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ORIGINAL RESEARCH PAPER

Volatiles from soybean flowers attract the Mexican soybean weevil, *Rhyssomatus nigerrimus* (Coleoptera: Curculionidae)

Mónica González‑Domínguez[1] · Guillermo López‑Guillén[2] · Leopoldo Cruz‑López[1]

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Abstract

The Mexican soybean weevil, *Rhyssomatus nigerrimus* Fahraeus (Coleoptera: Curculionidae), is a pest of soybeans. In this study we evaluate the volatiles of the soybean flower of the varieties FT-Cristalina-RCH and Flores as possible attractants for *R. nigerrimus*. Behavioral bioassays using an "Y" tube olfactometer and Electroantennography tests were performed to evaluate the responses of *R. nigerrimus* to the soybean flowers and their volatile extracts, in addition the volatiles were collected by dynamic aeration and identified using gas chromatography coupled with mass spectrometry (GC–MS). Bioassays showed that females and males were attracted by the flowers and volatile extracts of flowers of both varieties. However, females exhibited stronger antennal response than males to the volatile extracts of flowers of both varieties and their synthetic blends. The volatile extracts analysis showed the presence of 1-octen-3-one, 2-ethyl-1-hexanol, limonene, α-copaene, α-pinene, undecane, nonanal, octyl hexanoate, *trans*-α-bergamotene and calamanene. Quantitative differences in 1-octen-3-one, 2-ethyl-1-hexanol, *α*-pinene and limonene between the varieties were observed. In bioassays, males and females were attracted by α-copaene, 1-octen-3-ol α-pinene, and both synthetic blends. Females exhibited stronger antennal response than males to the synthetic compounds α-pinene, α-copaene, 1-octen-3-ol, nonanal and limonene.

Keywords Behavior · Herbivory · Antennal response · Floral volatiles · Synthetic volatiles · Curculionidae

Introduction

In Mexico, the main pest of soybean (*Glycine max* L.) is the Mexican soybean weevil *Rhyssomatus nigerrimus* Fahraeus (Coleoptera: Curculionidae) (López-Guillén et al. 2012b), which causes major damage during the vegetative and reproductive stages of soybeans. The female weevil deposits her eggs inside the pods and the larvae feed on the beans (Terán-Vargas and López Guillén 2014). It has been observed that *R. nigerrimus* also feeds on the flowers (López-Guillén et al. 2012a). The weevils locate the soybean plant possibly due to the volatiles that the plant produces. Generally, plants emit volatile compounds into the environment (Bautista-Lozada et al. 2012). These volatile compounds play an important role in the plant–insect interaction (Cantúa-Ayala et al. 2019; Hu et al. 2021; Karmakar et al. 2020; Zhang et al. 2016). Among these are the flowers volatiles. Floral volatiles have different functions in attraction, reproduction, dissuasion, and antagonism of herbivorous insects and pollinators (Hetherington-Rauth and Ramírez 2016; Schiestl 2015). Floral volatiles act as attractants, not only of pollinators, but also of herbivorous pests (Wang et al. 2018). In the case of curculionids, several species have been reported to interact with the flowers; for example, the apple blossom beetle *Anthonomus pomorum* (Linnaeus) (Coleoptera: Curculionidae) is attracted by apple flower buds (Collatz and Dorn 2013), whereas its allied *A. rubi* (Herbst) prefers strawberry plants with flowers over those without flowers (Mozūraitis et al. 2020) and females of *A. musculus* (Say) prefer open flowers of cranberry (*Vaccinium macrocarpon* L.) (Szendrei et al. 2009). Soybean plants are known to emit volatiles that attract herbivores; for example, *Riptortus pedestris* (Fabricius) (Hemiptera: Alydidae) is attracted by soybean volatiles (Song et al. 2022). It is

