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ORIGINAL ARTICLE

Attraction of the sugarcane billbug, *Sphenophorus levis*, to vinasse and its volatile composition

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Abstract

The expansion of sugarcane plantations in Brazil and the discarding of vinasse into the sugarcane field have been speculated to contribute to the growing population of the billbug *Sphenophorus levis*. This beetle attacks the root system and forms galleries in rhizomes, causing damage or even the death of host plants. It has been suspected that vinasse, a residue from ethanol production, can release volatiles that are attractive to the insect; however, no study has addressed this hypothesis so far. The aim of this study was to investigate the attractiveness of vinasse to *S. levis* adults and identify the volatile compounds released by this substance using gas chromatography–mass spectrometry (GC–MS). We found that vinasse was more attractive to *S. levis* than sugarcane stems, molasses and wastewater under laboratory conditions, but not than cane stems at field conditions. Our GC–MS analysis revealed the presence of primary alcohols, terpenes and organic carboxylic acids in vinasse. When a mixture of the commercial synthetic compounds identified in the chemical analysis was tested in the laboratory, a strong attraction of the insects to the mixture was observed. Our results help to explain how vinasse can contribute to the infestations of *S. levis* in sugarcane fields and shed new light on the development of strategies to control this pest using chemical attractants.

Keywords Coleoptera · Host search · Semiochemicals · Soil insects · Sugarcane byproducts

Introduction

Brazil is the world's largest producer of sugarcane, with an estimated annual production of 625.96 million tons and a harvested area of 8.61 million hectares (CONAB 2018). The sector has a very high level of technological investments in the production and processing of sugarcane due to the national and international demand for sugar and ethanol.

As a fuel alternative that has little impact on the environment, ethanol has been gaining popularity, and its production has been increasing yearly. During sugarcane processing, known as fractional distillation of the sugarcane juice, a liquid substance known as vinasse is produced; it is dark brown in appearance, has a strong and characteristic odour and is rich in nutrients and organic matter. Each litre of ethanol produced generates 13 L of vinasse (Paulino et al. 2011). Due to its richness in nutrients, vinasse is used in the fertigation of sugarcane, minimizing the consumption of water and synthetic fertilizers (Robles-González et al. 2012). The fermentation of vinasse with soil organic matter has promoted improvements in the soil microbiome (Wei et al. 2014).

With the expansion of sugarcane crops to new areas, an increase in some insect populations has also been observed. An example is the sugarcane billbug *Sphenophorus levis* (Coleoptera: Curculionidae). During the larval stage, this beetle can compromise the entire development of the plant by constructing channels in the rhizome, causing losses of up to 25 t ha^{-1} $year^{-1}$ (Precetti and Arrigoni 1990). Among the main reasons for the spread and establishment of this species in sugarcane is the transport and use of seedlings infested with the pest. It is also noted that in areas where vinasse is applied, there is an increase in the *S. levis* population (Precetti and Arrigoni 1990). The reasons are not clear, but it is likely that the increase in soil moisture due to vinasse applications plays

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an important role in the survival of the insect and its populations (Dinardo-Miranda 2018). Another hypothesis to explain this fact is that fermentation of vinasse can release volatile compounds that attract the insect, since a previous study identified the attractiveness of volatiles from vinasse to a different group of insect, the stable flies *Stomoxys calcitrans* (Diptera: Muscidae) (Jelvez Serra et al. 2017).

To date, no study has identified volatiles produced by vinasse and assessed the volatiles with respect to their ability to attract *S. levis*. Some studies have sought to develop sexual pheromones (Zarbin et al. 2004, 2009; Wadt 2016) for trap baiting, but there are still no conclusive results regarding the potential for use. The only olfactory cue known as an attractant to adults of *S. levis* is issued in baits prepared with sugarcane stems cut longitudinally in half and placed with the cut faces on the ground between rows. These bait traps are recommended to be 30 cm in length for monitoring of this insect (Almeida et al. 2008). However, no studies have been carried out to determine which length of the stem has the best cost/efficiency for insect attraction and which vinasse volume could be equivalent to that selected stem length.

In the present study, we investigated, under laboratory and field conditions, the olfactory response of *S. levis* adults to vinasse, cane stem, molasses and wastewater, as well as baits prepared with cane stems at different lengths. We also identified the volatile compounds emitted by vinasse by means of gas chromatography coupled to mass spectrometry (GC–MS) and tested the attraction of *S. levis* to synthetic analytical standards of the identified compounds. Our results may help to explain the higher populations of the sugarcane billbug in sugarcane areas that receive vinasse application. In addition, since the control of this species has been carried out with chemical insecticides, but with unsatisfactory results, the data presented here offer new insight for the development of strategies for monitoring and controlling insects based on chemical attractants.

Materials and methods

Insects

Adults of *S. levis* were collected weekly in sugarcane fields in São João da Boa Vista—SP-Brazil (21°55′33.84″ S, 46°55′26.49″ O). Bait traps of cane stems 30 cm in length, cut longitudinally, were distributed in the sugarcane field to collect the insects. Each bait trap consisted of two halves of the stem, placed at the base of the row with the cut side facing the ground, and covered with straw. The insects were collected every 3–7 days and taken to the Biological Control Laboratory at the "Advanced Research Center for Plant Protection and Animal Health—CAPPPSA", Biological Institute, Campinas—SP, where they were kept in trays at 25 °C and fed cane stems that were changed every 7 days. The insects were separated by gender according to the taxonomic characteristics of males and females (Vaurie 1978; Zorzenon et al. 2000).

Olfactometric assays with vinasse

To investigate the olfactory response of *S. levis* adults to vinasse, two studies were carried out: olfactory response analysis with vinasse, sugarcane stem, molasses or wastewater, and olfactory response analysis with vinasse at different volumes and different lengths of sugarcane stems. A two-way olfactometer made of polyvinyl chloride (95 cm long × 3 cm diameter) was used for this purpose (Filgueiras et al. 2016) (Fig. 1). At each end of the olfactometer, a pipe elbow was inserted to receive the treatments. To prevent the insects from reaching the treatments, a voile fabric was extended and fixed between the olfactometer set and each elbow. The first study aimed to assess the attraction of *S. levis* adults to sugarcane vinasse in the laboratory by comparing vinasse with cane stem, cane molasses, sugar industry wastewater, and distilled water (control). The following combinations of treatments were tested: (1) vinasse vs sugarcane stem; (2) vinasse vs molasses; (3) vinasse vs

Fig. 1 Two-way olfactometers for olfactory response analysis with vinasse (2 mL), sugarcane stem (5 cm), molasses (40 g) and wastewater (2 mL) (**a**) and with different volumes of vinasse (6, 12, 25, 50 and 100 mL) and different lengths of sugarcane stems (10, 15, 20, 25 and 30 cm) (**b**)

distilled water; (4) vinasse vs wastewater; and (5) distilled water vs distilled water.

