soil solution, depending only on the kinetics of dissolution of the active ingredient (Davis et al., 1996). Because of the MEIOSI planting system in field 2, with ratoon cane rows between planted cane rows, *S. levis* insects were attracted and detected earlier and in greater number than at field 1 and 3 (Figure 3) with the possibility of other sugarcane pests also being attracted and present earlier, including *Diatraeae saccharalis*, *Hyponeuma taltula*, canegrub and wireworm species. Thus, it is suggested that liquid applied insecticides in field 2 were quickly available to control a greater and earlier pest pressure whereas solid applied insecticides were not, explaining the higher plant yield trend.

On the other hand, although no meaningful differences across treatments were detected when observing the pooled data of all fields (Figure 7), an overall tendency of greater sugarcane yield with solid applied insecticides, except for *M. anisopliae*, was observed. Although not significant different, thiamethoxam solid applied, for example, provided 3.3 t ha^{-1} more yield than thiamethoxam liquid applied (Figure 7). This same tendency between solid and liquid applied insecticides were also noticed in the *S. levis* population results where solid thiamethoxam applications had less insects (733.3 insect ha^{-1}) than liquid thiamethoxam application (1160 insect ha^{-1}). These results indicate that solid insecticides may improve *S. levis* control and plant yield, depending on field characteristics, as supported by Ferreira (2022a) and as reported in different pests control studies (Buhler and Gibb, 1993; Roy et al., 2014; Ward, 2016; Allsopp, 2020, Pandey and Kumar, 2020).

CONCLUSION

Both liquid and solid applied insecticides presented low efficacy to control *S. levis*, reduce sugarcane injury levels and increase yield. However, when considering the mean results of insect control, a similar trend was noticed for all solid applied insecticides (thiamethoxam, fipronil, thiamethoxam + lambda-cyhalothrin and imidacloprid) which presented less *S. levis* insects and greater yield than liquid applied treatments.

In addition, *S. levis* monitoring results showed the absence of insects shortly after planting, 120 days after, and the increase of *S. levis* population at 360 days after planting, suggesting a potential insect migration between planted fields and nearby sheltering areas. In addition, a positive correlation indicated that one *S. levis* insect per cane row trench, on average, represented 12.4% of sugarcane injury at 360 DAA.

According to these results, insecticide application at planting for *S. levis* control should only be conducted if insects are detected prior or during planting. Ideally, insecticide treatment after planting should consider the best moment for application based on *S. levis* and cane injury monitoring results. Moreover, further studies with new insecticides, specific solid formulations and dosage are suggested.

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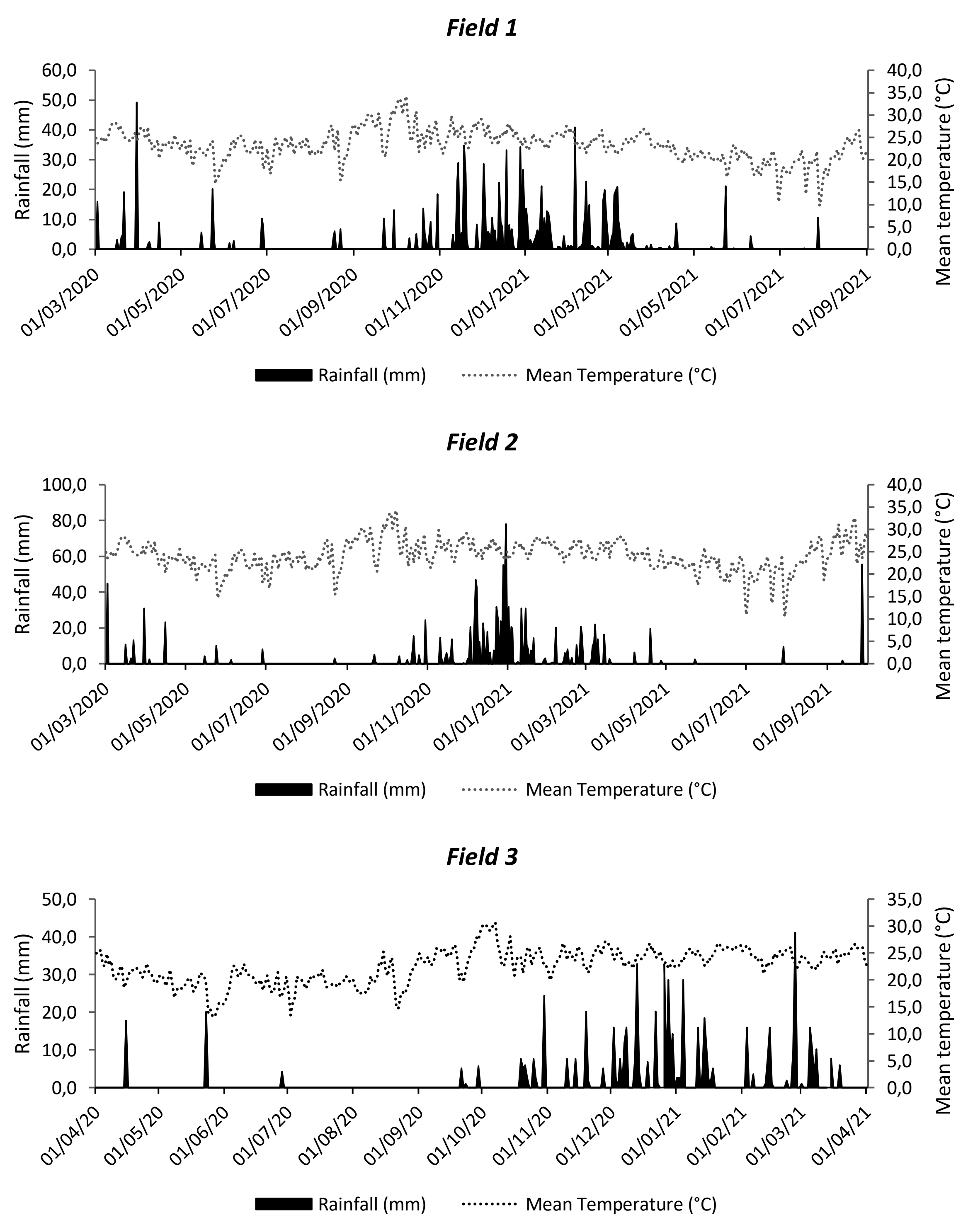
SUPPLEMENTARY MATERIAL



Mean daily rainfall and temperature data during the conduction of experiments in each field.

CHAPTER 6 - Sugarcane ratoon treatment with solid and liquid insecticide applications for *Sphenophorus levis* control

ABSTRACT - *Sphenophorus levis* is a hard to control soil pest in sugarcane because of its biology and difficulty to place insecticides close to the insect underneath the soil. Field experiments were conducted in three sugarcane sites in 2020 and 2021 to evaluate the effect of liquid and solid application of insecticides on *S. levis* control, sugarcane injury and yield. Liquid and solid applied treatments presented low efficacy to reduce *S. levis* population in relation to untreated sugarcane even though solid applications of thiamethoxam + lambada-cyhalothrin had lower insect population (8 insect ha^{-1}) than its liquid application (2517 insect ha^{-1}). Insecticide applications also had low efficacy to provide lower sugarcane injury than untreated plants despite some treatments lowering injury levels from 20% to 0% like the liquid applied thiamethoxam and solid applied thiamethoxam + lambda-cyhalothrin treatments. In addition, insect number and plant injury correlations indicated that 1 insect per soil sample in the sugarcane row represented 17.9% cane injury at 120 DAA. Insect monitoring results showed greater *S. levis* populations between June (200 DAA) and October (30 DAH). Liquid applications of thiamethoxam and *M. anisopliae* provided higher yields in one field while solid applications of thiamethoxam + lambda-cyhalothrin provided greater yield than untreated plants in another field. These data suggest overall low effectiveness of *S. levis* control with both liquid and solid applied insecticides. However, positive effects from solid insecticide applications were observed indicating potential benefits and the necessity of further studies with adequate solid formulations, new insecticides and dosages.

Keywords: billbug, soil pest, residual, granular, chemical

INTRODUCTION

The sugarcane weevil [*Sphenophorus levis* Vaurie, 1978 (Coleoptera: Curculionidae)] is an important soil pest in sugarcane (*Saccharum officinarum* L.). The insect's total life cycle is approximately 6 months long, with larvae stage of 35 days, pupae period of 10 days and adult stage of 120 days (Degaspari et al., 1987). In addition, *S. levis* is a gregarious pest with slow spatial distribution, ranging from 5.2 to 6.6 m month^{-1}, low flying capacity and nocturnal activity (Precetti and Arrigoni, 1990; Degaspari, 1978; Simi, 2014; Rosa, 2022, Ferreira, 2022a). Despite its low dispersion, *S. levis* causes great damage by reducing plant stand and longevity in which it has been reported yield losses of up to 40 t ha^{-1} and over 60% of sugarcane ratoon death (Terán and Precetti, 1982; Precetti and Arrigoni, 1990; Dinardo-Miranda et al., 2006). Yield losses are consequence of plant injuries caused by *S. levis* larvae feeding inside the sugarcane crown and tiller base forming tunnels with a white/yellow frass. Most plant injuries are located bellow the ground as researchers have described over 90% of plant injury and openings happening underneath the soil surface (Casteliani et al., 2020). Additionally, up to 95% of *S. levis* adults stay underneath the soil during the day, as observed by Ferreira (2022a).

