JOURNAL OF APPLIED ENTOMOLOGY

J. Appl. Entomol.

ORIGINAL CONTRIBUTION

The social wasp *Polybia fastidiosuscula* Saussure (Hymenoptera: Vespidae) uses herbivore-induced maize plant volatiles to locate its prey

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Keywords
corn plants, indirect defence, olfactometer, semichemical, social insect

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Received: July 6, 2016; accepted: October 26, 2016.

doi: 10.1111/jen.12378

Abstract

Social wasps in the *Polybia* genus are important for use as pest-control agents in agricultural systems. The objective of this study was to investigate the behavioural responses of *Polybia fastidiosuscula* Saussure (Hymenoptera: Vespidae) to volatiles from maize, both constitutive volatiles and those induced by the herbivory of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). To assess the behavioural response of *P. fastidiosuscula* to *S. frugiperda* larvae, undamaged plants, *S. frugiperda*-damaged plants, mechanically damaged plants, mechanically damaged plants plus regurgitant from larvae and extracts from various treatments, bioassays were conducted in a Y-olfactometer. In addition, the volatiles from plants subjected to different treatments were collected via aeration, and they were quantified and identified. The wasps showed a greater preference for plants with damage induced either by larval feeding or by being mechanically damaged plus regurgitant than for undamaged plants or either larvae alone or mechanically damaged plants. Wasps were more attracted to extracts from plants + *S. frugiperda* larvae and to an extract from mechanically damaged plants + the regurgitant of larvae compared to hexane. The primary compounds induced by herbivory for 5–6 h after the beginning of the damage or regurgitant treatment were identified as α-pinene, β-myrcene, (*Z*)-3-hexenyl acetate, limonene, (*E*)-ocimene, linalool, DMNT, (*E*)-β-farnesene, TMTT and indole. The results presented here show that the social wasp *P. fastidiosuscula* uses herbivore-induced plant volatiles from maize to locate its prey.

Introduction

The fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) is a major pest of maize, and it occurs in all the maize-producing regions in the Americas. This species attacks plants starting from its emergence until cob formation (Cruz 1995), and significant reductions in crop productivity rates have been observed every year (Araújo et al. 2001; Farinelli and Fornasieri-Filho 2006; Fernandes and Carneiro 2006; Michelotto et al. 2011). Traditionally, the management of this pest is conducted using pesticides that kill the pest's natural enemies in addition to the target pest itself because the pesticides are not selective (Toscano et al. 2012). However, significant progress has been made to identify and use natural enemies to control *S. frugiperda* effectively (Prezoto and Machado 1999; Wyckhuys and O'Neil 2006). Many social wasps, such as *Polybia* spp., prey on several groups of invertebrates and use protein

from these preys to feed their offspring (Prezoto et al. 1994, 2006; Giannotti et al. 1995; Andrade and Prezoto 2001). Thus, these social wasps can serve as pest-control agents in agricultural systems because their predation reduces herbivorous pest populations (Picanço et al. 2001; Hernandez et al. 2009; Santana et al. 2012).

Polybia fastidiosuscula Saussure (Hymenoptera: Vespidae) is a swarm-founding species with wide distribution in South America (Richards 1978). Its nests are large (approximately 30 cm long) and may remain active for several months. Recently, De Souza et al. (2010) investigated its homing ability and determined that *P. fastidiosuscula* workers cover an area of approximately 81 m from the nest to engage in foraging activities. According to Prezoto et al. (2008), similar to other species in the *Polybia* genus, this insect actively catches its preys, which are usually Lepidoptera larvae. The use of social wasps in the management of *S. frugiperda* on maize culture seems feasible, but their use in optimizing the biological control of maize pests requires an understanding of the foraging strategy that wasps use to locate their prey.

Plants constitutively emit volatile organic compounds (VOCs), which herbivores then exploit to locate a host (Jolivet 1998). Under attack by herbivores, plants emit a wide variety and quantity of herbivore-induced plant volatiles (HIPVs), which consist of specific tracks that are detectable by many natural enemies when locating their hosts/prey (Paré and Tumlinson 1999; Howe and Jander 2008). Interactions among plants, herbivores and natural enemies have been studied in recent decades (Kennedy 2003; Inbar and Gerling 2008; Tan and Liu 2014). Since the discovery that plants release HIPVs and thus recruit natural enemies, researchers have sought ways to harness this chemical communication system because it may assist in developing strategies to improve the efficiency of natural enemies and pest suppression (Schnee et al. 2006; Birkett and Pickett 2014).

The release of HIPVs generally follows a temporal pattern, with green leaf volatiles (GLVs) being issued first because they are released from damaged cell membranes (Hatanaka et al. 1987), followed by other volatiles that are synthesized and released by the plants in response to the perception of elicitors in the saliva or the regurgitant of insects (Paré and Tumlinson 1997; Turlings et al. 1998). These volatiles may be used by natural enemies of herbivores to locate plants that carry potential prey. The responses in some plants start within minutes after damage, while in other plants, the effects are observed only after years have passed (Baldwin 1994). Maize is known to respond to herbivore attacks within hours (Turlings and Tumlinson 1992). However, the times when the social predatory wasp is attracted to and has the ability to use *S. frugiperda*-induced maize volatiles to locate its prey are not known.