For the comparisons, the treatments were placed at the ends of the olfactometers by the openings of the "elbow" that were kept open. For the vinasse, wastewater and distilled water treatments, 2 mL of each product was applied with the aid of a pipette on a dental cotton wad 4 cm in length. For the molasses treatment, 40 g of the product was applied to the cotton. A stem was removed from the base of a sugarcane plant (variety CV6654) and cut longitudinally into two equal parts, and a 5 cm slice was used for each replicate. The 5 cm slice could generate a higher volume of vinasse compared to the 2 mL used on the cotton, but was chosen based on a previous test showing its lower ability to attract the insects compared to the 2 mL vinasse.

Olfactory responses were assessed separately for male and female adult insects, establishing 20 replicates for each gender. The insects were individually released in the center of the olfactometer. Pieces of aluminium foil were placed over the openings to prevent light ingress and, thus, avoid external interferences during bioassays.

After releasing an insect at the center of the olfactometer, it was considered a choice when the insect moved a distance that exceeded half of that of one side of the device (arm) within 25 min. Each insect and each source of odour was used only once and then discarded. In addition, after each replication, the olfactometer and the voile fabric were sanitized with running water plus neutral detergent, cleaned with alcohol and left to dry in natural conditions. The position of the olfactometer was inverted after each replicate to prevent external interference with the system. The tests were conducted under controlled conditions (25 °C ± 1 °C, in the dark).

A second study aimed to compare vinasse at different volumes with sugarcane stems at different lengths to determine which length of the stem and which volume of vinasse would have similar attraction to the insect in the laboratory condition. The following combinations were tested: (1) 2 mL vinasse vs a 10 cm stem; (2) 2 mL vinasse vs a 15 cm stem; (3) 2 mL vinasse vs a 20 cm stem; (4) 2 mL vinasse vs a 25 cm stem; (5) 2 mL vinasse vs a 30 cm stem; (6) 6 mL vinasse vs a 30 cm stem; (7) 12 mL vinasse vs a 30 cm stem; (8) 25 mL vinasse vs a 30 cm stem; (9) 50 mL vinasse vs a 30 cm stem; (10) 100 mL vinasse vs a 30 cm stem; (11) 100 mL distilled water vs a 30 cm stem; and (12) 100 mL vinasse vs 100 mL distilled water. The olfactory responses were, again, assessed separately for male and female adult insects, with 20 replicates each gender.

The same two-way olfactometers mentioned before were used also for the experiment cited above, but with some modifications to suit the treatments (Fig. 1). At each end, instead of an elbow, one "tube" of 40 cm long was attached to the "pipe fitting" to receive the treatments. To prevent the insects from reaching the treatments, a voile fabric was extended and fixed between the olfactometer set and each 40 cm tube. With the aid of pipettes, the 2 mL volume of vinasse was applied on a dental cotton wad 4 cm in length, the volume of 6 mL on a wad of 6 cm, the volume of 12 mL on a wad of 12 cm, and the volumes of 50 mL and 100 mL on a wad of 30 cm, the last of which was at the same length as the cane stem recommended for use in the field. Cane stems were tested at lengths of 10, 15, 20, 25 and 30 cm, for which the stems were removed from the base of a cane plant, cut at different lengths and divided longitudinally in the middle, with only one slice used in each bioassay.

The treatments were placed on the ends of the olfactometers inside the extra tubes, keeping the ends opened. The subsequent methodology was the same as previously described in the experiment "Bioassays of olfactory response to vinasse, sugarcane stem, molasses and wastewater".

Field experiments

A field study was carried out to evaluate cane stem, vinasse and combinations of cane stems and vinasse in their ability to attract adults of *S. levis*. An experiment was carried out in an field of sugarcane (variety CTC09) with plants that were approximately 3 m high and infested with sugarcane billbugs. This field was the only one avialable with a large infestation of *S. levis* adults, the reason why we used here a different variety of sugarcen from that tested in the lab experiments.

Four treatments were evaluated: (1) stem; (2) stem + vinasse; (3) distilled water; and (4) vinasse. The experiment was designed by randomized blocks, with four different treatments withing a block, and with a total of 24 blocks (replications). Treatments (baits) were spaced 6 m apart from each other in the blocks, and blocks were spaced 6 m or six cane rows apart from each other.

The treatment with the stem (1) consisted of the cane stem (variety RB867515) recommended for insect monitoring at a length of 30 cm, cut longitudinally in the middle and placed next to the cane row with the two cut sides facing the ground, covered with straw (Dinardo-Miranda 2014). Up to date, no variety is known as resistant and less attractive to *S. levis*, which explains again the use of one more different variety compared to the one used for the lab experiments.

Treatment (2) stem + vinasse consisted of the stem described in the previous treatment, on a 1000 mL plastic pot (15×9 cm^2) buried with its opening at the ground level, containing 100 mL of vinasse and covered with a screen (2×2 mm^2 mesh). The volume of 100 mL of vinasse was chosen because it is approximately the volume of vinasse generated by a stem of 30 cm, considering that the volume of vinasse generated by 1 ton of cane is 980 L. The stem was placed with the two cut sides down over the screen-covered

pot buried next to a row of sugarcane and later covered with straw. Treatment (3), with just vinasse, consisted of the pot buried next to the plant row, containing only vinasse at a volume of 100 mL, covered with straw to avoid drying the liquid. Treatment (4), of distilled water only, consisted of a pot buried next to the plant row containing 100 mL of distilled water and covered with straw to prevent the drying of the liquid.

The experiment was evaluated 3 days after its establishment. Adult insects found in the bait traps were collected and taken to the laboratory for quantification.