Thus, because of the difficulty of placing insecticides near the insect at proper and effective concentrations, most insecticides currently available for *S. levis* control have not been effective. Several authors have reported low insecticide efficacy against *S. levis*. In one research examining different insecticides for *S. levis* control, the average insecticide efficacy was of only 60% (Dinardo-Miranda et al., 2006). In another study assessing *S. levis* control with distinct insecticides, no significant treatment effect was also noted (Alencar, 2016). In addition, low efficacies with biological control products have also been observed, including applications with entomopathogenic fungus *Metarhizium anisopliae* and *Beauveria bassiana* and with entomopathogenic nematodes *Steinernema* sp. and *Heterorhabditis indica* (Tavares, 2006; Leite et al., 2012; Canassa, 2014; Simi, 2014).

Up to date, most insecticides and biological products used for *S. levis* control are applied either during sugarcane planting or during cane ratoon and, in both applications, treatments are usually liquid applied. On the other hand, solid applications of insecticides are not currently adopted for *S. levis* treatment despite several studies showing great pest control potential (Buhler and Gibb, 1993; Roy et al., 2014; Ward, 2016; Allsopp, 2020; Pandey and Kumar, 2020; Ferreira, 2022b; Ferreira, 2022c). Solid applications of thiamethoxam and lambda-cyhalothrin, for example, have shown great potential for *S. levis* control in comparison with liquid applications (Ferreira, 2022c). In addition, solid applied imidacloprid provided satisfactory control levels for Childers grub (*Antitrogus parvulus*) and Greyback canegrub (*Dermolepida albohirtum*) for up to 3 years after its application in sugarcane (Samson et al., 2010; Ward, 216). Moreover, solid applications of entomopathogenic fungus have also been described as adequate for pest control (Ekesi et al., 2005; Jaronski and Jackson, 2008).

Because solid applied insecticides are usually formulated with specific granule coating and thickness properties, an extended-release rate of the active ingredient can be achieved (Kimoto et al., 2007; Matthews et al., 2014) and as a consequence of this prolonged release rate, greater insecticide residual and pest control can be accomplished. In addition, when insecticide granules are applied, adequate available moisture is required to dissipate the active ingredient from the granule into the soil solution for later insect absorption (Davis et al., 1996). Liquid applied pesticides, however, can rapidly dissolve into the soil solution accelerating its availability but also accelerating its degradation in the environment through evaporation, photolysis, runoff, leaching and volatility (Davis et al., 1996; Fernández-Pérez, 2007).

Because current insecticide treatments through liquid applications have not been effective to control *S. levis* and because solid applications have proven to be effective against different pests and crops, further research comparing liquid and solid application of insecticides for *S. levis* control in sugarcane is required. Therefore, the present study's objective was to evaluate and compare the effect of liquid and solid application of different insecticides on *S. levis* control, plant injury level and yield in sugarcane ratoon fields.

MATERIALS AND METHODS

Three ratoon cane field sites with previous *S. levis* infestation history were used to evaluate the effect of liquid and solid insecticide application on insect efficacy and sugarcane yield. Field experiments were conducted in three sugarcane farming areas in Santa Ernestina, SP, Brazil (Field 1), in Pradópolis, SP, Brazil (Field 2) and Jaboticabal, SP, Brazil (Field 3). Experiments were installed in 2020 and evaluations were conducted in 2020 and 2021. Information of each field site is available in Table 1.). Field sites were located in areas with *S. levis* infestation history. Previous crop management operations in past ratoon cane and before sugarcane planting included conventional soil tillage in field 1, mechanical elimination of ratoon cane and conventional soil tillage in field 2 and conventional soil tillage in field 3. Information of each field site is available in Table 1.

The experimental design was a randomized complete block design with eleven treatments, four blocks and two replicates per block. Each sugarcane row was considered one replicate with each treatment composed of two rows of 50 m length and 1.5 m of row spacing. Five active ingredients and one entomopathogenic fungus were used in sugarcane ratoon drill operations for *S. levis* control with liquid and solid treatment application. Treatments were applied with a ratoon drill applicator directly over cane tillers (Figure 1a), except the untreated check (T1). Available insecticides with granular (WG and GR) and liquid formulation were selected to assess and compare its efficacy under solid and liquid applications. Insecticide physicochemical properties are available in Table 2.

Table 1. Field sites information including sugarcane variety, harvest date, ratoon number, soil specification, soil analysis and crop residue amount.

	Field Sites		
	Field 1	Field 2	Field 3
Coordinates	21° 27.707' S 48° 20.1142' W	21° 18.6232' S 48° 5.1001' W	21° 11.5574' S 48° 20.159' W
Sugarcane variety	CV6654	CTC4	CTC4
Harvest date	15/10/2020	04/09/2020	30/10/2020
Ratoon number	3° ratoon	3° ratoon	3° ratoon
Soil type	Oxisol	Oxisol	Oxisol
Texture	Medium Clay	Clay	Medium
Organic matter (g dm^{-3})	22	32	19
CEC ($mmol_c$ dm^{-3})	85	101	75
Base saturation (%)	67	57	66
pH ($CaCl_2$)	5.3	5.3	5.5
Residue amount (kg ha-1)	19,445.0	13,679.0	13,881.0

Table 2. Physicochemical properties of the active ingredient and entomopathogenic fungus used.

	Insecticide				
Physicochemical Property	thiamethoxam	fipronil	lambda-cyhalothrin	imidacloprid	*M. anisopliae*
solubility (mg L^{-1})	4100[a]	3.78[a]	0.005[a]	610[a]	NA[3]
adsorption (Koc)	56.2[a]	825 [b]	283707[a]	249 [c]	NA
volatility (mm Hg)	$4.9x10^{-11}$ [b]	$2.8x10^{-9}$ [b]	$1.5x10^{-9}$ [c]	$3x10^{-12}$ [c]	NA
photolysis[1] (days)	2.7[a]	0.33[a]	40[a]	0.2[a]	0.04 [d]
soil DT50-lab[2] (days)	50[a]	142[a]	175[a]	191[a]	50 [e]
soil DT50-field[2] (days)	39[a]	65[a]	26.9[a]	174[a]	NA

[1]aqueous photolysis; [2]degration time for 50% of a compound; [3]not available.
[a]Lewis et al. (2016); [b]Kim et al. (2019); [c]National Pesticide Informational Center (2019); [d]Zimmermann (1982); [e]Li and Holdom (1993).

Liquid application treatments (T7, T8, T9, T10 and T11) were applied with a ratoon drill applicator with one TP0006 solid stream jet nozzle (Spraying Systems Inc., Wheaton, IL, US) directed towards the rhizome and placed behind the drilling disc in each sugarcane row. The spray system was calibrated to deliver a spray volume of 200 L ha^{-1} at 214 kPa pressure with an application speed of 2.77 m s^{-1}. The application equipment for solid treatments was calibrated according to each insecticide dosage with a precision balance

(Kkmoon, Shenzhen TOMTOP Technology Co, Shenzhen, China). Solid application treatments (T2, T3, T4, T5 and T6) were delivered with two solid applicator equipment (FRS Equipamentos, Limeira, SP, Brazil) with a fluted rotor, placed at the rear of a ratoon drill applicator, one at each sugarcane row (Figure 1a). Feed hoses with 5 cm diameter linked the hopper with granular insecticide to ratoon rows. They were located behind the drilling discs in which insecticide was delivered through gravity once the equipment's electric motor was activated by an on/off switch control. Tractor batteries supplied energy for the electric motor of the solid applicator equipment. Solid treatments were applied at 2,77 m s^{-1}. Each treatment with adopted insecticide, trade name, dose and active ingredient rate is available on Table 3.