✉ Leopoldo Cruz-López
lcruz@ecosur.mx

1 El Colegio de la Frontera Sur – ECOSUR, Grupo Ecología de Artrópodos y Manejo de Plagas, Tapachula, Chiapas, Mexico

2 Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias – INIFAP, Campo Experimental Rosario Izapa, Tuxtla Chico, Chiapas, Mexico

also known that, when *R. nigerrimus* damages a soybean plant, induced volatiles are produced (Espadas-Pinacho et al. 2021). However, the composition of the volatiles from soybean flowers, which could attract *R. nigerrimus*, has not been reported, so it would be helpful to know the behavior of *R. nigerrimus* and develop a robust base of attractants for the control of this pest. For this reason, in this study we evaluate the response of *R. nigerrimus* to the volatile flower extracts of both varieties FT-Cristalina-RCH and Flores most frequently cultivated and the compounds that constitute the aroma of the soybean flowers were tentatively identified. Moreover, we tested behavioral and electrophysiological activities of individual compounds as well as blends of the compounds from flowers of both varieties to adults of *R. nigerrimus*.

Materials and methods

Biological materials

Adults of *R. nigerrimus* in active state and soybean flowers (varieties FT-Cristalina-RCH and Flores) in stage R2 (complete flowering) were collected in a soybean field located in the first section of the ejido Tinajas (N 14° 22′, W 092° 20′), Tapachula, Chiapas, Mexico. The flowers were placed in 250 mL Erlenmeyer flasks and kept at 5 °C until use. The weevils were sexed following the technique described by López-Guillén et al. (2016). Males and females were placed in separate 1 L plastic recipients. The insects were fed with pieces (0.5 mm × 0.5 mm) of sweet potato (*Ipomea batatas* L.) daily and kept in the insectarium of ECOSUR (El Colegio de la Frontera Sur), Mexico at a temperature of 25 ± 2 °C, 75 ± 5% relative humidity, and 12:12 h light:dark photoperiod.

Volatile collection

We collected the volatiles emitted by the flowers using the dynamic aeration technique. We placed 5 g in fresh weight of flowers in a 250 mL glass recipient. A current of air (previously purified through a filter with Tenax®) was made to pass over the flowers at a flow of 0.8 L/min. The volatiles were captured in a small glass column with Super Q (50–80 mesh; Water Associates, Milford, MA, USA). The collection process lasted 24 h. The volatiles were then extracted with 400 µL of dichloromethane, placed in 2 mL vials and stored at − 20 °C until analysis. The volatiles collection was performed at a temperature of 27 ± 2 °C, 75 ± 5% relative humidity, and photoperiod of 12:12 h light:dark. Ten replications were done for each variety of soybean.

Behavioral bioassays

The insects used in the bioassays were fasted for 24 h. We assessed the attraction response of *R. nigerrimus* to volatiles emitted by soybean flowers (var. FT-Cristalina-RCH and Flores) using a Y-shaped olfactometer placed horizontally on the table (main arm 15 cm long, lateral arms at 45° with 10 cm long and 2.3 cm internal diameter). Air, previously humidified and purified with an activated carbon filter, passed at a flow rate of 0.5 L/min through each arm of the olfactometer. In one of the olfactometer arms was placed 1 g of flowers, and the other remained empty as a control. We placed a group of 5 insects at the entrance of the main arm of the olfactometer and allowed them a maximum time of 5 min to select between the treatment and the control. We considered that the insect had made an election when it had entered 2 cm into one of the lateral arms. We changed the position of the Y-tube after five replications, interchanging the place of the glass adaptor (8 cm long, 1.3 cm internal diameter) every five replications to avoid response bias. For both males and females, 60 replications were performed for each soybean variety. The bioassays were carried out between 8:00 and 19:00 h. Before beginning and after each evaluation, the olfactometer and the adaptors were washed with water and neutral soap and placed in an oven at 120 °C for 60 min. The bioassays were conducted at a temperature 35 ± 2 °C, 75 ± 5% relative humidity and used artificial white light (1676 lx). For the bioassays with extracts, 20 µL of the extract or solvent was placed on a 5 cm × 5 cm piece of filter paper (Whatman No. 2, Maidstone, England) and left for 20 s to evaporate the solvent. We then placed a piece of filter paper with the extract in one of the arms of the olfactometer and a piece of filter paper with 20 µL of dichloromethane (control) in the opposite arm. We compared the response of the two sexes of weevils to the volatile extracts of the two soybean varieties simultaneously in the olfactometer. This was replicated 30 times for each volatile extract and each sex.