The objective of this study was to investigate the behavioural response of *P. fastidiosuscula* to constitutively emitted volatiles of maize and their inductions, at different times, by *S. frugiperda* herbivory.

Materials and Methods

Plants and insects

Maize seeds (30F35 conventional hybrid) were sown individually in 200-ml plastic cups containing clay soil collected in the field (consisting of 59% clay, 5% silt and 36% sand). To correct the soil acidity, dolomitic calcarium (RTNP 90%) was applied. The plants were kept in a greenhouse (at an average temperature of 27.5 ± 4°C) at the Embrapa Dairy Cattle Research Station (Juiz de Fora, MG, Brazil). When the plants reached 16 days of age (with an average height of 28 cm), they were used for bioassays and extract collection.

Spodoptera frugiperda individuals were obtained from a laboratory colony maintained at the Embrapa Maize and Sorghum Research Station (Sete Lagoas, MG, Brazil); this colony was started with the insects collected from one maize field. The eggs from this colony were used to establish a new colony at the Entomology Laboratory of Embrapa Dairy Cattle, which was used in this research. After hatching, the larvae were individually placed in plastic pots (measuring 3 cm diameter × 8 cm height) and maintained on an artificial diet, as adapted from Parra (2001). The larvae were maintained in climate-controlled chambers (phytotron-type) at 24 ± 1°C, 70 ± 10% R. H., and a 12:12-h L : D photoperiod. Third- and fourth-instar larvae were used in the experiments.

Polybia fastidiosuscula wasps were collected in the area surrounding the Federal University of Juiz de Fora in the city of Juiz de Fora, Brazil. The capture was performed by sweep netting during the hottest hours of the day (10:30 a.m. to 2:30 p.m.), when the wasps return to the nest after foraging. The wasps were isolated in plastic pots (3 cm diameter × 8 cm height) and transported to the laboratory. Before the behavioural bioassays, the wasps were held for 1 h in a room in which the olfactometry bioassays were performed for acclimatization.

Collecting *Spodoptera frugiperda* regurgitant

To collect *S. frugiperda* regurgitant, third- to fourth-instar larvae were kept on the maize plants for 24 h. After this period, the larvae were placed on a Petri dish, on which their heads were pressed with forceps for regurgitation. The regurgitant was then collected with a 2-ml Pasteur glass pipette that was connected to a silicone septum attached to a vacuum pump (Turlings et al. 1993). After suction, the regurgitant was transferred to a 1.5-ml Eppendorf® tube that was ready to use. Then, 10 μl of regurgitant was used to treat the plants.

Odour sources used in the bioassays

The induction of maize plants was performed using three *S. frugiperda* third- and fourth-instar larvae, or the plants were mechanically damaged with scissors (three fully expanded leaves per plant were cut at 25% of the distance from the apex to the base). Bioassays were performed using the following odour sources (OS):

OS1: clean air;
OS2: undamaged maize plants;
OS3: three *S. frugiperda* larvae;
OS4: plants + *S. frugiperda* larvae during the 1–2-h period after adding the larvae, without removing the larvae during the bioassay;
OS5: plants + *S. frugiperda* larvae during the 5–6-h period after the addition of larvae, without removing the larvae during the bioassay;
OS6: plants + *S. frugiperda* larvae during the 24–25-h period after adding the larvae, without removing the larvae during the bioassay;
OS7: plants treated with *S. frugiperda* larvae for 1 h and used in the bioassay at 5–6 h after removal of the larvae;
OS8: plants treated with *S. frugiperda* larvae for 1 h and used in the bioassay at 24–25 h after removal of the larvae;
OS9: plants that were mechanically damaged and used in the bioassay at 1–2 h after the damage;
OS10: plants that were mechanically damaged and used in the bioassay at 5–6 h after the damage;
OS11: plants that were mechanically damaged and used in the bioassay at 24–25 h after the damage;
OS12: plants that were mechanically damaged + treated with regurgitant and used in the bioassay at 5–6 h after treatment;
OS13: plants that were mechanically damaged + treated with regurgitant and used in the bioassay at 24–25 h after treatment;
OS14: plants that were undamaged + treated with regurgitant and used in the bioassay at 5–6 h after treatment;
OS15: plants that were mechanically damaged + H_2O; and
OS16: plants that were undamaged + H_2O.

Olfactometry bioassays with maize plants

The olfactometry bioassays were performed at the Entomological Laboratory of Embrapa Dairy Cattle using a Y-olfactometer (diameter: 3.5 cm, main arm: 30 cm length and side arms: 23 cm length each), which operated with a continuous air flow of 1.0 l/min; the air was pre-humidified, filtered with activated carbon and calibrated through a flow meter. Each arm of the olfactometer was connected via silicone tubing to two glass chambers (30 cm high × 8 cm wide) containing the odour sources.