Headspace collection, analysis and olfactometric assays with the volatile compounds

This study aimed to determine the volatiles of vinasse and to evaluate the olfactory responses of *S. levis* adults to these compounds. For volatile collection, 2 mL of vinasse was applied to a cotton roll that was inserted into a cylindrical glass chamber (25 cm long × 6 cm i.d.) and exposed to an air flow of 0.6 L/min for 2 h. Volatiles in the headspace were trapped on 30 mg of Super Q® (Supelco, Bellefonte, PA, USA) in a glass pipette (8.5 cm long × 0.5 cm i.d.) with the adsorbent held in place with glass wool plugs, and, after aeration, the polymer was eluted with 500 µL of dichloromethane (Supelco, Bellefonte, PA, USA). The final solution was concentrated to 200 µL. Four replicates, consisting each by one cylindrical glass chamber, were performed for volatiles collection ($N = 4$).

Qualitative analysis of the vinasse compounds was initially performed by gas chromatography-flame ionization detection (GC-FID, Shimadzu GC-2010, Kyoto, Japan) using an HP-1 capillary column (Agilent Scientific, Santa Clara, CA,USA; 30 m × 0.25 mm × 0.25 µm) and helium as the carrier gas. Aliquots of 1 µL were injected, with an injector temperature of 240 °C. The column temperature was maintained at 60 °C for 1 min, increased at a rate of 15 °C per minute until reaching 320 °C and maintained at that temperature for 10 min. Compound identification was done with gas chromatography-mass spectrometry (GC–MS, Varian 4000, Palo Alto, CA, USA), equipped with an HP5-MS capillary column (Agilent Scientific, Santa Clara, CA, USA; 30 m × 0.25 mm i.d. × 0.25 µm). Helium was used as the carrier gas and the column temperature programme was the same as that described for the GC-FID procedure above. Identification of the compounds present in vinasse was performed by comparing the retention times and the acquired mass spectra with those available in the NIST 98 library (Mass Spectral Library).

Another study aimed to evaluate, under laboratory conditions, the olfactory response of *S. levis* adults to commercially obtained standards of the identified volatile chemical compounds of vinasse: propanoic acid (Sigma®, purity 99.5%), butyric acid (Sigma®, purity 99%), 1-pentanol (Sigma®, purity 99.5%), limonene (Sigma®, purity 97%), caproic acid (Sigma®, purity 99%), valeric acid (Sigma®, purity 99%), isobutyl acetate (Sigma®, purity 98%) and cyclohexanemethanol (Sigma®, purity 99%). A mixture was tested at three concentrations, and mineral oil was used as the solvent and alone as the control. The following combinations were tested: (1) 1000 ppm mixture of the overall compounds vs mineral oil; (2) 100 ppm mixture of the overall compounds vs mineral oil; (3) 10 ppm mixture of the overall compounds vs mineral oil; and (4) mineral oil vs mineral oil.

To obtain the three above-cited concentrations, the standards of the compounds identified in our analysis were diluted to 1000 ppm (1 µL of the pure product and 1 mL of solvent mineral oil), called the stock solution, to 100 ppm (100 µL stock solution and 900 µL solvent) and to 10 ppm (10 µL stock solution and 990 µL solvent).

Olfactory responses were evaluated separately for male and female adult insects, establishing 20 replications each. Two-way olfactometers (95 cm in length and 3 cm in diameter) were used as described in the study "Bioassays of olfactory response to vinasse, cane stem, molasses and wastewater". For each replication, 2 mL of each solution was applied to a cotton wad. The rest of the methodology was the same as for previous bioassays.

Statistical analysis

Data from the laboratory-conducted olfactometer bioassays were evaluated by a binomial test in R software, version 3.0.2 (Vienna 2018). The data of the field experiments were submitted to analysis of variance (ANOVA), in which the raw data were transformed into Log $x + 1$ and compared by the Tukey test at the 5% level of significance. The data were processed in SPSS (Statistical Package for Social Sciences) software version 15.0.

Results

Attraction of *S. levis* to vinasse under laboratory conditions

In the comparisons of vinasse with the stem, molasses, wastewater and distilled water (negative control), the adults of *S. levis* were significantly more attracted to vinasse than to the other treatments. Comparing vinasse with the stem, 90% of the males and 75% of the females responded to vinasse. Vinasse was also more attractive than molasses and wastewater, attracting 90% and 85% for both sexes, respectively (Fig. 2).

Comparing vinasse at different volumes, stems at different lengths and the control (water), male and female adults