Chemical analysis

The volatile extracts were analyzed in a gas chromatograph coupled with a mass spectrometer (Shimadzu GC-2010 Plus, Tokyo, Japan) equipped with a 5% phenyl-methyl-silicone column (DB5-MS) 30 m × 0.25 mm internal diameter and 0.25 µm film thickness. The oven temperature program consisted of an initial temperature 50 °C (for 2 min) with increments of 15 °C/min up to a final temperature of 280 °C (for 10 min). The analyzed aliquot was 1 µL per extract of soybean extract obtained by dynamic aeration.

Volatile compounds were tentatively identified retention indexes, comparing the spectral data of each compound with the NIST database and then confirmed with the use of reference synthetics when possible. We used the percentage of the peak areas to know their relative proportions and the quantification of selected compounds was carried out using the external standard method, which consisted of preparing a set of solutions of each compound at different concentrations (100 ng/µL, 75 ng/µL, 50 ng/µL, 25 ng/µL and 1 ng/µL), then a calibration curve was constructed with the areas obtained for each compound and related to the concentrations.

Electroantennography (EAG)

We evaluated the antennal response of *R. nigerrimus* males and females to the volatile extracts from both soybean flower varieties, synthetic blends and individual synthetic compounds through EAG tests. Each weevil was exposed to cold until anesthetized for 1 min to facilitate manipulation. We cut off the insect's head and inserted the reference electrode at the base, using a glass capillary filled with Ringer solution (NaCl 0.35 g, $CaCl_2$ 0.21 g, KCl 0.35 g, and $NaHCO_3$ 0.2 g dissolved in 1 L water). The distal tip of the antenna was inserted into the tip of the glass capillary placed in the recording electrode. We used a standard aliquot (1 µL) of the extract, blend, or compound (solution prepared with dichloromethane HPLC grade at a concentration of 1 µg/µL) on a piece of filter paper (0.5 × 1.0 cm, Whatman No.1, Whatman International, Maidstone, United Kingdom) and exposed it to air for 20 s on a glass Petri dish to allow the solvent to evaporate. A glass Pasteur pipette or sample cartridge was then introduced. A new cartridge was prepared for each antenna replicate. As a control, we used a piece of filter paper loaded with 1 µL dichloromethane at the beginning, middle and end of each assay. To present the stimulus, we inserted the tip of the pipette into an orifice located in the upper middle part of a glass tube (10 mm diameter) and passed a flow of air (0.5 L/min) through by activating a pedal connected to a stimulus controller (Syntech CS-05, Hilversum, The Netherlands). The duration of the stimulus was 1 s. A current of purified humid air (0.7 L/min) was constantly directed toward the antenna through a glass tube to eliminate the odors in the system. The signals generated by the antenna were amplified using a controller of intelligent data acquisition (Syntech IDAC-02, Hilversum, The Netherlands) connected to a computer and visualized in a monitor using the software Syntech EAG v.2.7. The response to the different compounds was measured consecutively in the same antenna in a random manner. We evaluated 30 males *R. nigerrimus* antennae and 30 females *R. nigerrimus* antennae.

Bioassays with synthetic compounds

Response of adult *R. nigerrimus* to synthetic compounds

The compounds *α*-pinene, 1-octen-3-one, 1-octen-3-ol, 2-ethyl-1-hexanol, limonene, undecane, nonanal, and *α*-copaene were assessed individually at a concentration of 1 µg/µL and blends of the eight compounds from both varieties of flowers were prepared in accord with the concentrations of the aeration extracts (Fig. 4): FT-Cristalina-RCH (6.5, 8.5, 23.0, 49.0, 7.0, 7.0, 14.0, 82.0 ng, respectively) and Flores (4.5, 22.5, 25.0, 25.0, 4.5, 8.0, 11.0, 80.0 ng, respectively). The bioassays were conducted following the methodology described previously in a Y-shaped olfactometer using 1 µL of the synthetic blend to be evaluated and placing as a control a piece of filter paper with 1 µL of dichloromethane, leaving it to evaporate for 20 s before commencing each replication. The two blends were assessed separately with each sex, and also the two synthetic blends were also assessed simultaneously in the Y-shaped olfactometer. We performed 30 replications with *R. nigerrimus* males and females.