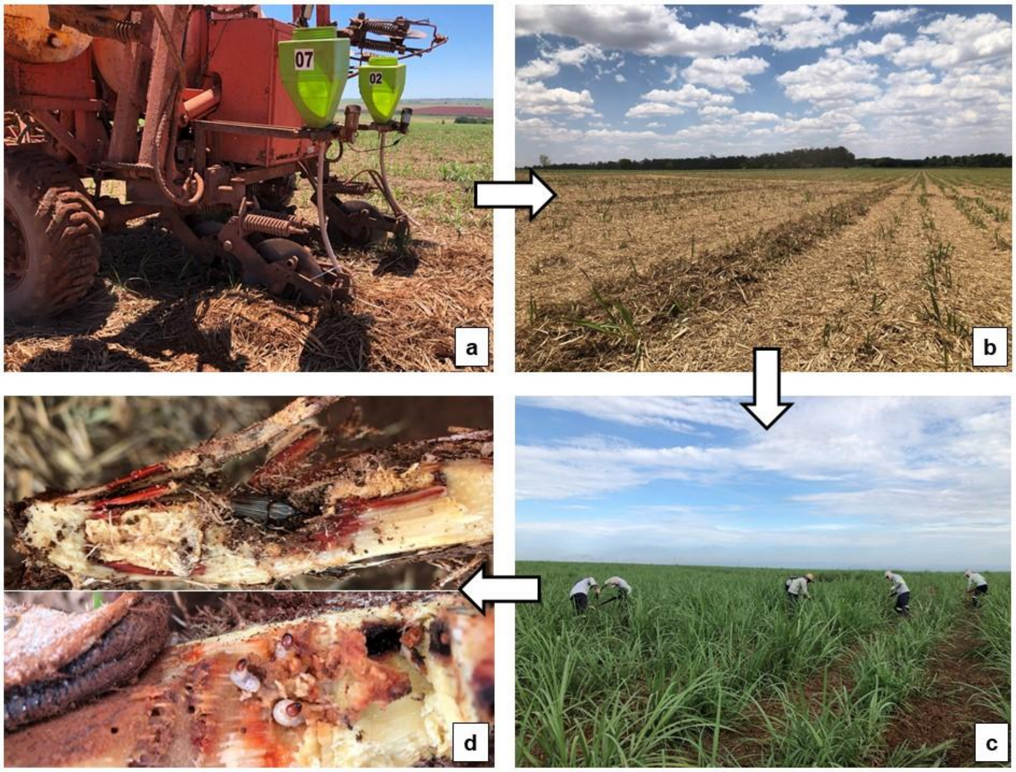


Figure 1. Ratoon drill applicator with solid and liquid insecticide application system (a); sugarcane ratoon rows after insecticide-drill application (b); field evaluations (c); *S. levis* adult and larvae and rhizome injury symptoms (d).

Table 3. Application method treatments including insecticides and trade names, dose, and active ingredient application rate.

Application	Treatment	Trade name	Dose kg ha^{-1}	a.i. Rate g ha^{-1}
------	T1 – untreated	---------------------	--------------	----------------
solid	T2 – thiamethoxam	[1]Actara® 250 WG	1.128	282
	T3 – fipronil	[2]Regent® 20 GR	22.5	450
	T4 – thiamethoxam + lambda-cyhalothrin	[3]Kaiso Sorbie BR GR + Actara® 250 WG	0.883 + 1.128	282 + 212
	T5 – imidacloprid	[4]Warrant® 700 WG	1.5	1200
	T6 – *M. anisopliae* IBCB 425	[5]Metarriz® GR	10	1000
liquid	T7 – thiamethoxam	Actara® 250 WG	1.128	282
	T8 – fipronil	[2]Regent® 800 WG	0.562	450
	T9 – thiamethoxam + lambda-cyhalothrin	[1]Engeo Pleno™ S	2.0 L ha^{-1}	282 + 212
	T10 – imidacloprid	Warrant® 700 WG	1.5	1200
	T11 – *M. anisopliae* IBCB 425	[5]Metarriz® Plus WP	1.11	1000

[1]Syngenta, Basel, Switzerland; [2]BASF SE., Ludwigshafen, Germany; [3]Nufarm Limited, Laverton North, VIC, Australia., [4]FMC Química do Brasil Ltda, SP, Brazil; [5]Biocontrol Sistema de Controle Biológico Ltda, SP, Brazil;

Soil sampling was conducted with four samples per field site and sent for soil analysis at the Soil Fertility Laboratory at UNESP following methodology of Raij et al. (2001) for organic matter (OM) content, cation exchange capacity (CEC), base saturation and soil pH (Table 1). Crop residue estimation was conducted in all field sites prior to treatment application with a 1 square meter frame and by selecting four random points and collecting all residue from each point. Residue collection was conducted after a minimum of 7 days without rain to ensure crop residue dryness and was estimated using a portable electronic scale BM-A06 (Guangzhou Wei Heng Electronics Co., Guangzhou, China). Weather data of each field was obtained for the experiment duration and included rainfall, relative humidity and average temperature data. Temperature and relative humidity were measured during treatment application with a digital thermo hygrometer

Jprolab (JProlab, São José dos Pinhais, PR, Brazil). Wind speed was quantified with an anemometer Kestrel 3000 (Kestrel Instruments, Boothwyn, PA, USA). Soil temperature was measured with a digital thermometer TE-400 (Instrutherm, São Paulo, SP, Brazil) and soil humidity was measured with ph-2500 pH/soil humidity meter (Instrutherm, São Paulo, SP, Brazil). Meteorological and soil conditions are available in Table 4.

Table 4. Information of treatment application including date, average meteorological and soil conditions and first rainfall event after planting application.

	Field Sites		
	Field 1	Field 2	Field 3
Application date	05/11/2020	04/12/2020	21/11/2020
Application time	09:00 am	10:00 am	15:00 pm
Temperature (°C)	31.5	35.5	31.9
Relative humidity (%)	29.6	55.5	38.0
Wind speed (m s^{-1})	3.97	0.88	2.62
Soil humidity (%)	43	48	52
Soil temperature (°C)	23.0	25.6	25.1
First rain after application/rain amount	5 DAA[1] / 4.9 mm	2 DAA / 20 mm	7 DAA / 5.1 mm

[1]DAA - days after application.

Evaluations of *S. levis* insect count and sugarcane injury (Figure 1c and 1d) were taken at 60, 120 and 200 days after application (DAA) and 30 days after harvest (DAH). Insect count assessment was conducted by counting the number of *S. levis* larvae, pupae, and adult discovered per replication. Cane injury evaluations consisted of counting the total number of rhizomes and the number of injured rhizomes by *S. levis* per replication. Soil trenches of 0.50 x 0.50 m x 0.30 m were dug for both insect count and plant damage evaluations in the center of each row (replication). Sugarcane rhizomes were separated and subterranean shoots and steams were cut for assessment of insect presence in soil/plant and cane injury symptoms.

Yield was estimated at 200 DAA by counting sugarcane plants in 10 m of each replication, manually harvesting 10 cane stems and weighting harvested stems with a portable electronic scale BM-A06 (Guangzhou Wei Heng Electronics Co., Guangzhou, China) (Adapted from Arizono et al., 1998; Landell et al., 1999). Weather data including rainfall, humidity and temperature during the study period was obtained for each field and is available in supplementary material section.

Data analysis

Treatment and evaluation period effects were analyzed for individual and pooled field site data to observe specific and general effects across field sites. Descriptive analysis and model fitness were conducted for insect count (all insect development stages), sugarcane injury and yield data in R version 1.4.1717 software (RStudioTeam, 2022). Before model selection, different model error distributions and link functions were tested and adjusted correcting overdispersion and assessing goodness of fit. Model's performances were compared by half-normal plots with simulation envelopes using the hnp package in R software (Moral et al., 2017) and based on Akaike information criterion (AIC) and residual deviance values to select the best model.

Insect count data was treated as dependent variable in a negative binomial generalized linear model of a two-way interaction of independent variables (treatment x evaluation period) using the MASS package (Venables and Ripley, 2002). Cane injury data was treated as dependent variable in zero-inflated negative binomial models of a two-way interaction of independent variables (treatment x evaluation period). The zero-inflated model was used to compensate for excessive zeros and overdispersion using the pscl package (Jackman, 2020) and zeroinfl function (Zeileis et al., 2008). Sugarcane yield data was treated as dependent variable in a gaussian generalized linear model with treatment as the independent variable.

After model selection, results of *S. levis* count, cane injury and yield were submitted to an analysis of deviance (type II Wald chi-square tests) considering two-factor interaction and main effect, respectively. Significant interactions and main effects were analyzed using the emmeans package with Sidak's test at $p < 0.05$ (Lenth, 2019) to determine significant differences between treatments. Mean values of insect count (insect per trench) were converted to number of insects per hectare considering a trench square area of 0.5 m^2 and row spacing between plants of 1.5 m.

To assess the relationship between sugarcane injury levels and number of insects at each evaluation period, correlations were conducted in R software with the Spearman's rank correlation method for nonparametric data. Field results were pooled to observe the overall relationship across field sites. If treatment effect was not significant, results were also pooled to observe the overall relation despite of treatment effect. When moderate to strong correlations ($r > 0.5$) were detected, a zero-inflated negative binomial model, considering cane injury as dependent variable and insect count as the independent variable, was adopted to account for overdispersion and excessive number of zeros using the pscl package (Jackman, 2020) and zeroinfl function (Zeileis et al., 2008). Predicted cane injury levels were estimated using the predict function in R considering the adopted zero-inflated negative binomial model.

RESULTS

Treatment and evaluation period effects: *S. levis* population

In general, both liquid and solid applied treatments showed low efficacy to reduce *S. levis* population. Although the interaction of treatments and evaluation period was significant to influence its population ($p = 0.0060$), no treatment was more effective than another including the untreated control, during the same period (Figure 2). During different periods times, however, it possible to see an increment of *S. levis* population at 200 DAA compared to previous evaluations, where the liquid and solid applied thiamethoxam, thiamethoxam + lambda-cyhalothrin, the solid applied *M. anisopliae* and the liquid applied imidacloprid had higher number of insects than at earlier evaluations (Figure 2).