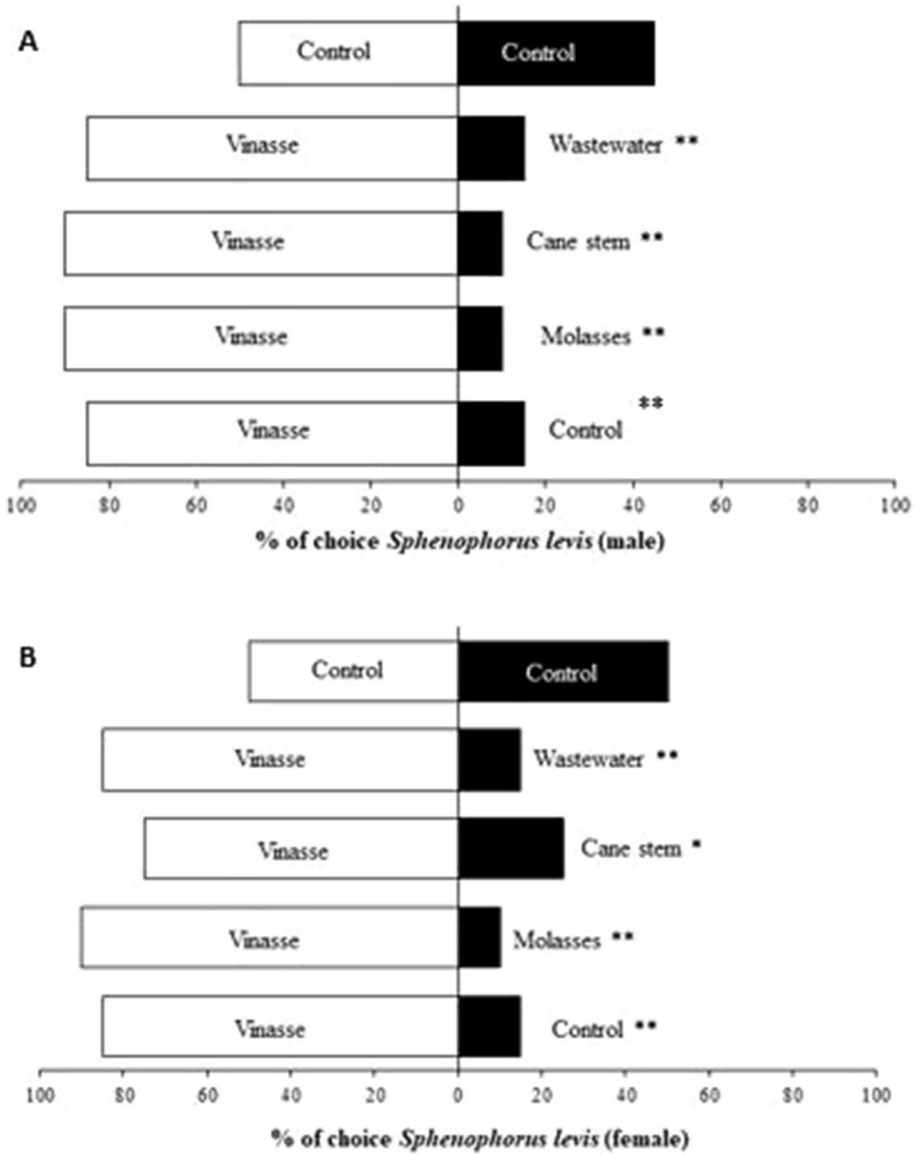


Fig. 2 Response of adult male (**a**) and female (**b**) *Sphenophorus levis* in a two-way olfactometric analysis to odours produced in the following comparisons: 2 mL vinasse vs 2 mL distilled water (control); 2 mL vinasse vs 40 g molasses; 2 mL vinasse vs 5 cm stem; 2 mL vinasse vs 2 mL wastewater; and 2 mL distilled water (control) vs 2 mL distilled water (control). The bars represent the initial response of the insects (when the insect reached one of the arms/edges of the olfactometer in 25 min). Asterisks indicate significant differences between treatments according to the binomial test (*$p < 0.05$ and **$p < 0.01$) $N = 20$

were significantly more attracted to vinasse at a volume of 100 mL and to stems at a length of 30 cm than to water (control). For males, adults were significantly also more attracted to vinasse at a volume of 2 mL than to stems at lengths of 10 and 15 cm (Fig. 3). For females, adults were also significantly more attracted to vinasse at a volume of 2 mL than to stems at lengths of 10, 15 and 20 cm (Fig. 3).

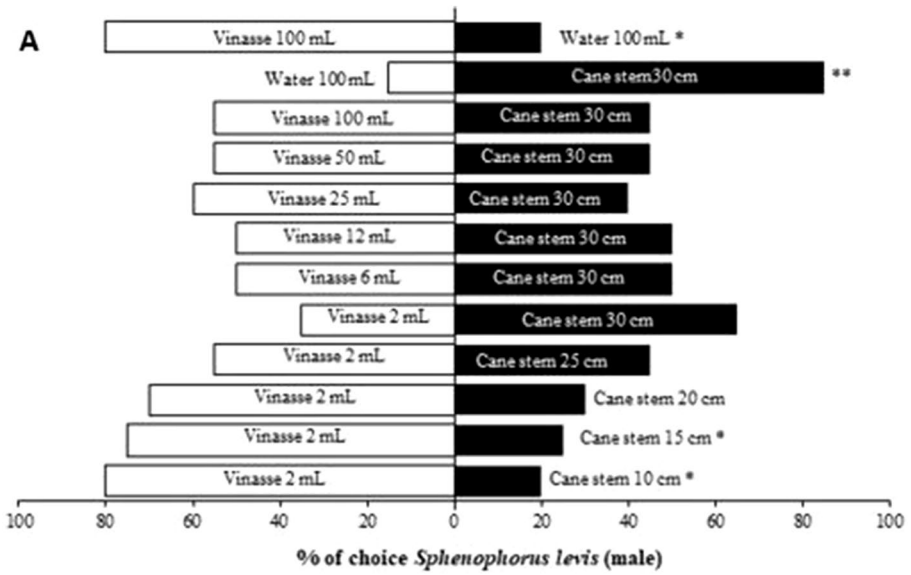
For vinasse at a volume of 2 mL compared to stems at different lengths, there was a gradual increase in the attraction to the stem as its length increased, with stems measuring 20–30 cm long for male and 25 and 30 cm for female showing similar attractiveness compared to that of vinasse. Vinasse at volumes of 6, 12, 25, 50 and 100 mL showed similar attractiveness to stem at a length of 30 cm (Fig. 3).

Field experiment

Vinasse was more attractive than the control (water), but it was less attractive than the combination of stem + vinasse and the stem alone ($F_{3,47} = 104.251$; $P < 0.001$) (Fig. 4).

Volatile collection and analysis

Using a headspace volatile collection system and GC–MS, we identified organic carboxylic acids, primary alcohols, an ester and a terpene as the volatiles released by vinasse. The organic carboxylic acids detected were propanoic acid, butyric acid, valeric acid and caproic acid. The primary alcohols detected were 1-pentanol and cyclohexanemethanol, as