There were two steps to the bioassays for assessing the olfactory response of the social wasp *P. fastidiosuscula* as follows: the evaluation of the wasp response to plants damaged by *S. frugiperda* larvae (first) and the evaluation of the wasp response to mechanically damaged plants (second).

During the first step, the odour sources used as stimulus were as follows: 1 – OS1 vs. OS1 (to check for any biases in the bioassay setup), 2 – OS1 vs. OS2, 3 – OS1 vs. OS3, 4 – OS2 vs. OS4, 5 – OS2 vs. OS5 and 6 – OS2 vs. OS6. In combinations 4, 5 and 6, the larvae were allowed to feed on the plants during the entire bioassay. There were also 7 – OS2 vs. OS7 and 8 – OS2 vs. OS8. In combinations 7 and 8, the larvae were allowed to feed on the plants for only 1 h.

During the second step, the odour sources used as stimuli were as follows: 1 – OS2 vs. OS9, 2 – OS2 vs. OS10, 3 – OS2 vs. OS11, 4 – OS12 vs. OS15, 5 – OS13 vs. OS15 and 6 – OS14 vs. OS16.

For each treatment, at least 40 *P. fastidiosuscula* workers were tested, and for all the bioassays, each individual was tested once and considered as one replicate. The wasps that walked upwind and reached the end of an odour source-containing arm within 10 min were recorded as a response. Individuals that did not walk upwind of any odour source within 10 min were recorded as having no response. The position of the olfactometer arms was reversed for every tested wasp to avoid any outside interference. After every 5 wasps were tested, the olfactometer was cleaned with 96° GL ethyl alcohol (Jales Machado, Goianésia, GO, Brazil) and kept in an oven at 50°C for 10 min. After every 10 wasps were tested, the plants

(odour sources) were exchanged, and the olfactometer was washed with water, cleaned with 96° GL ethyl alcohol and placed in an oven at 50°C for 20 min. All the olfactometry tests were conducted between 10:00 a.m. and 04:00 p.m., the time when the wasps are most active (Elisei et al. 2010).

Trapping of maize plant volatiles and olfactometry bioassays

Volatile collection was performed by air entrainment. To collect the plant volatiles, plastic cups (200 ml) containing maize plants were wrapped in aluminium foil from the base of the cup to the base of the stem, thereby minimizing contamination with volatiles from the soil in which the plants were grown (fertilized soil) or from other organisms (bacteria, fungi, etc.). Each maize plant was placed in a glass chamber (42 cm long × 16 cm wide), and the volatiles emitted were trapped in a glass tube (11 cm long × 1 cm diameter) containing 0.5 g of adsorbent polymer (HayeSep® D 80/100; Supelco, Belfonte, PA), as described by Zarbin (2001). A pushed humidified and charcoal filtered airstream (1 l/min) was maintained throughout the aeration system.

Because *P. fastidiosuscula* was attracted to volatiles released by plants with larval damage and regurgitant-treated plants, the volatile collections were conducted only with the following treatments: OS5, OS7, OS12 and OS2 (control). For all of the treatments, the volatiles released by the plants were collected over 3 h.

The compounds were desorbed from the adsorbent using 4 ml of distilled *n*-hexane (J.T. Baker® – 95% hexane) (Sovereign, Taboão da Serra, SP, Brazil) into borosilicate glass vials. The samples were pre-concentrated under a nitrogen flow (with a purity of 99.99%) to approximately 100 μl and placed in a freezer (−25°C) for later use in bioassays and chemical analyses. The samples used for chemical analysis were spiked with 1 μl of tetracosane, which was used as an internal standard for quantitative analysis (see below in chemical analysis).

The wasp behavioural responses to the volatiles collected from the plants with herbivore or mechanical damage were tested using a Y-olfactometer as described above. To evaluate the attractiveness of the volatile compounds collected from the maize plants, a piece of filter paper (3 cm × 2 cm) was impregnated with 10 μl of the collected volatile compound or *n*-hexane (control) and then placed at the base of each olfactometer arm. The volatiles used as stimuli were 1 – OS5 vs. *n*-hexane, 2 – OS7 vs. *n*-hexane and 3 – OS12 vs. *n*-hexane.

For each treatment, at least 40 *P. fastidiosuscula* workers were tested, and for all the bioassays, each individual was tested once.

Analyses of the extracts released from the maize

The air-entrainment extracts containing the volatile compounds released from the maize plants were quantified by gas chromatography coupled to flame ionization detection (GC-FID) (7890A GC; Agilent Technologies Inc., Wilmington, DE) using a DB-5MS column (30 m × 0.25 mm, 0.25 μm film thickness, Supelco, Bellefonte, PA). The following temperature program was used for this analysis: an oven temperature initially held at 50°C for 1 min, then raised to 180°C at 5°C per min and held for 0.1 min, followed by an increase of 10°C per min to 250°C and holding for 10 min. One microlitre of each sample was injected in splitless mode with the injector temperature at 250°C. Helium was used as the carrier gas, and FID was performed at 270°C. Quantification was performed by internal standard method by comparing each peak area to the area of the tetracosane internal standard at a final concentration of 10 ng/μl. The detector response factor was considered equal to 1 for all the compounds.