Statistical analysis

We analyzed the percentage of the peak areas of the compounds from flowers of each soybean variety and the quantities of each compound with the Kruskal–Wallis test. We analyzed all the data of the behavioral bioassays using the G test, previously transformed to square root to achieve normality of the data. The insects that did not respond in the bioassays were not included in the analysis. We conducted an analysis of variance (ANOVA) of multiple factors, considering the electrophysiological response to synthetic compounds individually and by *R. nigerrimus* sex, followed by the Tukey test ($\alpha = 0.05$) to compare treatment means. The statistical analyses were conducted with R software, version 4.3.1, and the Rcmdr package version 2.8.0 (Fox and Bouchet-Valat 2022).

Results

Behavioral bioassays with flowers and extracts

In the bioassays, adult of *R. nigerrimus* males were more attracted by flowers of both varieties FT-Cristalina-RCH ($G = 209.92$, $df = 1$, $p < 0.001$) and Flores ($G = 2014.50$, $df = 1$, $p < 0.001$), compared to the control. Likewise, *R. nigerrimus* females preferred the flowers of the variety Flores ($G = 152.59$, $df = 1$, $p < 0.001$) and FT-Cristalina-RCH

($G = 153.70$, $df = 1$, $p < 0.001$) over the control. However, when the flowers were tested simultaneously there was no significant difference with males ($G = 1.11$, $df = 1$, $p = 0.29$), but there was a significant difference with females ($G = 4.24$, $df = 1$, $p < 0.05$) (Fig. 1a, b).

R. nigerrimus males preferred the volatile extracts from the varieties FT-Cristalina-RCH ($G = 5.77$, $df = 1$, $p < 0.01$) and Flores ($G = 15.55$, $df = 1$, $p < 0.001$) over the control. Females showed the same response pattern, preferring the volatile extracts from both varieties ($G = 11.77$, $df = 1$, $p < 0.001$ in FT-Cristalina-RCH; $G = 13.70$, $df = 1$, $p < 0.001$ in Flores) over the controls. Between males and females, there were no significant differences when we tested the two volatile extracts simultaneously ($G = 1.00$, $df = 1$, $p = 0.31$ in males and $G = 1.22$, $df = 1$, $p = 0.26$ in females) (Fig. 2a, b).

Identification of volatile compounds

The analysis using gas chromatography coupled with mass spectrometry (GC–MS) showed that the composition of the volatiles emitted by the flowers of the two varieties includes at least 11 compounds: α-copaene, 1-octen-3-ol, 2-ethyl-1-hexanol, α-pinene, limonene, undecane, nonanal, 1-octen-3-one, octyl hexanoate, *trans*-α-bergamotene and calamanene (Table 1). The compounds 1-octen-3-one ($H = 6.81$, $df = 1$, $p < 0.01$), 2-ethyl-1-hexanol ($H = 3.93$, $df = 1$, $p < 0.05$), α-pinene ($H = 3.93$ $df = 1$, $p < 0.05$), 1-octen-3-one ($H = 5.77$ $df = 1$, $p < 0.01$), 2-ethyl-1-hexanol ($H = 6.81$ $df = 1$ $p < 0.001$) and limonene ($H = 5.77$ $df = 1$, $p < 0.01$) vary quantitatively between the two varieties of soybean flowers (Figs. 3, 4).

Electroantennography (EAG)

The antennal response of *R. nigerrimus* females was more intense than that of males to each of the evaluated treatments ($F = 17.88$, $df = 12$, $p < 0.001$). The following treatments stand out: volatile extract from both soybean flower varieties, synthetic blends, and the compounds α-pinene, 1-octen-3-ol, limonene, nonanal and α-copaene. In general, we observed stronger antennal response from females than from males ($F = 128.27$, $df = 1$, $p < 0.001$) (Fig. 5).