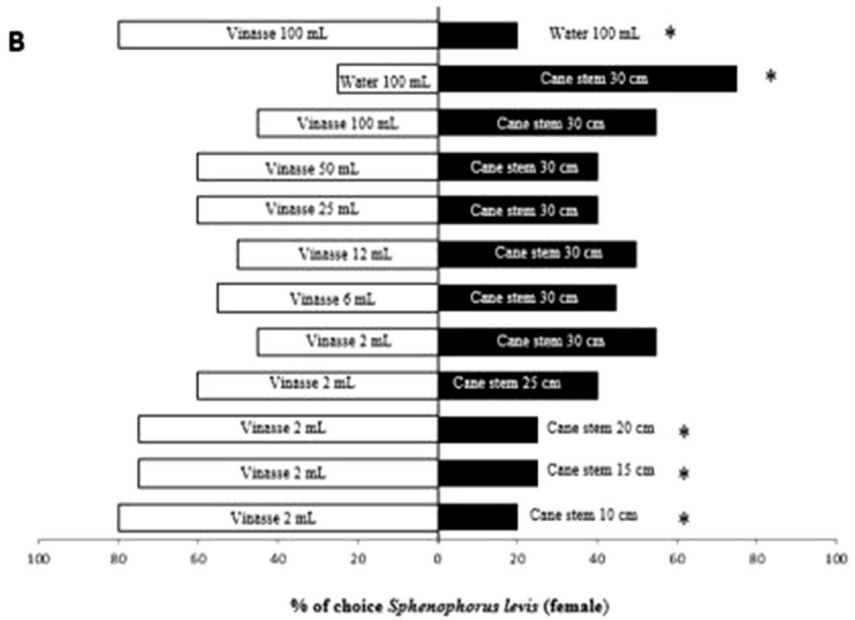


Fig. 3 Response of adult male (**a**) and female (**b**) *Sphenophorus levis* in a two-way olfactometer to odours produced in the following comparisons: 100 mL vinasse vs 100 mL water (control); 100 mL distilled water (control) vs s 30 cm stem; different volumes of vinasse compared to 30 cm stem; and 2 mL vinasse compared to different lengths of stems. The bars represent the initial response of the insects (when the insect reached one of the arms/edge of the olfactometer in 25 min). Asterisks indicate significant differences between treatments according to the binomial test (*$P<0.05$ and **$P<0.01$) $N=20$

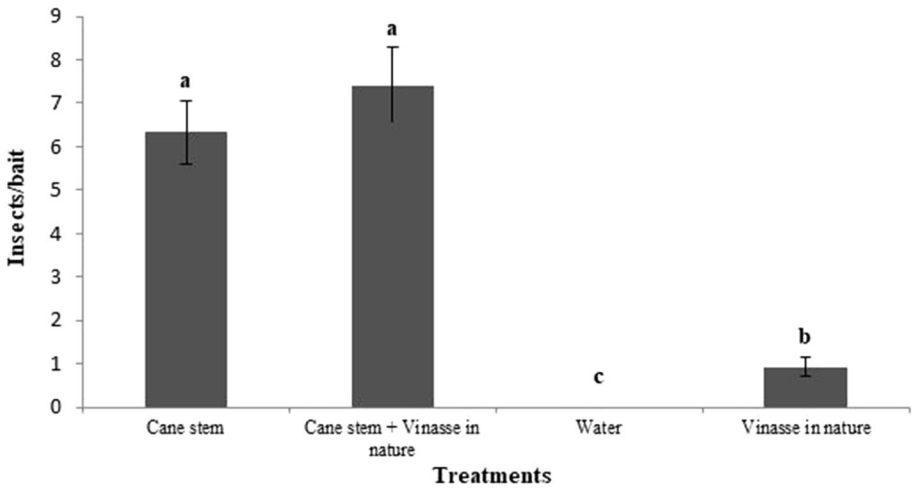


Fig. 4 Attraction of *Sphenophorus levis* adults to vinasse (100 mL), sugarcane stem (30 cm), stem (30 cm) + vinasse (100 mL) and distilled water (100 mL) (control) in field trials. Bars represent the mean ± SD. Different letters on the bars indicate differences between treatments (Tukey, $P<0.05$) $N=24$ blocks

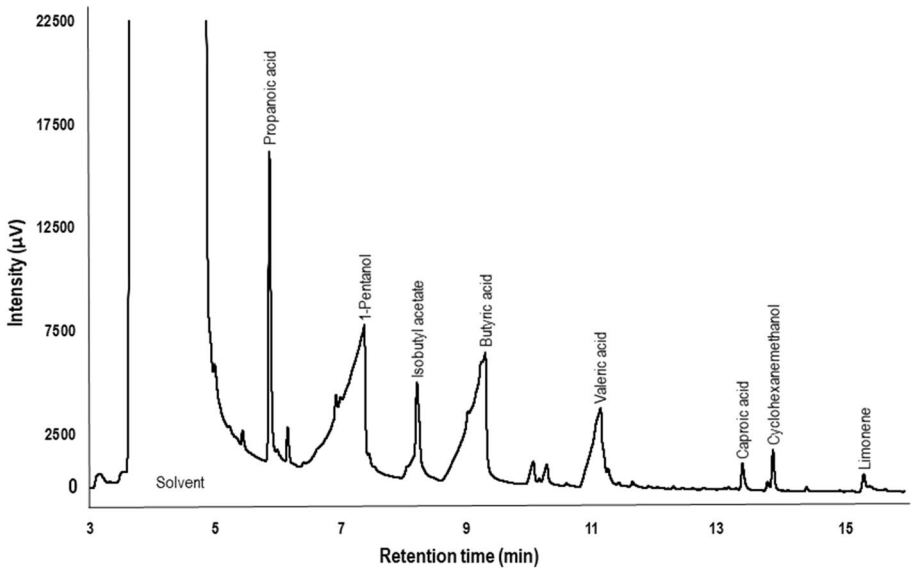


Fig. 5 Vinasse volatile compounds detected by GC–MS analysis. Peaks indicate the intensity, in microvolts, of each identified compound

well as the ester isobutyl acetate and the terpene limonene (Fig. 5).

Bioassay using the chemical compounds

The compound mixture significantly attracted male insects when tested at concentrations of 1000 and 100 ppm, attracting 80% and 75% of the insects released, respectively (Fig. 6). For females, the mixture significantly attracted insects only at the highest concentration of 1000 ppm, attracting 85% of the insects (Fig. 6).

Discussion

The two-way olfactometer was efficient for assessing the response of *S. levis* to the tested olfactory stimuli. The insect easily moved in the system and showed no tendency to choose a particular side based on some exogenous source of attractiveness, such as light or temperature. The responses were similar when we tested only the controls on both sides of the olfactometer (distilled water).

Vinasse was the most attractive substance for *S. levis* compared to water, wastewater and molasses according to the laboratory tests. This may help to explain why the largest infestations of this insect were in sugarcane areas treated with this substance, as previously observed and proposed (Dinardo-Miranda 2018). Recent studies have shown that vinasse is also an attractant for other insects, such as the stable fly *Stomoxys calcitrans* (Jelvez Serra et al. 2017) and other important pests of sugarcane.