Selected air-entrainment extracts from maize plants were analysed by gas chromatography coupled to mass spectrometry (GC-MS) using an Agilent 5975C inert XL EI/CI MSD device, with a triple axis detector that was directly coupled to an Agilent 7890A (Agilent Technologies Inc.) gas chromatograph. The gas chromatograph was equipped with a DB-5 column (30 m × 0.25 mm d, 0.25 μm film thickness; Supelco), and the analysis was performed using the same temperature program described above for GC-FID. Helium was used as the carrier gas. Ionization was performed by electron impact (70 eV, source temperature 230°C). One microlitre of each sample was injected in splitless mode. The resulting data were collected and evaluated using ChemStation software. The volatile compounds of the aeration were identified by comparing the fragmentation pattern with library data (NIST 2011) and confirmed by analyses of authentic chemical standards. The retention index (RI) for each compound was calculated to aid in the identification of compounds, and the resulting value was compared with published data available online at Pherobase and NIST Chemistry WebBook (2011).

Chemicals

HayeSep® (80–100 mesh) was purchased from Supelco. Hexane for the HPLC (≥97% and redistilled),

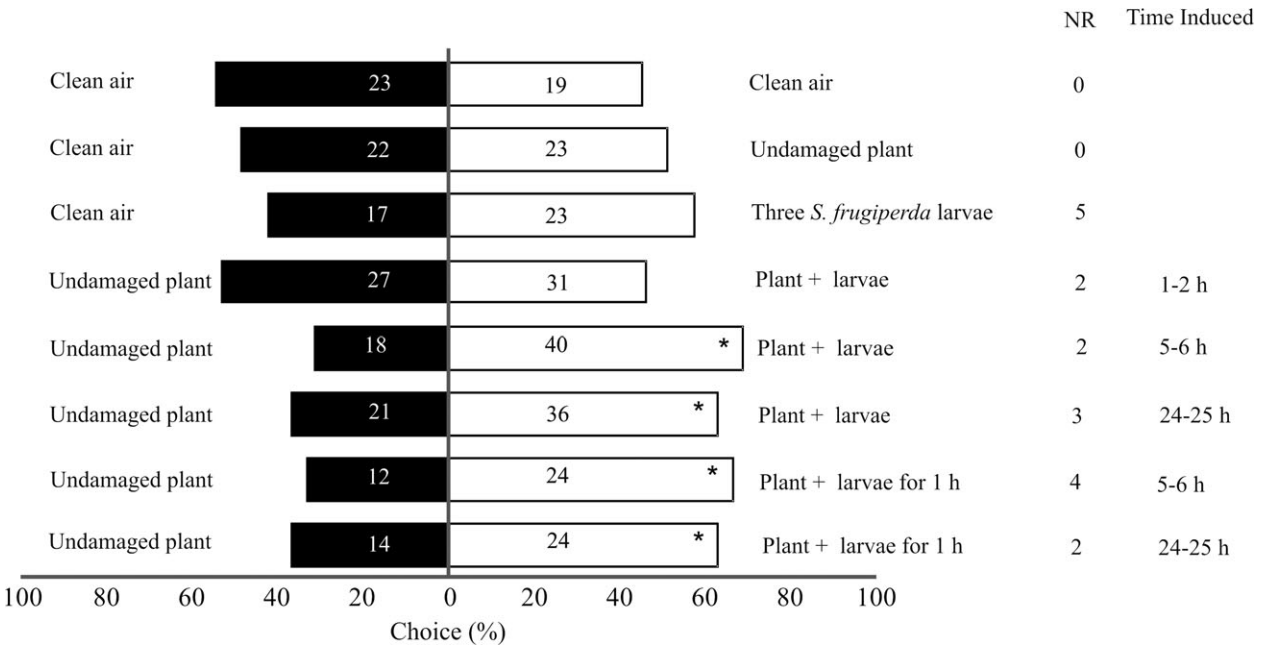

Fig. 1 Behavioural responses of the wasp *Polybia fastidiosuscula* in a Y-olfactometer to maize plants volatiles induced by *Spodoptera frugiperda* larvae or to undamaged plants. The numbers inside the bars are the total numbers of wasps that responded to each treatment. *Significant differences in choices between treatments, chi-square test, $P < 0.05$. No response – NR.

nonane, decane, undecane, tridecane, tetradecane, pentadecane, α-pinene (98%), benzothiazole (96%), β-pinene (99%), myrcene (90%), (*Z*)-3-hexenyl acetate (98%), ocimene [a mix of isomers (*E*) 70% and (*Z*) 30%], benzaldehyde (99%), indole (98.5%) and α-copaene (90%) were purchased from Sigma-Aldrich (Steinheim, Germany). Linalool, α-humulene (96%) and limonene (97%) were purchased from TCI-America (Portland, USA). Geranylacetone (a mixture of isomers) (96%) and (*E*)-β-farnesene were purchased from TCI (Tokyo, Japan). (*E*)-4,8-dimethyl-1,3,7 nonatriene (DMNT) and (*E*,*E*)-4,8,12-trimethyl-trideca-1,3,7,11-tetraene (TMTT) were kindly provided by Dr. Michael A. Birkett from Rothamsted Research (UK).