The treatment effect alone was only significant to have an influence on *S. levis* population in field 2 ($p < 0.0001$) as shown in Figure 3. However, no treatment was more effective than the untreated control. In fact, the treatments thiamethoxam and imidacloprid solid applied, and the liquid applied thiamethoxam alone, thiamethoxam + lambda-cyhalothrin, imidacloprid and *M. anisopliae* had more *S. levis* insects than the untreated control. However, the number of insects from the solid application of thiamethoxam +

lambada-cyhalothrin, 0.0006 insect trench^{-1} or 8 insect ha^{-1}, considering only insects found underneath the soil and inside cane rhizomes in trenches (0.5 x 0.5 x 0.3 m) dug over canes row with rows spacing of 1.5 m, was significantly lower than its liquid application, 0.18 insect trench^{-1} or 2517 insect ha^{-1} (Figure 3). In addition, despite no significant, both fipronil applications had low insect numbers with fewer than 200 insect ha^{-1}.

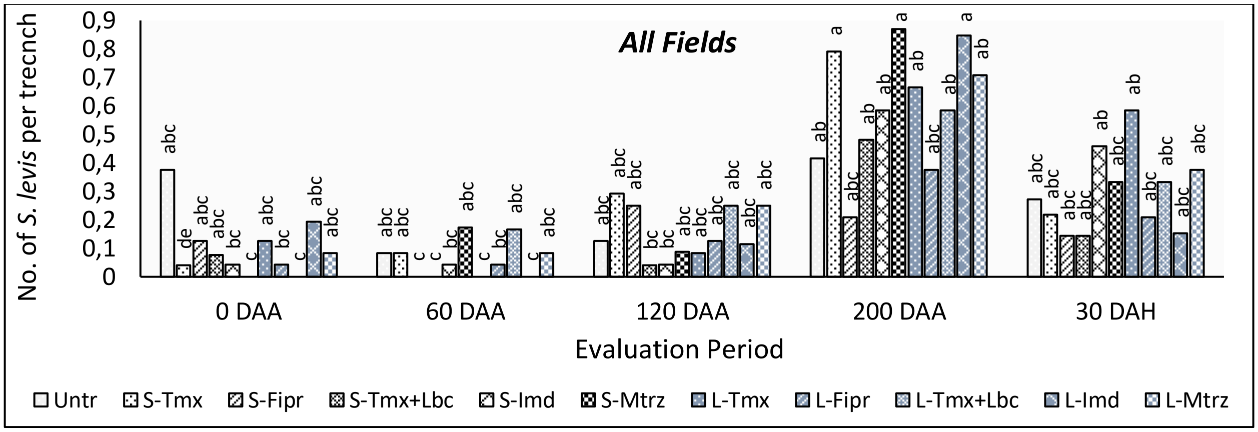


Figure 2. Mean number of *S. levis* insects per evaluation period of all fields for each treatment. Bars with same letter are not significantly different at $p \leq 0.05$. Untr-untreated; S-Solid application; L-Liquid application; Tmx-thiamethoxam; Fipr-fipronil; Tmx+Lbc-thiamethoxam+lambda-cyhalothrin; Imd-imidacloprid; Mtrz-*Metharizium anisopliae*.

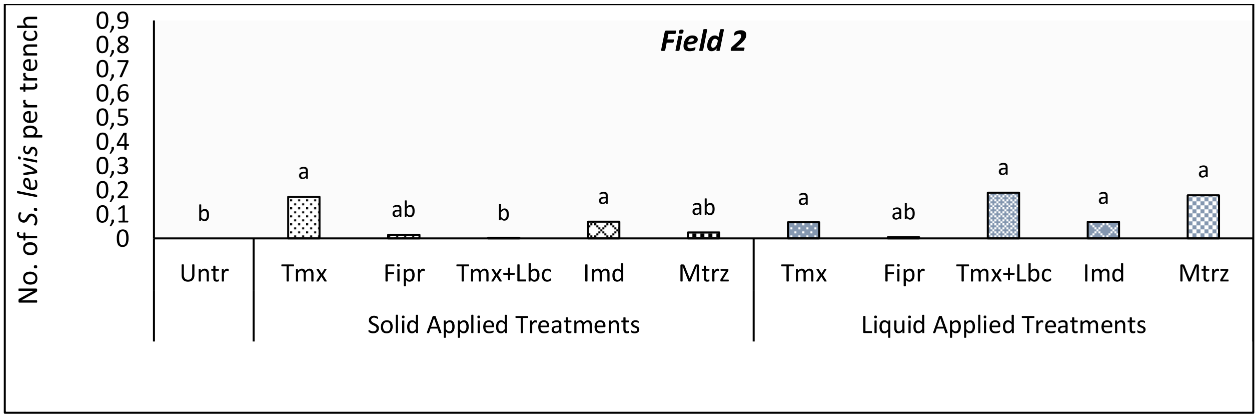


Figure 3. Number of *S. levis* insects per treatment in field 2, regardless of evaluation period. Bars with same letter are not significantly different at $p \leq 0.05$. Untr-untreated; Tmx-thiamethoxam; Fipr-fipronil; Tmx+Lbc-thiamethoxam+lambda-cyhalothrin; Imd-imidacloprid; Mtrz-*Metharizium anisopliae*.

The effect of the evaluation period over *S. levis* population was observed in all fields (Figure 4). In field 1, an increase of insect number at 200 DAA, in June, was noticed ($p < 0.0001$). In field 2, *S. levis* population started increasing at 120 DAA, in April, until 200 DAA, in July, with a slight decrease at 30 DAH in September ($p < 0.0001$). In field 3, insects were more present at 200 DAA in July ($p < 0.0001$). When considering the mean results of all fields, an overall small increment of *S. levis* insect at 120 DAA was noticed, followed by a greater number of insects at 200 DAA and by a population reduction at 30 DAH ($p < 0.0001$), as shown in Figure 4.

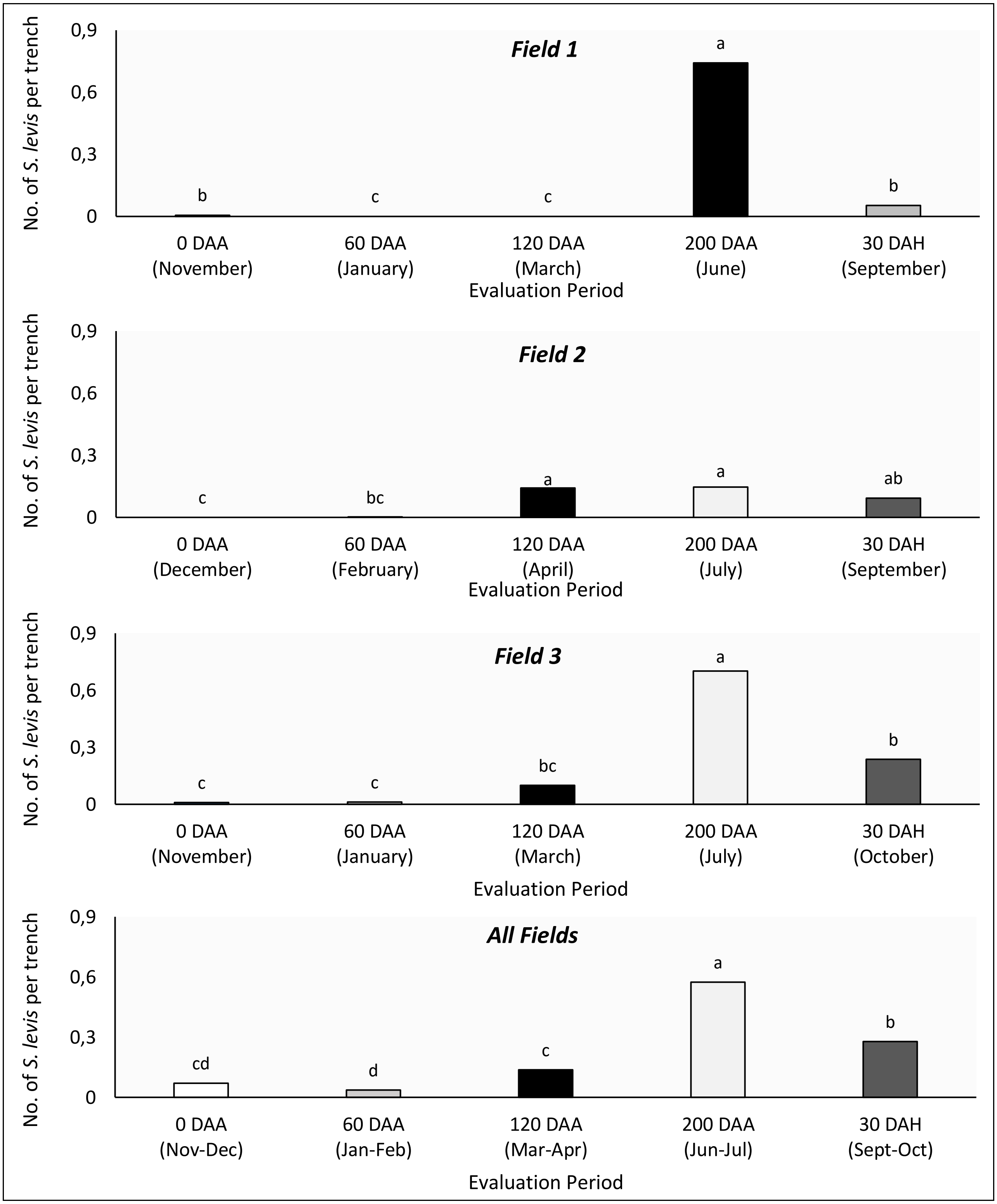


Figure 4. Number of *S. levis* insects per evaluation period regardless of treatment, considering each and all fields. Bars with same letter are not significantly different at $p \leq 0.05$; *ns* – not significant.