According to Tinzaara et al. (2002), the olfactory attraction of insects to plant substrates (hosts) depends on the insect species, but some similarity in the responses may occur for different species depending on the presence of odours, degree of fermentation and eating habits of the insects. In a field experiment, adults of *Metamasius hemipterus* showed a higher preference for substrates of fermented pineapple and sugarcane than for their unfermented counterparts. In the present study, the greater attraction of *S. levis* adults to vinasse may be due to the higher degree of fermentation and, consequently, the greater concentration of volatile compounds in vinasse.

Vinasse was a better attractant for *S. levis* than wastewater, possibly because the sugarcane wastewater was used to wash sugarcane before milling, presenting less fermentation than vinasse. Likewise, vinasse was a better attractant than molasses, a byproduct of sugar production that has been recommended for use in association with cane stems to increase the attraction of insects to the bait in the field. In studies under laboratory conditions, cane stems combined with molasses and fermented for 24 and 48 h attracted 90% and 85% of *S. levis* adults, respectively, whereas cane stems combined with water and fermented for 24, 48 and 72 h attracted less than 50% of the insects (Girón-Pérez et al. 2009).

In general, the male and female adult insects showed the same trends in responses to the different natural compounds in all laboratory-conducted bioassays, suggesting little difference in their attraction to the compounds based on insect gender. In the tests with vinasse at a volume of 100 mL and stems at a length of 30 cm, compared with water, both insect genders were more attracted to vinasse and to the stem. Likewise, comparing the mixture of the chemical compounds with the mineral oil, both genders were significantly more attracted to a concentration of 1000 ppm, but only the males were more attracted to a concentration of 100 ppm than to water. This suggested a higher preference of males for the

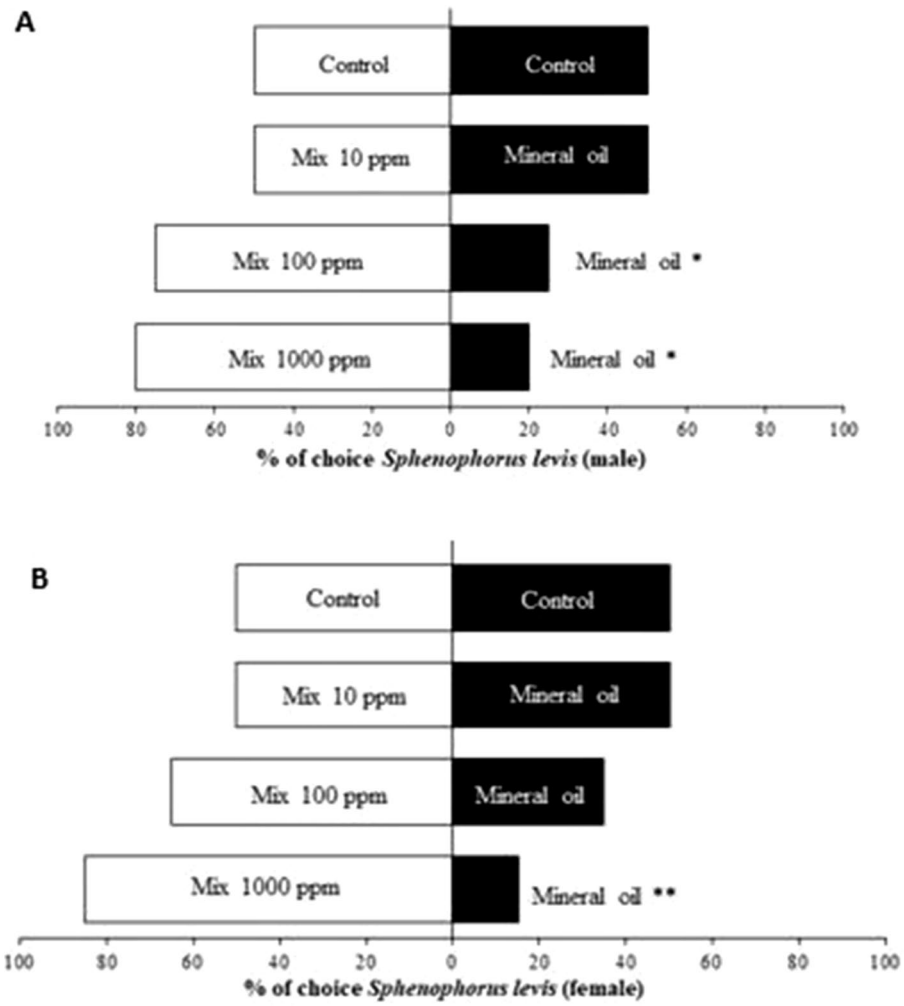


Fig. 6 Response of adult male (**a**) and female (**b**) *Sphenophorus levis* in a two-way olfactometric analysis to odours produced in the following comparisons: 1000 ppm mix vs mineral oil; 100 ppm mix vs mineral oil; 10 ppm mix vs mineral oil; and mineral oil (control) vs mineral oil (control). The bars represent the initial response of the insects (when the insect reached one of the arms/edge of the olfactometer in 25 min). Asterisks indicate significant differences between treatments according to the binomial test (*$P < 0.05$ and **$P < 0.01$) $N = 20$. Mix = mixture of all the compounds identified in vinasse

mixture of the chemical compounds, but further studies should be carried out to confirm this assumption. According to Girón-Pérez et al. (2009), male and female adults of *S. levis* showed similar attraction to sugarcane stems associated or not with molasses and to pineapple obtained after different fermentation periods. Jaffé et al. (1993) also found similar attractions of male and female adults of the palm weevil *Rhynchophorus palmarum* (Coleoptera: Curculionidae) to pineapple, banana and coconut palm.

The cane stem was less attractant than 2 mL of vinasse only at lengths shorter than 25 cm for females and 20 cm for males, suggesting a recommendation for using lengths greater than 25 cm for the collection, monitoring or control of these insects in the field. At a length of 30 cm, as recommended by Almeida et al. (2008), the stem was a better attractant for adults of *S. levis* than water. Increasing vinasse volumes up to 100 mL did not increase the attraction of *S. levis* compared to that of the stem 30 cm in length, probably due to saturation of the volatiles released by vinasse (mainly in larger volumes) distanced 40 cm from the insect release point, confusing the treatment choice of the insect.