Statistical analyses

The choices made by the wasps were analysed using the chi-square test in the BioEstat statistical program (Ayres et al. 2003). Insects that did not choose any of the arms were excluded from statistical analysis.

Data on the chemical profiles of maize volatiles subjected to different treatments did not obey any distribution available in the R program of the GLM (generalized linear model), even after they were transformed with a logarithmic function or arcsine. Therefore, a Kruskal–Wallis nonparametric test was performed using the Agricolae library in R at a significance level of 5%, and the means were compared by Dunn's method ($\alpha = 0.05$).

Results

Wasps had no significant preference for any arm of the olfactometer when clean air was tested in both arms ($\chi^2 = 0.906$, d.f. = 1, $P = 0.3942$), indicating that there was no positional bias in the olfactometer (fig. 1). Similarly, wasps showed no attraction to undamaged plants ($\chi^2 = 0.049$, d.f. = 1, $P = 0.9029$) or *S. frugiperda* larvae ($\chi^2 = 2.25$, d.f. = 1, $P = 0.1615$) compared to clean air. However, the wasps significantly preferred odours emitted by plants + *S. frugiperda* larvae during the 5–6-hour period after the larvae started feeding on the plants ($\chi^2 = 13.904$, d.f. = 1, $P = 0.0003$) and odours emitted by plants + *S. frugiperda* larvae during the 24–25-hour period after the larvae started feeding on the plants ($\chi^2 = 6.927$, d.f. = 1, $P = 0.0113$) compared to odours emitted by undamaged plants. The wasps showed no increased attraction to plants + *S. frugiperda* larvae during the 1–2 h after the larvae started feeding on these plants compared to undamaged plants ($\chi^2 = 0.476$, d.f. = 1, $P = 0.5552$). Wasps had a significant preference for plants that had been subjected to *S. frugiperda* for 1 h and tested 5–6 h after

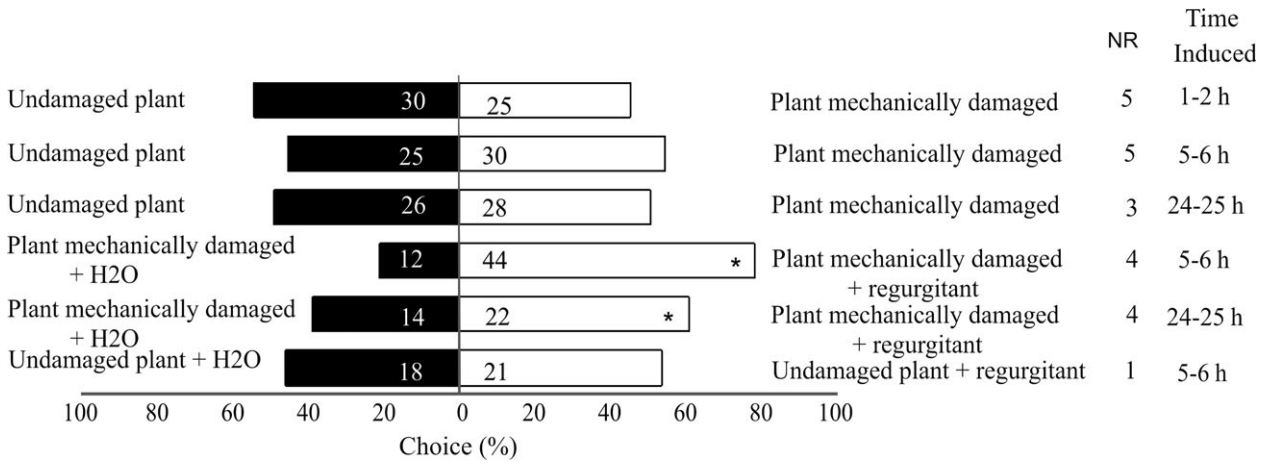

Fig. 2 Behavioural response of the wasp *Polybia fastidiosuscula* in the Y-olfactometer to maize plant volatiles induced by mechanical damage with or without *Spodoptera frugiperda* larvae regurgitant. The numbers inside the bars are the total numbers of wasps that responded to each treatment. *Significant differences, chi-square test, $P < 0.05$. No response – NR.

the larvae started feeding on the plants ($\chi^2 = 11.116$, d.f. = 1, P = 0.0012) or at 24–25 h after the larvae started feeding on the plants ($\chi^2 = 6.927$, d.f. = 1, P = 0.0113) compared to undamaged plants.