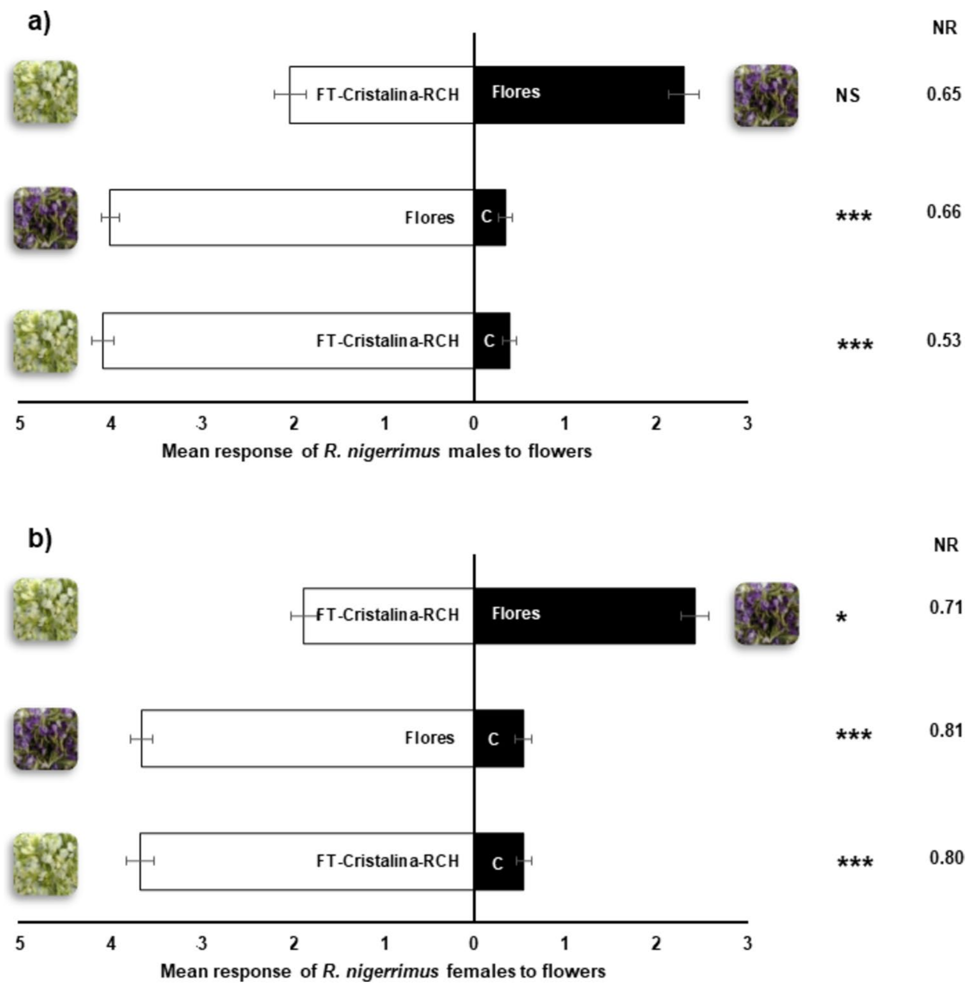

Fig. 1 Mean response (± SE) of *R. nigerrimus* **a** males and **b** females to soybean flowers (*G. max* L.) variety FT-Cristalina-RCH, Flores, and both flower varieties in bioassays conducted in a Y-tube olfactometer. The asterisk (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) indicates significant differences between evaluated treatments (G test). *C* control, *NR* non-responding, *NS* non-significant