Treatment and evaluation period effects: sugarcane injury

Treatments presented low efficacy to reduce sugarcane injury caused by *S. levis* in comparison with the untreated control. A significant interaction between effects of treatment and evaluation period was detected only in field 1 ($p < 0.0149$) as shown in Figure 5. Before treatment applications in field 1 at 0 DAA, the only difference of injury levels among treatments was seen in plots where the solid application of thiamethoxam would be applied (Figure 5). After applications, however, no differences across treatments at the same evaluation period were observed.

However, when comparing different evaluation times in field 1, significant reductions of injury levels were observed. The solid application of thiamethoxam + lambda-cyhalothrin, for example, reduced the injury level from 19.75% to 0%, in relation to 0, 60 and 120 DAA periods (Figure 5). In addition, the liquid application of thiamethoxam also reduced the injury percentage of 20.5% at 0 DAA, to 0% at 120 DAA. On the other hand, at 200 DAA, a cane injury increment was noticed for some treatments, including thiamethoxam and imidacloprid solid and liquid applied, and thiamethoxam + lambda-cyhalothrin and *M. anisopliae* solid applied (Figure 5).

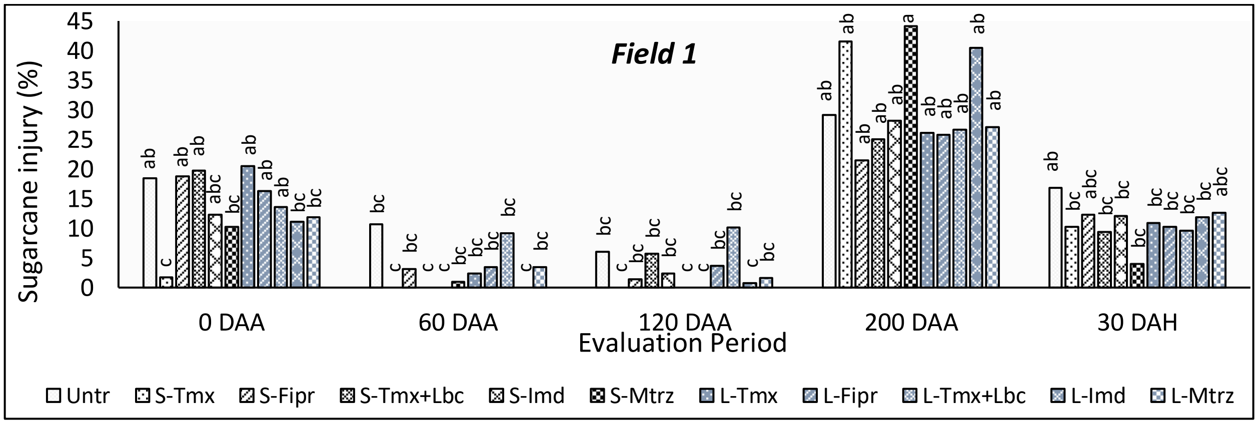


Figure 5. Sugarcane injury level (%) per evaluation period in field 1, for each treatment. Bars with same letter are not significantly different at $p \leq 0.05$. Untr-untreated; S-Solid application; L-Liquid application; Tmx-thiamethoxam; Fipr-fipronil; Tmx+Lbc-thiamethoxam+lambda-cyhalothrin; Imd-imidacloprid; Mtrz-*Metharizium anisopliae*.

A significant effect of evaluation period, regardless of treatment application on sugarcane injury was observed in all fields (Figure 6). In field 1, for example, high injury levels at 200 DAA in June (30.5%) and at 0 DAA and 30 DAH were seen whereas low levels at 60 DAA (3%) and 120 DAA (2.8%) were detected ($p < 0.0001$). In field 2, the level of injured cane started increasing in April, at 120 DAA, followed by an increment in July (21.5%), at 200 DAA, and similar injury level in September at 30 DAH, whereas earlier dates in December and February (0 DAA and 60 DAA) presented lower injury percentages with 6.7% and 3.5%, respectively ($p < 0.0001$). Similarly, in field 3, the highest percentage of plant injury was also detected in July, at 200 DAA (12.2%), and in October at 30 DAH (9.7%) while at earlier evaluations cane injury levels were less than 1.5% ($p < 0.0231$). When considering the pooled results of all fields, the overall effect of evaluation period was also characterized by great injury levels at 200 DAA, between June and July, and low cane injury at 60 DAA, between January and February ($p < 0.0001$).

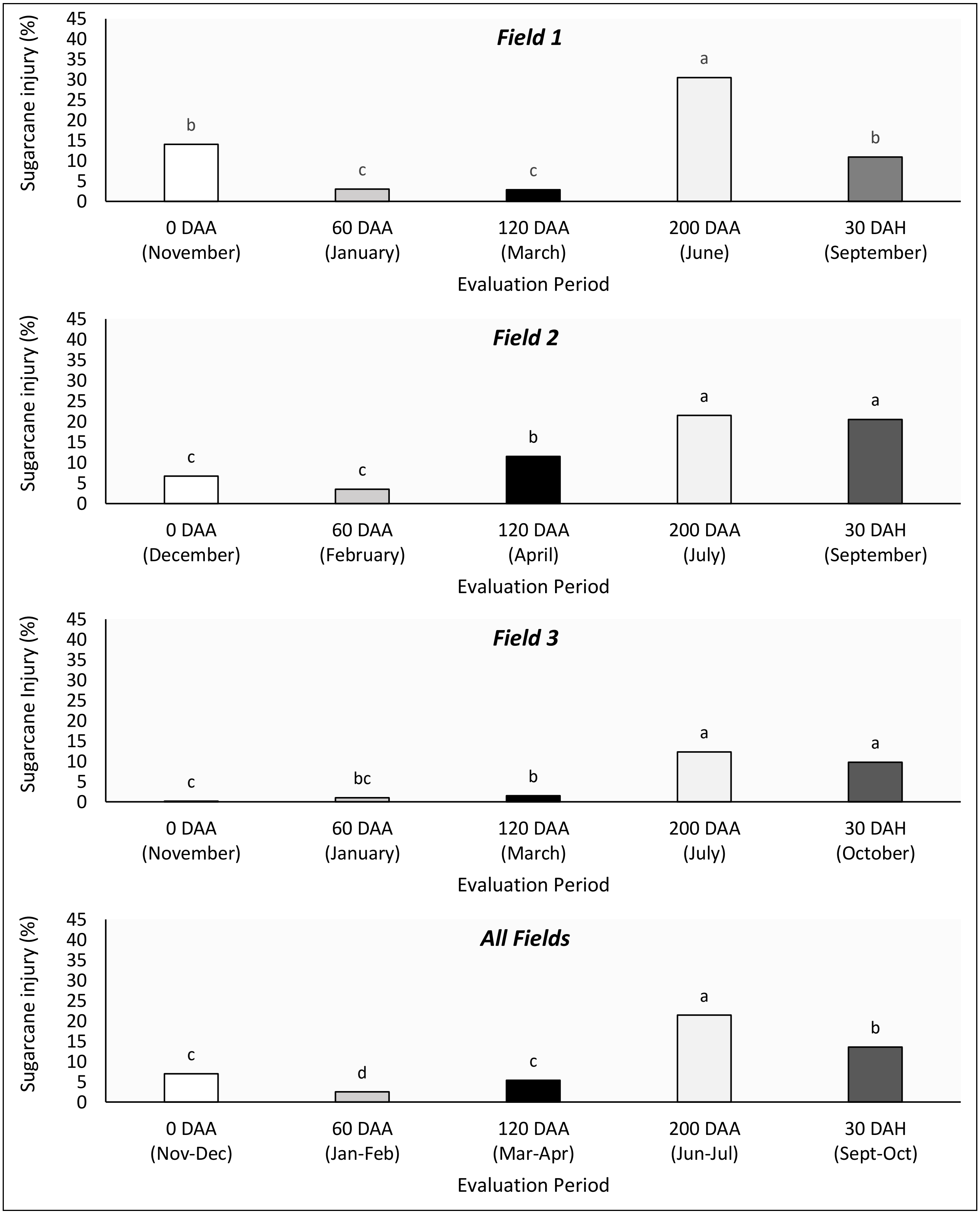


Figure 6. Sugarcane injury (%) per evaluation period regardless of treatment, considering each and all fields. Bars with same letter are not significantly different at $p \leq 0.05$.