Polybia fastidiosuscula were not significantly attracted to mechanically damaged plants that were tested after 1–2 h ($\chi^2 = 0.828$, d.f. = 1, P = 0.4179), 5–6 h ($\chi^2 = 0.828$, d.f. = 1, P = 0.4179) or 24–25 h ($\chi^2 = 0.031$, d.f. = 1, P = 0.9394) compared to undamaged plants (fig. 2). Nonetheless, the wasps significantly preferred mechanically damaged plants + regurgitant when tested 5–6 h after the larvae started feeding on the plants ($\chi^2 = 32.65$, d.f. = 1, P = 0.0001) or 24–25 h after the larvae started feeding on the plants ($\chi^2 = 4.937$, d.f. = 1, P = 0.0338) compared to mechanically damaged plants + H_2O. However, the wasps did not show a significant preference for undamaged plants + regurgitant when tested after 5–6 h compared to undamaged plants + H_2O ($\chi^2 = 0.593$, d.f. = 1, P = 0.5029).

Wasps were significantly attracted to extract from plants with induced damage because 75.44% of the insects showed a significant preference for plant extract + *S. frugiperda* larvae during the period of 5–6 h ($\chi^2 = 25.888$, d.f. = 1, P = 0.0001), 75% showed a significant preference for extract from plants exposed to *S. frugiperda* larvae for 1 h and tested 5–6 h later ($\chi^2 = 25.00$, d.f. = 1, P = 0.0001), and 83.33% showed a significant preference for extract from mechanically damaged plants + larvae regurgitant ($\chi^2 = 44.436$, d.f. = 1, P = 0.0001) compared to hexane (fig. 3).

The major compounds identified in the extracts of maize plants subjected to the various treatments were α-pinene (RI = 934), myrcene (RI = 989), (*Z*)-3-hexenyl acetate (RI = 1014), limonene (RI = 1030), (*E*)-ocimene (RI = 1046), linalool (RI = 1099), DMNT (RI = 1113), unknown monoterpene (RI = 1179), benzothiazole (RI = 1227), indole (RI = 1292), α-copaene (RI = 1373), (*E*)-geranylacetone (RI = 1447), (*E*)-β-farnesene (RI = 1452) and TMTT (RI = 1573).

There was a quantitative difference in the compound emissions among the treatments (table 1). The compounds (*Z*)-3-hexenyl acetate (H = 16.31, d.f. = 3, P = 0.0009), linalool (H = 14.40, d.f. = 3, P = 0.002), (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) (H = 16.12, d.f. = 3, P = 0.001), (*E*)-β-farnesene (H = 9.69, d.f. = 3, P = 0.021) and indole (H = 12.01, d.f. = 3, P = 0.007) were released at higher amounts by plants that were damaged by *S. frugiperda* herbivory or that were mechanically damaged and treated with regurgitant compared to untreated plants (table 1). The compounds α-pinene (H = 11.72, d.f. = 3, P = 0.008), myrcene (H = 12.22, d.f. = 3, P = 0.006), limonene (H = 7.62, d.f. = 3, P = 0.06), (*E*)-ocimene (H = 7.67, d.f. = 3, P = 0.06), (*E*)-β-farnesene (H = 9.69, d.f. = 3, P = 0.021) and (*E*, *E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) (H = 7.16, d.f. = 3, P = 0.067) were released in greater amounts by plants subjected to herbivory by the larvae compared to the control plants.

The release of α-copaene (H = 5.4, d.f. = 3, P = 0.144), (*E*)-geranylacetone (H = 3.45, d.f. = 3, P = 0.326), an unknown monoterpene (H = 1.99, d.f. = 3, P = 0.573) and benzothiazole (H = 1.06, d.f. = 3, P = 0.786) did not differ among the treatments (table 1).