In the field, unlike in the lab experiments, the cane stem proved to be more efficient for insect attraction compared to vinasse, probably due to the different methodologies used for the studies conducted under the lab and field conditions. In the lab, vinasse showed higher or similar attraction ability compared to that of the stems because both were still fresh in the olfactometer. In the field, vinasse smelled much weeker after 1 or 2 days of exposure to the natural environment, while the stem remained whole for at least 1 or 2 weeks. According to Lima et al. (2017), vinasse contain mainly alcohol (30.12%), which can be quickly volatized and drag other compounds. As for the 30 cm stem, Precetti and Terán (1983) proved it remains attractive to *S. levis* adults for more than 21 days after its set in the field, with peak of attractiveness in the 6th day. Moreover, adults that fell in the vinasse pots died, while those attracted to the stem remained alive, feeding on the stem, attracting even more adults by the release of pheromones (Zarbin et al. 2003, 2004, 2009).

Vinasse alone was a better attractant than water, confirming the results of the laboratory tests. Vinasse at a volume of 100 mL showed a much lower attraction ability compared to

that of the stem at a length of 30 cm but may attract much more insects when applied in the field as "fertigation" at volumes higher than 30 m^3 per hectare or 3 L per metre of cane row.

Cane stems have been recommended for the monitoring of *S. levis* and *M. hemipterus* in sugarcane fields, demonstrating the efficiency of attracting and capturing insects since the insects remain attached to the stem. They have also been recommended for the monitoring and control of *R. palmarum* in palm fields associated with the pheromone (Tiglia et al. 1998).

Previous reports tested four types of baits in a field experiment, consisting of cane stem, cane stem + cane syrup, cane stem + port wine and cane stem + cane molasses (El-Sayed et al. 2005). All were good attractants for *Graphania mutans* and *Tmetolophota* spp. The cane stem and cane stem + cane syrup groups were better attractants than cane stem + cane molasses and cane stem + port wine. Based on this study by El-Sayed et al. (2005) and on the present study, the use of cane stem associated with a by-product does not increase its bait efficiency compared to that of the stem alone.

In the present study, organic carboxylic acids, primary alcohols, an ester and a terpene were detected in vinasse, attracting adults of *S. levis* when the compounds were mixed and the overall mixture was tried at concentrations of 100 and 1000 ppm for male, and at a concentration of 1000 ppm for female. However, some of the compounds identified (e.g., cyclohexanmethanol) might be contaminants since negative control samples (empty cotton roll in glass chamber) were not collected. Thus, the bioassays with the mixture might be biased since contaminansts might had being included and had influenced the behavior of insects (attractants or repellents). In another study (Jelvez Serra et al. 2017), in addition to organic carboxylic acids and primary alcohols, phenols, ketones, aldehydes, and other compounds were also detected in vinasse by GC–MS analysis. Moreover, fatty acids, alcohols and esters were also identified as the volatile compounds present in vinasse using GC–MS analysis (Lima et al. 2017). The lower number of compounds found in the present study, using the same methodology (GC–MS analysis), was probably related to natural variations in vinasse samples obtained from one industry to another, as the results obtained by Jelvez Serra et al. (2017) were also different from those of Lima et al. (2017) with respect to the number and type of compounds detected.

The high content of short-chain organic acids gives vinasse a characteristic odour that can be perceived from a distance (Jelvez Serra et al. 2017) and may explain the strong odour from the mixture of compounds in the present study that resembled that of vinasse. These volatile acids include propanoic acid, butyric acid, valeric acid and caproic acid according to a previous study that reported the last three compounds (in vinasse) and according to the present study, which reported all listed compounds. All of these compounds were also detected in horse and cow manure according to previous studies (Jeanbourquin and Guerin 2007), showing an attraction ability to *S. calcitrans* (stable fly) in olfactory response tests as well as in studies of the physiological response of the antenna.

In general, vinasse consists of 93% water and 7% organic and inorganic compounds (Rolim et al. 2013). The variety of chemical compounds present in vinasse depends on the distillation processes to which it has been subjected. Different volatiles detected in two different vinasse samples attracted *S. calcitrans*, suggesting that vinasse is composed of a variety of chemical stimulants that can function as attractants irrespective of their distillation processes (Jelvez Serra et al. 2017).

The present study is the first to confirm the attraction ability of vinasse for *S. levis* under laboratory and field conditions. This highlights the industrial byproduct vinasse as a factor that is likely responsible for the increased infestations of the insect in areas of sugarcane treated with vinasse, as previously proposed (Precetti and Arrigoni 1990; Dinardo-Miranda 2018). Further laboratory and field studies are suggested to identify the volatiles produced by cane stems, which are highly attractive to *S. levis* in the field. In addition, combinations of pheromones, sugarcane stem and volatile compounds could be assessed, seeking the development of new technologies for monitoring, capturing and controlling *S. levis*. Other components, such as hydrolysed protein, could also be assessed for their ability to attract *S. levis*. Greater attraction of *C. sordidus* and *Metamasius* sp. adults to banana pseudocoel baits was observed when the baits were associated with the hydrolysed protein of the trademark Bio Fruit (Pavarini et al. 2018).