S. levis insect monitoring for sugarcane injury estimation

A positive correlation (r = 0.51) was observed between the level of sugarcane injury and the mean number of *S. levis* per soil sample in March and April of 2021, at the evaluation period of 120 DAA, with a significant effect of insect number on injury level at 120 DAA ($p < 0.0001$). Higher mean number of insects per soil sample represented higher injury levels. For example, an average of 1 *S. levis* per soil sample or 13333 insects ha^{-1}, considering only insects found underneath the soil and inside cane rhizomes in trenches (0.5 x 0.5 x 0.3 m) dug over canes row with 1.5 of row spacing, was estimated to represent 17.9% of sugarcane injury at 120 DAA (Figure 7) while a mean number of 2 insect per soil sample (26666 insects ha^{-1}) represented 26.6% of injury level (Figure 7). During the remaining evaluation periods (0, 60 and 200 DAA and 30 DAH) week positive correlations (r < 0.40) were detected between sugarcane injury and number of *S. levis* insects, and, therefore, no relationship was considered to further analysis.

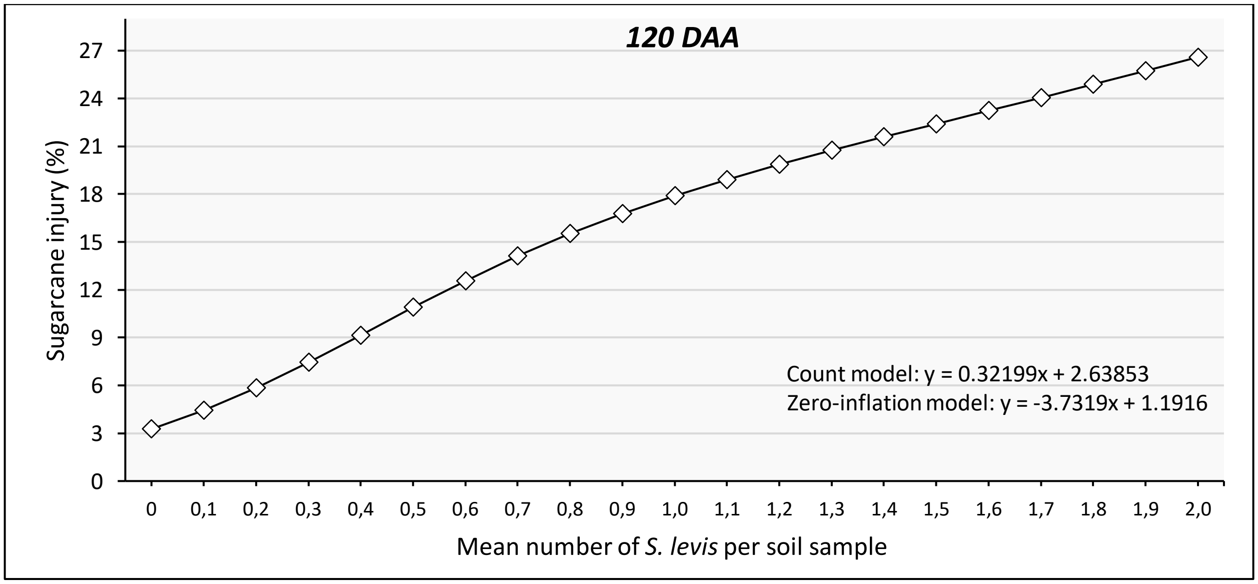


Figure 7. Estimated sugarcane injury prediction based on the mean number of *S. levis* per soil sample from evaluations conducted at 120 days after application (155 days ratoon cane. Predicted plant injury values were calculated using a zero-inflated negative binomial model with count and zero-inflated coefficients, and with insect number effect significant at $p \leq 0.05$.

Treatment effects: sugarcane yield

Treatments affected sugarcane yield in two out of three experimental fields. In field 1, both liquid applications of thiamethoxam (111.7 t ha^{-1}) and *M. anisopliae* (111.6 t ha^{-1}) provided higher yields than the untreated control, fipronil treatments and solid applied *M. anisopliae* ($p < 0.0001$) as shown in Figure 8. In field 2, no differences were observed among treatments ($p = 0.0692$) (Figure 8). In field 3, the solid application of thiamethoxam + lambda-cyhalothrin provided greater sugarcane yield, 83.6 t ha^{-1}, than the untreated control with 52.9 t ha^{-1} ($p = 0.0437$) (Figure 8). Moreover, when considering the mean result of all fields, treatments had no impact over sugarcane yield ($p = 0.5703$) as represented in Figure 8.

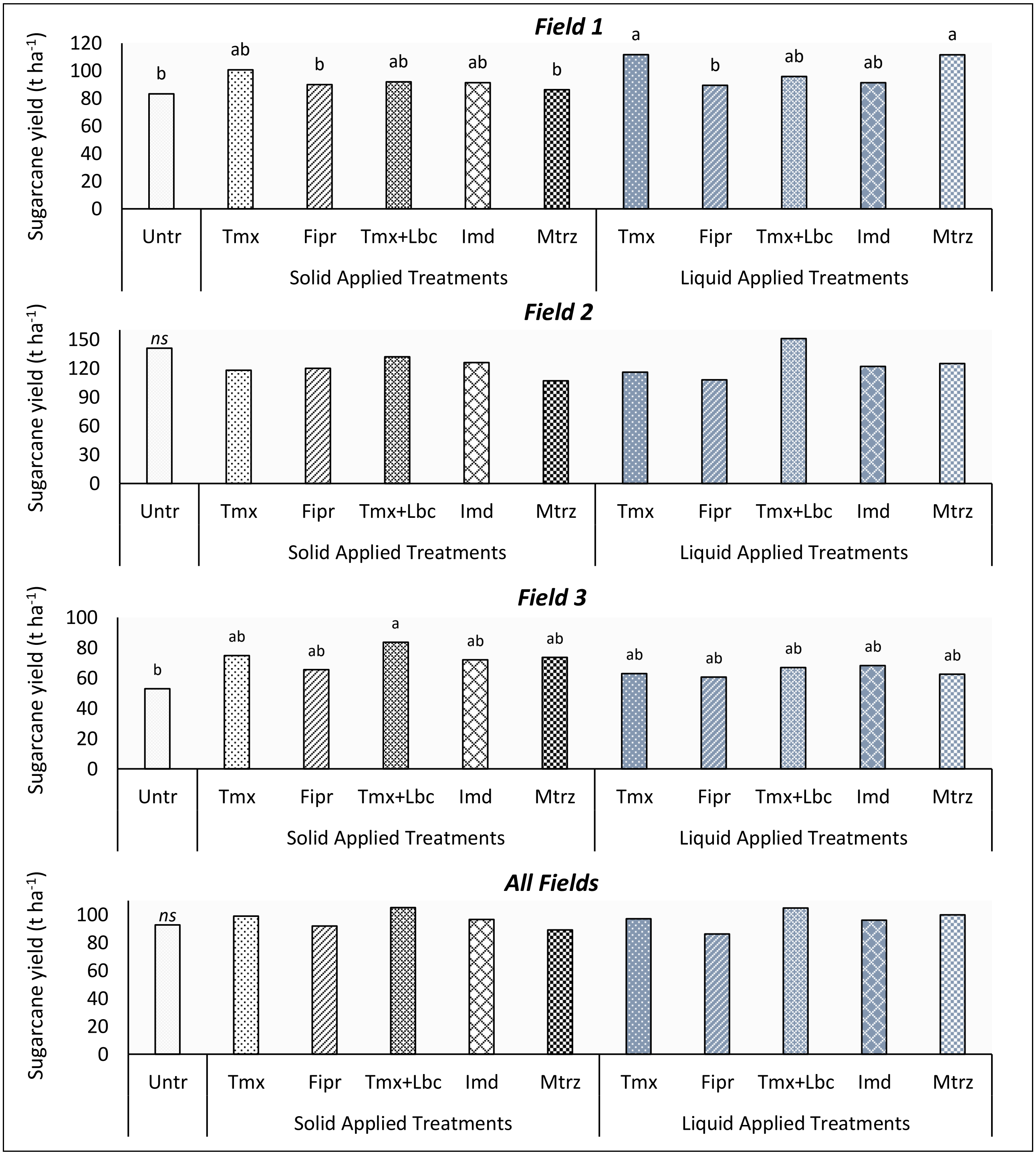


Figure 8. Sugarcane yield (t ha^{-1}) per treatment, in each field and considering pooled data of all fields. Bars with same letter are not significantly different at $p \leq 0.05$. *ns* – not significant; Untr-untreated; Tmx-thiamethoxam; Fipr-fipronil; Tmx+Lbc-thiamethoxam+lambda-cyhalothrin; Imd-imidacloprid; Mtrz-*Metharizium anisopliae*.