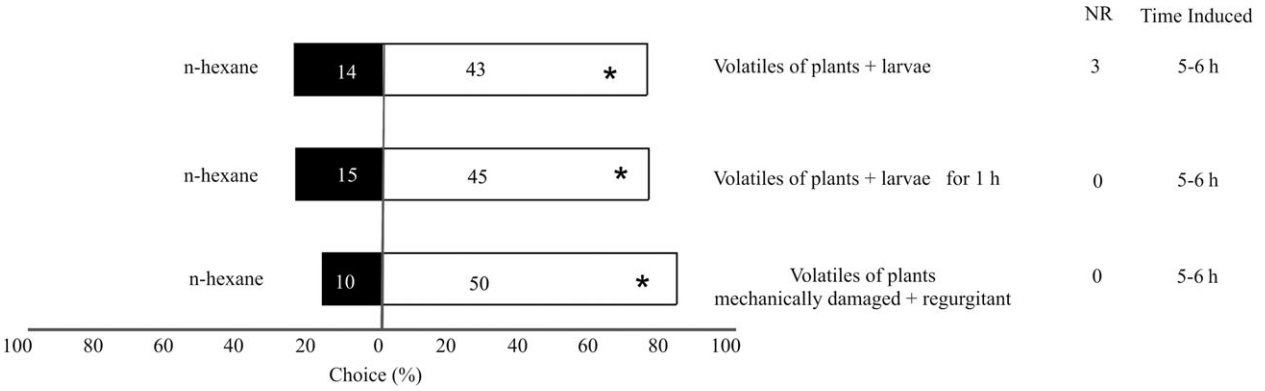

Fig. 3 Behavioural response of the wasp *Polybia fastidiosuscula* in the Y-olfactometer to *n*-hexane (control) or to maize volatiles collected from *Spodoptera frugiperda* larvae-treated plants or mechanically damaged plants + *S. frugiperda* larvae regurgitant. The numbers inside the bars are the total numbers of wasps that responded to each treatment. *Significant differences, chi-square test, $P < 0.05$. No response – NR.

Table 1 Mean ± standard error (ng/3 h) of volatile compounds identified from air-entrainment extracts of maize submitted to different treatments: plants + *Spodoptera frugiperda* larvae at 5–6 h after the addition of the larvae without removal of larvae (OS5), plants + *S. frugiperda* larvae for 1 h analysed at 5–6 h after removal of the larvae (OS7), plants treated with mechanical damage + regurgitate and analysed at 5–6 h after the damage (OS12), undamaged plants (OS2)

Compounds	RI DB-5MS	Plants + larvae for 1 h (OS7)	Plants + larvae for 5–6 h (OS5)	Plants mechanically damaged + regurgitant (OS12)	OS2 Undamaged plant
α-pinene	934	8.45 ± 2.49 ab	6.91 ± 1.67 a	2.36 ± 0.29 c	4.31 ± 1.28 bc
β-myrcene	989	18.99 ± 7.55 b	5.09 ± 1.10 a	3.47 ± 0.38 bc	3.21 ± 1.76 c
(*Z*)-3-hexenyl acetate	1014	79.69 ± 20.98 b	140.78 ± 23.64 a	25.79 ± 4.27 c	0
Limonene	1030	302.06 ± 225.81 a	25.82 ± 4.89 ab	13.14 ± 2.38 bc	12.12 ± 3.53 c
(*E*)-ocimene	1046	21.38 ± 5.93 a	13.79 ± 4.63 a	8.16 ± 1.48 ab	1.66 ± 1.85 b
Linalool	1099	91.19 ± 30.10 a	97.52 ± 27.54 a	44.23 ± 12.38 b	0
DMNT*	1113	177.46 ± 36.83 a	147.34 ± 12.82 a	42.82 ± 10.59 b	0.37 ± 0.26 c
Monoterpene	1179	2.52 ± 1.21 a	1.94 ± 0.22 a	2.84 ± 0.79 a	2.21 ± 1.02 a
Benzothiazole	1227	11.72 ± 7.98 a	66.44 ± 65.17 a	8.11 ± 2.10 a	20.66 ± 15.53 a
Indole	1292	174.53 ± 86.07 a	118.06 ± 37.72 ab	24.66 ± 3.94 b	0.91 ± 0.95 c
α-copaene	1373	24.63 ± 4.34 a	34.29 ± 16.27 a	16.11 ± 2.30 a	19.44 ± 8.15 a
Geranylacetone	1447	12.93 ± 2.26 a	11.57 ± 3.02 a	18.55 ± 5.72 a	10.71 ± 409 a
(*E*)-β-farnesene	1452	143.91 ± 50.12 a	147.09 ± 66.53 ab	57.83 ± 13.19 bc	16.50 ± 18.44 c
TMTT*	1573	13.62 ± 4.26 a	7.62 ± 1.98 a	6.39 ± 3.58 ab	1.41 ± 0.69 b

*(*E*)-4,8-Dimethylnona-1,3,7-triene (DMNT) and (*E*,*E*)-4,8,12-trimethyl-trideca-1,3,7,11-tetraene (TMTT).
Different letters indicate significant differences between treatments (Kruskal Wallis test and Dunn test with confidence level 95%, n = 5).

Discussion

Social wasps (Hymenoptera: Vespidae) are opportunistic generalists when they are hunting, and they use a variety of mechanisms to locate and choose prey (Raveret Richter 2000). Their response to a particular cue may vary among different situations and different wasp species. Thus, the patterns of variability in hunting behaviours within and among social wasp species remain to be explored. In the current research, the wasp *P. fastidiosuscula* showed stronger attraction to maize plants that were injured by *S. frugiperda* larvae or plants that were mechanically damaged and treated with larval regurgitant than to undamaged plants or larvae alone. These results suggest that this species may use specific herbivore-induced plant volatiles (HIPVs) when foraging for prey.