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Author contributions LdFM, MT and LGL were responsible for setting up the bait traps and catching insects in the field. LdFM conducted all the bioassays carried out in the laboratory and in the field. MT and JMSB were involved with the chromatographic analysis. CJB and LGL helped with the field bioassays and statistical analyses. All authors helped with the writing and reviewing of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

Almeida LC, Stingel E, Arrigoni EB (2008) Monitoramento e controle de pragas da cana-de-açúcar. Piracicaba, Centro de Tecnologia Canavieira, p 15

CONAB (2018). Acompanhamento da Safra Brasileira Cana-de-açúcar—V.5—SAFRA 2018/19—N.1—Primeiro levantamento—Maio 2018, Brasília: Conab, Boletim Cana 1, 66 p. https://www.conab.gov.br/info-agro/safras/cana/boletim-da-safra-de-cana-de-acucar

Dinardo-Miranda LL (2018) Nematoides e pragas da cana-de-açúcar., 2.ª. Atual, Campinas: Instituto Agronômico

Dinardo-Miranda LL (2014) Nematoides e pragas da cana-de-açúcar. 2.a. Atual. Instituto Agronômico, Campinas

El-Sayed AM, Heppelthwaite VJ, Manning LM et al (2005) Volatile constituents of fermented sugar baits and their attraction to lepidopteran species. J Agric Food Chem 53:953–958. https://doi.org/10.1021/jf048521j

Filgueiras CC, Willett DS, Pereira RV et al (2016) Eliciting maize defense pathways aboveground attracts belowground biocontrol agents. Sci Rep 6:1–9. https://doi.org/10.1038/srep36484

Girón-Pérez K, Nakano O, Silva AC, Oda-Souza M (2009) Atração de adultos de Sphenophorus levis Vaurie (Coleoptera: Curculionidae) a fragmentos vegetais em diferentes estados de conservação. Neotrop Entomol 38:842–846. https://doi.org/10.1590/S1519-566X2009000600019

Jaffé K, Sánchez P, Cerda H et al (1993) Chemical ecology of the palm weevil *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae): attraction to host plants and to a male-produced aggregation pheromone. J Chem Ecol 19:1703–1720. https://doi.org/10.1007/BF00982302

Jeanbourquin P, Guerin PM (2007) Chemostimuli implicated in selection of oviposition substrates by the stable fly Stomoxys calcitrans. Med Vet Entomol 21:209–216. https://doi.org/10.1111/j.1365-2915.2007.00685.x

Jelvez Serra NS, Goulart HF, Triana MF et al (2017) Identification of stable fly attractant compounds in vinasse, a byproduct of sugarcane–ethanol distillation. Med Vet Entomol 31:381–391. https://doi.org/10.1111/mve.12246

Lima AVA, Barbosa MAS, Cunha LCS et al (2017) Volatile compounds obtained by the hydrodistillation of sugarcane vinasse, a residue from ethanol production. Rev Virtual Quim 9:764–773. https://doi.org/10.21577/1984-6835.20170047

Paulino J, Zolin CA, Bertonha A et al (2011) Estudo exploratório do uso da vinhaça ao longo do tempo: II. Características da cana-de-açúcar. Rev Bras Eng Agrícola e Ambient 15:244–249. https://doi.org/10.1590/S1415-43662011000300004

Pavarini GMP, Demonari CG, Pavarini R, Souza IV (2018) Atração de adultos de Cosmopolites sordidus e Metamasius sp. (Coleoptera: Curculionidae) por armadilhas associadas a inseticida e proteina hidrolisada. Cult Agronômica 27:148–159

Precetti AACM, Arrigoni EDB (1990) Aspectos bioecológicos e controle do besouro Sphenophorus levis (Vaurie, 1978) (Coleoptera, Curculionidae) em cana-de-açúcar. Bol Técnico Copersucar 1–15

Precetti, A.A.C.M.; Terán, F.O. (1983) Gorgulho da cnana-de-açúcar, Sphenophorus levis Vaurie, 1978, e Metamasius hemipterus (L., 1765) (Col., Curculionidae). Reun Técnica Agronômica 32–37

Robles-González V, Galíndez-Mayer J, Rinderknecht-Seijas N, Poggi-Varaldo HM (2012) Treatment of mezcal vinasses: a review. J Biotechnol 157:524–546. https://doi.org/10.1016/j.jbiotec.2011.09.006

Rolim MM, Lyra MRCC, Duarte ASD et al (2013) Influência de uma lagoa de distribuição de vinhaça na qualidade da água freática. Rev Ambient Água Interdiscip J Appl Sci 8:155–171. https://doi.org/10.4136/1980-993X

Tiglia EA, Vilela EF, Moura JIL, Anjos N (1998) Eficiência de armadilhas com feromônio de agregação e cana-de-açúcar na captura de *Rhynchophorus palmarum* (L.). An da Soc Entomológica do Bras 27:177–183. https://doi.org/10.1590/S0301-80591998000200002

Tinzaara W, Dicke M, van Huis A, Gold CS (2002) Use of infochemicals in pest management with special reference to the Banana Weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). Int J Trop Insect Sci 22:241–261. https://doi.org/10.1017/S1742758400020877

Vaurie P (1978) Revision of the genus Sphenophorus in South America. American Museum Novitates, New York, pp 1–32

Vienna 2013 (2018) R development core team. R 3.0.2.R development core team. R 3.0.2

Wadt L (2016) Morfometria, amostragem populacional e reinvestigação do feromônio de Sphenophorus levis (Coleoptera : Curculionidae). Tese versão simplificada (Doutorado em Entomologia)—Escola Superior de Agricultura "Luiz de Queiroz". Piracicaba 2016:1–22

Wei MM, Xu GP, Guo HR et al (2014) Effect of vinasse application on soil in sugarcane fields. Appl Mech Mater 700:270–275. https://doi.org/10.4028/www.scientific.net/AMM.700.270

Zarbin PHG, Arrigoni EB, Reckziegel A, Moreira JA, Baraldi PT, Vieira PC (2003) Identification of male-specific chiral compound from the sugarcane weevil *Sphenophorus levis*. J Chem Ecol 29(2):377–386

Zarbin PHG, Princival JL, Santos AA, De Oliveira ARM (2004) Synthesis of (S)-(+)-2-Methyl-4-octanol: male-specific compound released by sugarcane weevil *Sphenophorus levis* (Coleoptera: Curculionidae). J Braz Chem Soc 15:331–334

Zarbin PHG, Rodrigues MACM, Lima ER (2009) Feromonios de insetos: Tecnologia e desafios para uma agricultura competitiva no Brasil. Quim Nova 32:722–731. https://doi.org/10.1590/S0100-40422009000300016

Zorzenon FJ, Bergmann EC, Bicudo JEA (2000) Primeira ocorrência de Metamasius hemipterus (Linnaeus, 1758) e Metamasius ensirostris (Germar, 1824) (Coleoptera, Curculionidae) em palmiteiros dos gêneros Euterpe e Bactris (Arecaceae) no Brasil. Arq Inst Biol 67:265–268