DISCUSSION

Sphenophorus levis population results indicated an overall low number of insects found per trench with mean number values ranging from 0 to 0.87 insect per trench or from 0 to 11600 insect ha^{-1}, considering only insects found underneath the soil and inside cane rhizomes in trenches (0.5 x 0.5 x 0.3 m) dug over canes row and with row spacing of 1.5 m, as shown in Figure 2. Similarly, in other *S. levis* studies, authors observed insect values varying from 0 to 1.6 insect trench^{-1} and from 0 to 1.8 insect trench^{-1} (Alencar, 2016; Ferreira, 2022b). Low mean number of insects per soil sample are a result of a high amount of zero values when no insects are found, common to ecological count datasets with field variability and heterogeneity of pest distribution (Sileshi, 2006). Despite apparent low insect count values, an average of one single insect per soil sample (13333 insects ha^{-1}) at 120 DAA represented up to 17.9% of sugarcane injury (Figure 7) demonstrating the great damage potential of *S. levis* insects in sugarcane. In fact, based on previous studies, every 1% of sugarcane injury from *S. levis* represents 1 t ha^{-1} yield loss (Casteliani et al. 2020). Therefore, considering this damage potential, a mean number of one insect per trench, which characterized an injury level of 17.9%, may represent yield losses of 17.9 t ha^{-1}. In addition, because of its great damage potential and to prevent further losses, the current adopted control threshold for *S. levis* in sugarcane has been the detection of 1 insect per field or 5% of sugarcane injury (Dinardo-Miranda et al., 2008; Dinardo-Miranda, 2014).

Regardless of the number of *S. levis* detected during pest monitoring, treatments were generally low effective to control *S. levis* in relation to the untreated control during all evaluation periods although there were some specific high efficacy results. Overall low efficacy is in accordance with reported results from several authors (Dinardo-Miranda et al., 2006; Tavares, 2006; Alencar, 2016). The insect's biology, behavior and habitat below the soil surface (Precetti and Arrigoni, 1990; Ferreira, 2022a) are one of the main reasons insecticides may not be adequately deposited and absorbed by insects with consequent poor control. In fact, in a study describing *S. levis* adult behavior, the author observed over 95% of adults hidden underneath the soil surface during the day (Ferreira, 2022a).

Some treatments, however, provided full insect control depending on field location and period (e.g., solid applied fipronil and thiamethoxam + lambda-cyhalothrin, liquid applied thiamethoxam and imidacloprid, at 60 DAA in field 2) despite not different than the untreated control (Figure 2). Similarly, in another study evaluating insecticide efficacy for *S. levis* control, insecticides like thiodicarb + imidacloprid also reduced the number of insects, from 1.2 to 0.1 insect per trench (0 DAA to 90 DAA), but were also not different than the untreated control with 0.8 insects per trench (Alencar, 2016). Moreover, when only considering the treatment effect on *S. levis* control in field 2, the solid application of thiamethoxam + lambda-cyhalothrin presented fewer insects (8 insects ha^{-1}) than its liquid application (2517 insects ha^{-1}). Considering there was a high amount of rain following treatment applications in field 2 with over 109 mm only 4 days after application (supplementary material section), and there was an earlier incidence (April) of *S. levis* than in other fields (June-July), it is suggested that the liquid applied thiamethoxam + lambda-cyhalothrin was less available than its solid application to control *S. levis* because of its degradation in the environment, especially through leaching. Thiamethoxam is a very soluble insecticides (4100 mg L^{-1}) with low adsorption (Koc = 56.2) known for its high mobility in soil (Lewis et al., 2016; Mörtl et al., 2016). In fact, low thiamethoxam efficacy was also accounted for its high mobility when evaluating insecticide activity in irrigated sugarcane for *S. levis* adult mortality (Ferreira, 2022c). On the other hand, lambda-cyhalothrin is strongly adsorptive to soil colloids (Koc = 283707) (Lewis et al., 2016), and was probably not available for late *S. levis* control regardless of the application method. Moreover, potential benefits from solid applications of thiamethoxam + lambda-cyhalothrin for *S. levis* control have also been reported by Ferreira (2022c), in which granular applications provided 52.4% *S. levis* adult mortality against 40.6% adult mortality from its liquid application. In the same study, further mortality levels were observed when increasing the solid applied thiamethoxam + lambda-cyhalothrin dosage, providing up to 76.7% *S. levis* adult mortality (Ferreira, 2022c). Several authors have also reported benefits of insecticide solid applications. Studying the effectiveness insecticides for banana borer (*Cosmopolites sordidus*) control, authors observed great insect control with granular applications of terbufos and carbofuran (Barbosa et al., 2004). In another study, testing insecticides for wireworm (*Melanotus communis*) control in sugarcane, authors noticed better insect control with the granular phorate treatment (Larsen et al., 2015).

Complementary to *S. levis* insect control results, thiamethoxam + lambda-cyhalothrin solid applied also helped to reduce initial sugarcane injury levels in field 1, from 19.7% to 0% at 60 DAA, while its liquid application only reduced plant injury from 13.6% to 9.2%. Considering insect and injury control improvements were obtained even though no adequate solid insecticide formulations were used for soil applications, promising results may be expected when adopting proper formulations. A specific controlled release (CR) formulation for soil application, for example, provided up to 4-year control to different canegrub species in sugarcane (Ward, 2016).

At 120 DAA, the liquid application of thiamethoxam also reduced initial cane injury percentage in field 1, from 20.5% to 0%. Considering that liquid applied insecticides are usually readily available for plant/insect uptake after reaching the soil solution (Davis et al., 1996), faster injury reductions from the liquid applied thiamethoxam would be expected. In contrast, an extended effect of liquid applied thiamethoxam was observed in field 1, despite its high solubility. Meanwhile, no similar effect was seen in all remaining fields 2 and 3 and, therefore, such result may be accounted due to field variability and plant injury distribution. In addition, as also stated in another *S. levis* control study, the sampling method for insect population and injury level may not always be precise enough to demonstrate the real effect on injury level (Dinardo-Mirada and Fracasso, 2010).

In addition, because the present study only included four insecticide's active ingredients (thiamethoxam, fipronil, lambda-cyhalothrin, imidacloprid) and one entomopathogenic fungus (*M. anisopliae*) due to solid formulations availability at the time, future studies with different registered insecticides for *S. levis* control (e.g., chlorantraniliprole, alphacypermethrin, bifenthrin and carbosulfan) and other entomopathogenic products (e.g., *Beauveria bassiana*), solid and liquid applied, may present different *S. levis* control results. However, considering current *S. levis* control results and based on previous studies with different insecticides (Dinardo-Miranda, 2006; Dinardo-Miranda, 2010; Canassa, 2014; Simi, 2014; Alencar, 2016; Evangelista et al., 2017; Ferreira, 2022b), it is possible to conclude that an overall low efficacy of tested insecticides it is still evident.

Significant improvement of *S. levis* control may be achieved when adopting an effective integrated pest management (IPM) including mechanical control methods like the destruction of volunteer cane with infested *S. levis*; the adoption of cultural methods like bare fallow, crop rotation and usage of high-quality variety and seed cane billets; behavioral methods for insect control/monitoring and different biological and chemical control methods. Nevertheless, some alternatives for chemical and biological insecticide applications can potentially help to improve *S. levis* control.

Increasing insecticide dosage in site-specific infested areas, for example, may significantly improve product efficacy as observed by Ferreira (2022c), in which *S. levis* mortality increased from 58.8 to 76.7% when doubling the dosage. In addition, new product formulations allowing extended insecticide activity, like controlled-released (CR) granular formulations, may further improve *S. levis* control. One imidacloprid CR formulation, for example, provided up to 4 years of canegrub control in sugarcane (Ward, 2016) while one granular formulation of *M. anisopliae* provided fruit fly larvae control after 668 of soil inoculation (Ekesi et al., 2005). Furthermore, although current soil drill application methods have shown to be more effective than other methods for *S. levis* ratoon treatment as described by Dinardo-Miranda (2014) and by Ferreira (2022b), nocturnal band-spraying targeting *S. levis* adults may also improve insecticide efficacy. Because *S. levis* are nocturnal insects with adult activity peaks happening from 18:00 pm until 2:00 am, insecticide applications at night could be recommended (Ferreira, 2022a). Moreover, continuous monitoring evaluations of *S. levis* can help sugarcane farmers programing insecticide applications at the best time.

For instance, a clear effect of the evaluation period was noticed from current results. An overall tendency of few *S. levis* detected in early evaluations after applications (0 and 60 DAA) between November and February was observed while a great number of insects was detected in later evaluations (200 DAA and 30 DAH) between June and October. Similarly, sugarcane injury level was more evident during June and July and between September and October (Figure 6). Several authors have reported greater *S.*

levis populations, specially of larvae and pupae, during June, July and August (Degaspari et al., 1987; Precetti and Arrigoni, 1990; Canassa, 2014; Izeppi et al. 2014, Ferreira, 2022b) which are in accordance with current results. In addition, *S. levis* monitoring results can help to determine the best moment for precise and effective applications. Thus, further insecticide applications could be recommended when monitoring results indicate an increase of *S. levis* population. For example, a second application could be recommended based on the monitoring results of 120 DAA between March and April, for most experimental fields (Figure 4) where there is an evident indication of *S. levis* population increment. Applications during this period should be more effective than preventive applications without *S. levis* presence. In fact, greater control of sugarcane borer [Diatraea saccharalis (Lepidoptera: Crambidae)] was reported in a study when insecticides were applied shortly after insect presence in comparison with preventive applications without insects (Wilson et al., 2022).