According to Turlings et al. (1990), terpenoids may be produced in defence against herbivores, but they may also have a secondary function in attracting the enemies of herbivores. These authors also showed that artificially damaged seedlings do not release volatiles in significant amounts unless oral secretions from the caterpillars *Spodoptera exigua* (Hübner)

(Lepidoptera: Noctuidae) are applied to the damaged sites. They also found that undamaged leaves do not release detectable amounts of the terpenoids, regardless of whether they are treated with oral secretions. These results are consistent with the present study. By contrast, three species of social wasps (*Polybia ignobilis*, *Polistes satan* and *Polistes versicolor*) were attracted to the odour of artificially freshly cut leaves. These social wasp species employed this odour to select which leaves to inspect, and they also remembered a place to which they were recently strongly attracted so they could return to hunt there. Preliminary results suggest that *P. fastidiosuscula* might be attracted to the recently cut leaves of *Crotalaria* species, although insufficient data were collected for statistical analysis (Raw 1998). This author mentions that a specialist predator might prefer the particular odour that a leaf emits as a result of damage caused by its herbivorous prey, whereas a generalist might be attracted to the odour following any mechanical damage.

Research has shown that green leaf volatiles (GLVs) are chemical substances emitted by plants following the first injury caused by mechanical damage or herbivory, and these compounds attract natural enemies (James 2005; Scala et al. 2013). Moreover, GLVs are an important group of HIPVs that act primarily as priming agents (Paré et al. 2005). Although exposure to synthetic GLVs primes plants in the field, the effect is not great enough to attract natural enemies (von Mérey et al. 2011). In the current research, the profile of chemical volatiles released by plants during the early hours (1–2 h) of herbivory damage is not involved in attracting the predatory wasps. However, the wasps responded to maize volatiles only 5–6 h after the onset of the damage. The chemical profile of these volatiles was composed largely of monoterpenes and sesquiterpenes, and only one GLV, namely (*Z*)-3-hexenyl acetate, was present. A chemical analysis of the air-entrainment extract at 1–2 h after the onset of damage was not performed, making it impossible to understand whether the maize plants release higher amounts of GLVs at 5–6 h or whether the earlier time window was too short for the plant to begin emitting the HIPVs.

According to Baldwin et al. (2006), plant response times to an injury can vary considerably; in some plants, a significant change in the chemical profile can start within minutes, whereas in other plants, the effect can take hours or even years. Studies conducted by Turlings et al. (1998) with *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) showed that the response time during the period of maximum volatile emission by maize plants varies among the cultivars, with a maximum of 12 h after treatment with the larvae regurgitate, and at 5 h after induction, maize plants emit large amounts of volatiles. The results presented here showed that the volatiles that are attractive to predatory wasps continued to be attracted even after the herbivory-related damage had ceased.

As in other studies conducted with maize (D'Alessandro et al. 2006, 2009; Erb et al. 2009), the major compounds induced by herbivores were α-pinene, myrcene, (*Z*)-3-hexenyl acetate, limonene, (*E*)-ocimene, linalool, DMNT, (*E*)-β-farnesene, TMTT and indole. However, compounds that were present in minor amounts and background odours could also be important for attracting the wasps (Bruce and Pickett 2011; Michereff et al. 2013; Mc Cormick et al. 2014). Therefore, the specific blend of compounds that are important for wasp attraction remains to be identified.

After 5–6 h of induction in the mechanically injured maize plants that were treated with larval regurgitant, maize plants that were continuously damaged by larvae or plants in the presence of larvae for only 1 h released higher amounts of linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene, indole and (*E*)-β-farnesene compared to undamaged plants. However, maize plants that experienced the continuous presence of larvae emitted a higher amount of (*Z*)-3-hexenyl acetate than plants that were in the presence of the larvae for 1 h, confirming that the plants continued to emit GLVs while emitting terpenes, as this (*Z*)-3-hexenyl acetate compound is among the GLVs that are released immediately after the initial injury. This difference can be explained by the continued presence of the larvae on the maize plant, while bioassays were conducted. The results of this study show that even without the presence of the herbivore, the plant continues to release volatiles that are attractive to social wasps, but not in the same way that it emits volatiles in the continuous presence of the herbivore. Nevertheless, this difference does not seem to be crucial for the long-range attraction of wasps, which are attracted to damaged plants after 5–6 h or to continuously damaged plants within up to 24 h of injury. This finding may indicate that the presence of (*Z*)-3-hexenyl acetate in the volatiles mixture is not crucial for wasp attraction. Future studies could indicate which compounds are important for the perception and attraction of the wasps.

This is the first study to show the ability of the social predatory wasp *P. fastidiosuscula* to use HIPVs to locate herbivorous preys. We wish to explore this phenomenon further to determine which of the volatiles in the blend induced by *S. frugiperda* larvae are most important for attraction. This knowledge may aid in the development of strategies for integrated pest management.

Acknowledgements

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Brazil) for supporting our research, and we thank Simone Mendes Oliveira of Embrapa Maize and Sorghum for the initial *S. frugiperda* colony.

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