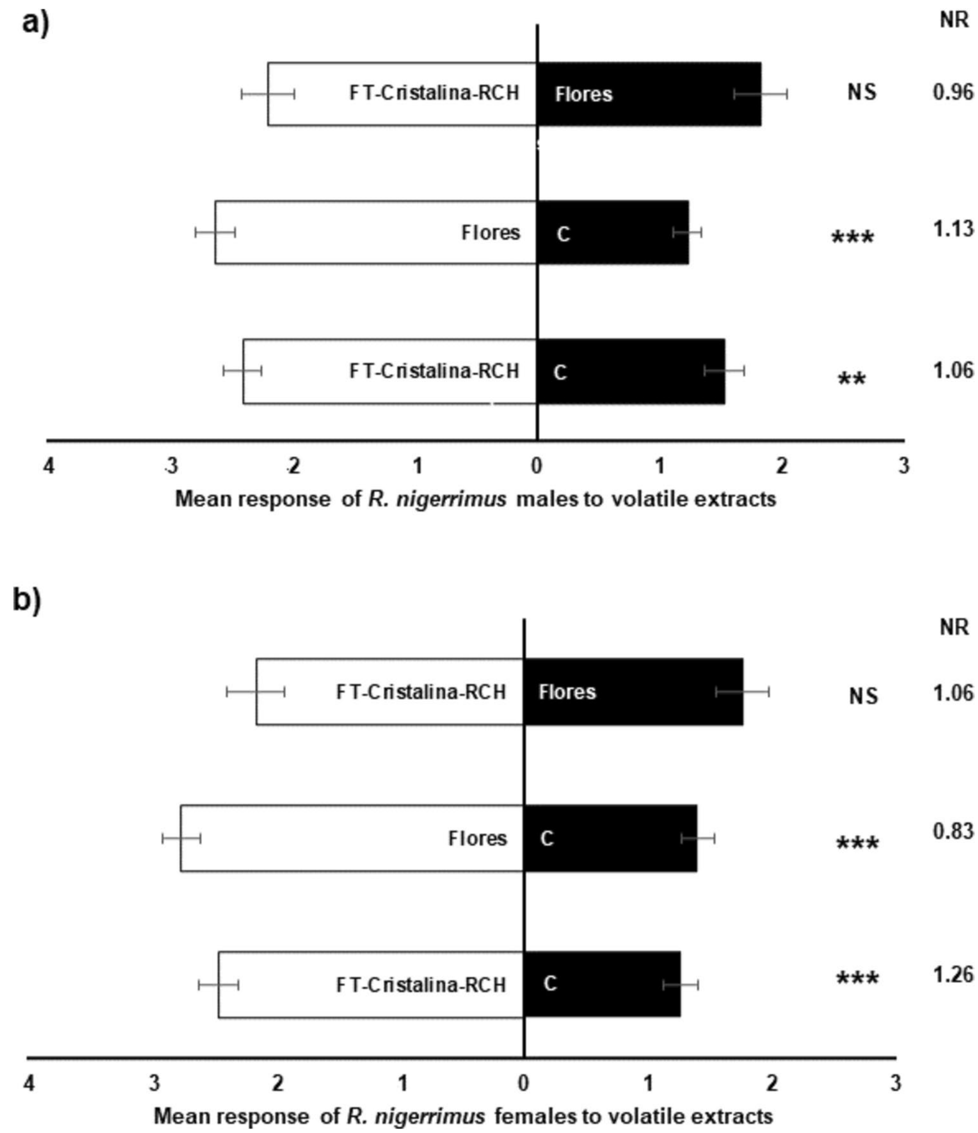

Fig. 2 Mean response (± SE) of *R. nigerrimus* **a** males and **b** females to volatile extracts from var. FT-Cristalina-RCH and Flores soybean flowers (*G. max* L.) and from both varieties in bioassays conducted in a Y-tube olfactometer. The asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) indicate significant differences between the evaluated treatments (G test). *C* control, *NR* non-responding, *NS* non-significant

Table 1 Volatile compounds identified in soybean flowers of the varieties FT-Cristalina-RCH and Flores (Percentage of peak area ± SE)