Regarding yield effects, application treatments were effective increase sugarcane yield in two fields. In field 1 both liquid applications of thiamethoxam and *M. anisopliae* provided greater yield despite no significant *S. levis* control. Yield benefits were also reported in one study of thiamethoxam's influence on plant yield through early root development, alteration of photoassimilates distribution, total soluble protein concentration increment, reduction of nitrate reductase activity and by phenylalanine ammonia-lyase activity increase (Macedo et al., 2011). Liquid applied *M. anisopliae* has also shown to improve sugarcane yield in another study (Chelvi et al., 2011). In addition, in field 3, the solid application of thiamethoxam + lambda-cyhalothrin provided greater yield than untreated plants followed by high yields from solid applied thiamethoxam plots, despite no significant *S. levis* control or sugarcane injury reduction. The positive yield potential of thiamethoxam, described by Macedo et al. (2011), was possibly extended by its solid application ensuring prolonged plant development through longer thiamethoxam release and residual. However, further studies to examine the effect on *S. levis* control and cane yield should consider, for example, different insecticides, higher dosages, nocturnal and sequential applications to better assess applications efficacy with liquid and solid insecticides.

CONCLUSION

Individual field results have shown some positive effects from solid application of insecticides for *S. levis* control, cane injury reduction and yield despite an overall low efficacy among treatments. Solid applications of thiamethoxam + lambda-cyhalothrin, for example, provided lower *S. levis* population than its liquid application in field 2, reduced sugarcane injury levels to zero at 60 DAA in field 1 and improved sugarcane yield in field 3, indicating potential benefits of solid applied insecticides for *S. levis* management. Thus, developing proper granular formulations for soil applications with adequate active ingredient concentration should improve insecticide performance for *S. levis* control.

In addition, *S. levis* population and sugarcane injury was more evident in June-July and in September-October demonstrating the importance of monitoring *S. levis* populations to recommend the best time of application with potential benefits of a second application during these periods.

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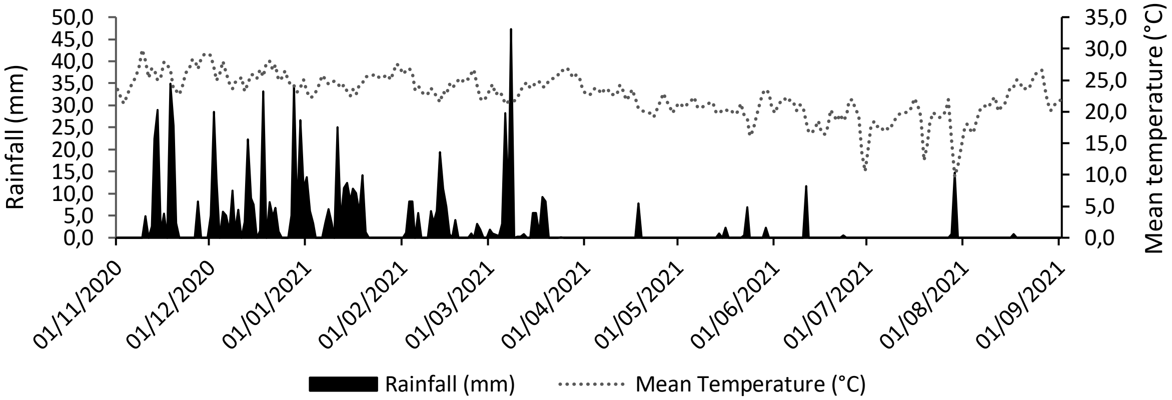
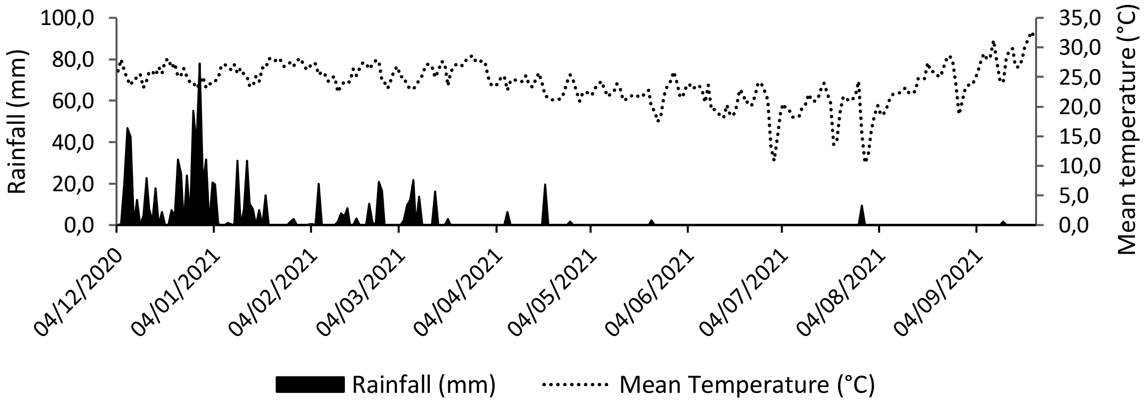
SUPPLEMENTARY MATERIAL

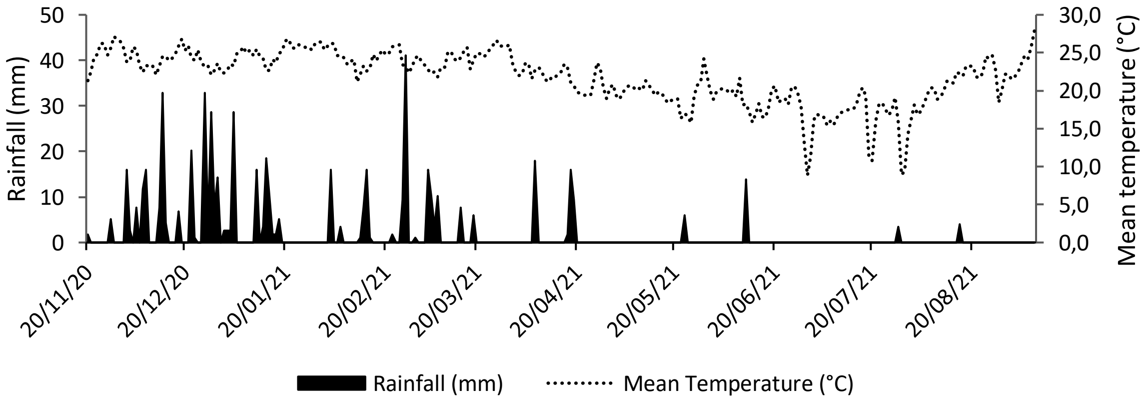
Field 1



Field 2



Field 3



Mean daily rainfall and temperature data during the experiment conduction in field 1, field 2 and field 3.

CHAPTER 7 – FINAL CONSIDERATIONS

Considering current results and the extensive support from literature, *Sphenophorus levis* is an extremely hard to control soil pest and new options for pest management must be adopted to improve its control.

The location, behavior and activity of *Sphenophorus levis* are directly associated with its difficulty of control by insecticide applications. Results showed that not only larvae and pupae of *S. levis* were exclusively located underneath the soil and inside the sugarcane crown obstructing insecticide deposit, but also, adults of *S. levis* were mostly located under the soil surface, especially during the day.

As reported for other Curculionidae species, *S. levis* was confirmed to present a nocturnal behavior with adults being more active and exposed in the soil surface at night. However, current insecticide treatments do not target *S. levis* adults while most applications happen during the day. Considering the low efficacy of *S. levis* control and the difficulty of placing effective insecticide concentrations near the hidden insect underneath the soil, nocturnal applications are a great opportunity to reach exposed *S. levis* adults and should be conducted for pest control improvements.

Further efficacy improvements for *S. levis* control could be accomplished when applying solid insecticides. Present laboratory and field results showed the potential of solid applications for *S. levis* control. Even with inappropriate granular formulations, some solid applied insecticides were effective to reduce *S. levis* population, reduce sugarcane injury and improve yield due to an extended residual. As described in the literature, specific granular formulations of insecticides can slowly release its active ingredient and can promote longer insect control than liquid applied insecticides. In addition, the dosage increment of insecticides (thiamethoxam + lambda-cyhalothrin) was also shown to increase *S. levis* mortality suggesting the necessity of higher insecticide concentrations. Therefore, the development of adequate insecticide granular formulations for soil applications with appropriate active ingredient concentration should optimize *S. levis* control. These results should encourage the pesticide industry to develop new granular formulations for soil applications aiming *S. levis* control.

In addition, field results have showed the importance of S. *levis* monitoring evaluations to observe the population fluctuation and to determine the necessity and best moment for insecticide treatments. During sugarcane planting, insecticide applications for S. *levis* control should only be conducted if insects are detected prior or at planting unless specific granular insecticide formulations are available. Best control of *S. levis* control after planting may be achieved when conducting applications after the detection of insect presence. In ratoon sugarcane treatments, a second insecticide application based on pest monitoring evaluations may also improve *S. levis* control.

Therefore, in despite of the overall difficulty of effectively controlling *S. levis*, the present study supports new *S. levis* management approaches in sugarcane. These proposed methods, including nocturnal applications, solid applications with adequate granular insecticide formulations, dosage increment, frequent pest monitoring and sequential insecticide applications, when properly associated with an effective Integrated Pest Management (IPM) program, should improve the control of *S. levis* in sugarcane.