No	Compounds	RI	FT-Cristalina-RCH	Flores
1	*α*-Pinene*	938	2.56 ± 0.24	1.95 ± 0.40
2	1-Octen-3-one*	979	5.84 ± 2.31	16.62 ± 3.61
3	1-Octen-3-ol*	982	24.41 ± 5.81	25.02 ± 6.21
4	2-Ethyl-1-hexanol*	1031	18.03 ± 1.32	9.80 ± 1.11
5	Limonene*	1037	2.35 ± 0.26	1.51 ± 0.19
6	Undecane*	1100	2.31 ± 0.56	2.54 ± 0.70
7	Nonanal*	1108	5.95 ± 2.97	4.30 ± 1.26
8	Octyl hexanoate	1192	1.50 ± 0.37	2.12 ± 0.22
9	α-Copaene*	1394	27.19 ± 2.21	28.00 ± 4.94
10	*trans*-α-Bergamotene	1427	1.09 ± 0.14	1.43 ± 0.32
11	Calamanene	1542	8.72 ± 0.48	6.65 ± 1.24

RI retention index, *confirmed with synthetics

Bioassays with synthetic compounds

Males were more attracted by *α*-copaene ($G = 10.60$, $df = 1$, $p < 0.001$), 1-octen-3-ol ($G = 7.98$, $df = 1$, $p < 0.01$), and *α*-pinene ($G = 10.68$, $df = 1$, $p < 0.001$) than by the controls (Fig. 6a), while females were attracted by the compounds *α*-pinene ($G = 35.97$, $df = 1$, $p < 0.001$), 1-octen-3-ol ($G = 4.52$, $df = 1$, $p < 0.05$) and *α*-copaene ($G = 31.07$, $df = 1$, $p < 0.001$) (Fig. 6b).

Male and female weevils significantly preferred the eight compounds blends of the varieties FT-Cristalina-RCH ($G = 17.76$, $df = 1$, $p < 0.001$ in males and $G = 13.47$, $df = 1$, $p < 0.001$ in females) and Flores ($G = 17.81$, $df = 1$, $p < 0.001$ in males and $G = 10.96$, $df = 1$, $p < 0.001$ in females) over the controls in all the bioassays. However, we found no significant differences when we evaluated

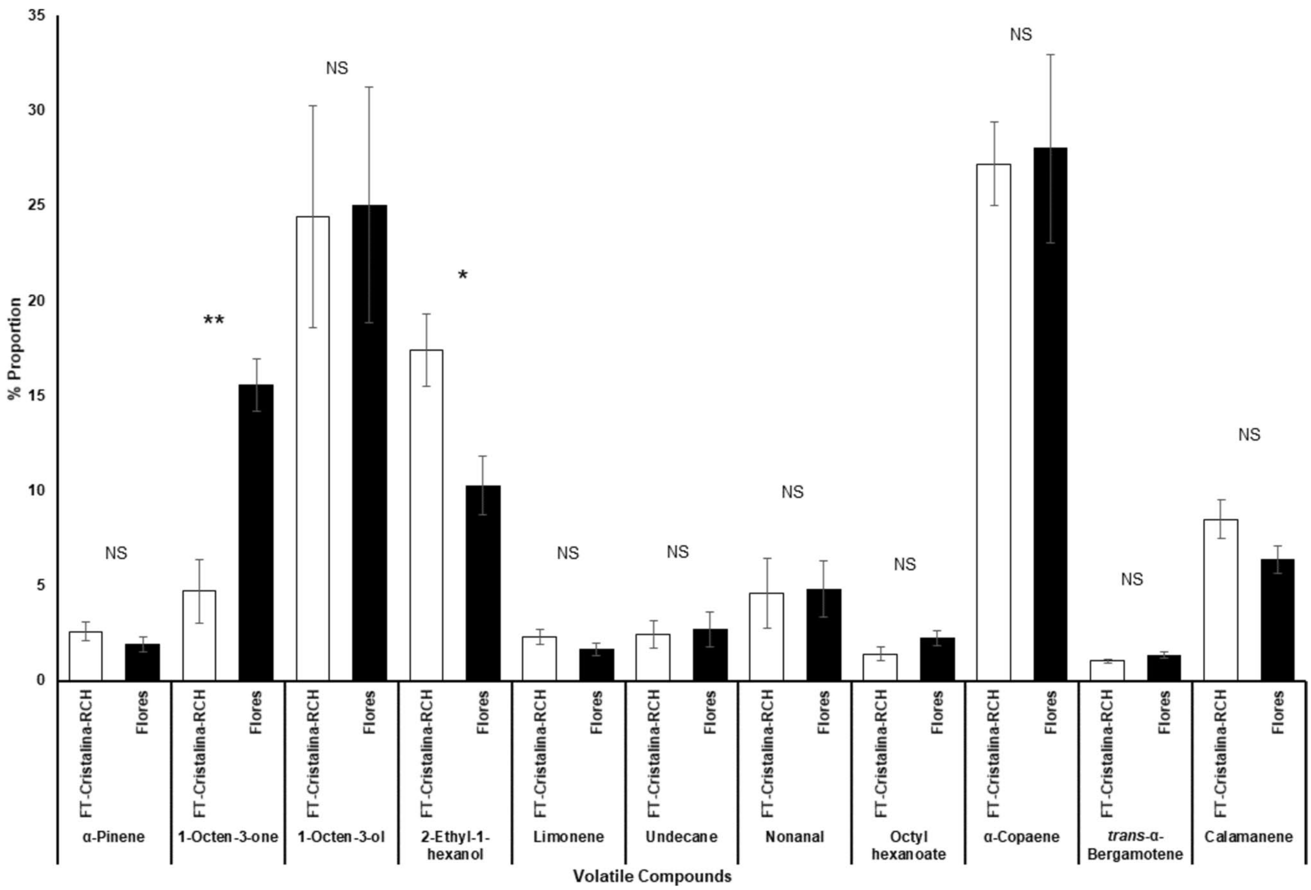

Fig. 3 Percentage of peak areas (±SE) of the volatile compounds emitted by flowers of soybean varieties FT-Cristalina-RCH and Flores. Comparisons at $p<0.05$ were significant (Kruskal–Wallis test). *NS* non-significant

both synthetic blends ($G=2.00$, $df=1$, $p=0.15$ in males and $G=0.11$, $df=1$, $p=0.73$ in females) (Fig. 7a, b).

Discussion

In this study we found that the Mexican soybean weevil *R. nigerrimus* is attracted to volatiles emitted by the flowers of the soybean plant and their aeration extracts. These results are congruent with the knowledge that many phytophagous species and pollinators are attracted by volatiles emitted by flowers (Baldelomar et al. 2018; Bouwmeester et al. 2019; Dudareva et al. 2013; Hetherington-Rauth and Ramírez 2016; Junker et al. 2010; Marín-Loaiza and Céspedes 2007). Like *R. nigerrimus*, other weevils such as *A. pomorum* (L.). (Coleoptera: Curculionidae) and its allied species prefer their host plants in flowering stage (Collatz and Dorn 2013; Mozūraitis et al. 2020). Other insects, such as the small green plant bug *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae), prefer host plants also in the flowering stage (Pan et al. 2015).

The two varieties of flowers evaluated in this study emit the same mixture of volatiles. However, they vary quantitatively. Floral aromas vary not only among species but also within a single plant species, as described by Campbell et al. (2019) and Delle-Vedove et al. (2017). However, there are few studies that center on intraspecific variation of floral aromas in a given geographic site, rather, they focus on comparing populations at different spatial scales and type of pollination (Aceves-Chong et al. 2018; Dötterl et al. 2005; Farré-Armengol et al. 2015; Wang et al. 2019). Our study permits us to appreciate how the volatile profiles of the flowers of the two soybean varieties differed: the compounds 1-octen-3-one and 2-ethyl-1-hexanol differed significantly in proportions, while *α*-pinene, 1-octen-3-one, limonene and 2-ethyl-1-hexanol differed in quantities. Nevertheless, the two mixtures of volatiles attract *R. nigerrimus*. However, we do not exclude the possibility that *R. nigerrimus* uses other factor to find its host as the flower color, size and shape, since when both physical flowers were evaluated simultaneously in bioassays, females preferring the "Flores" variety and when evaluating both volatile extracts did not

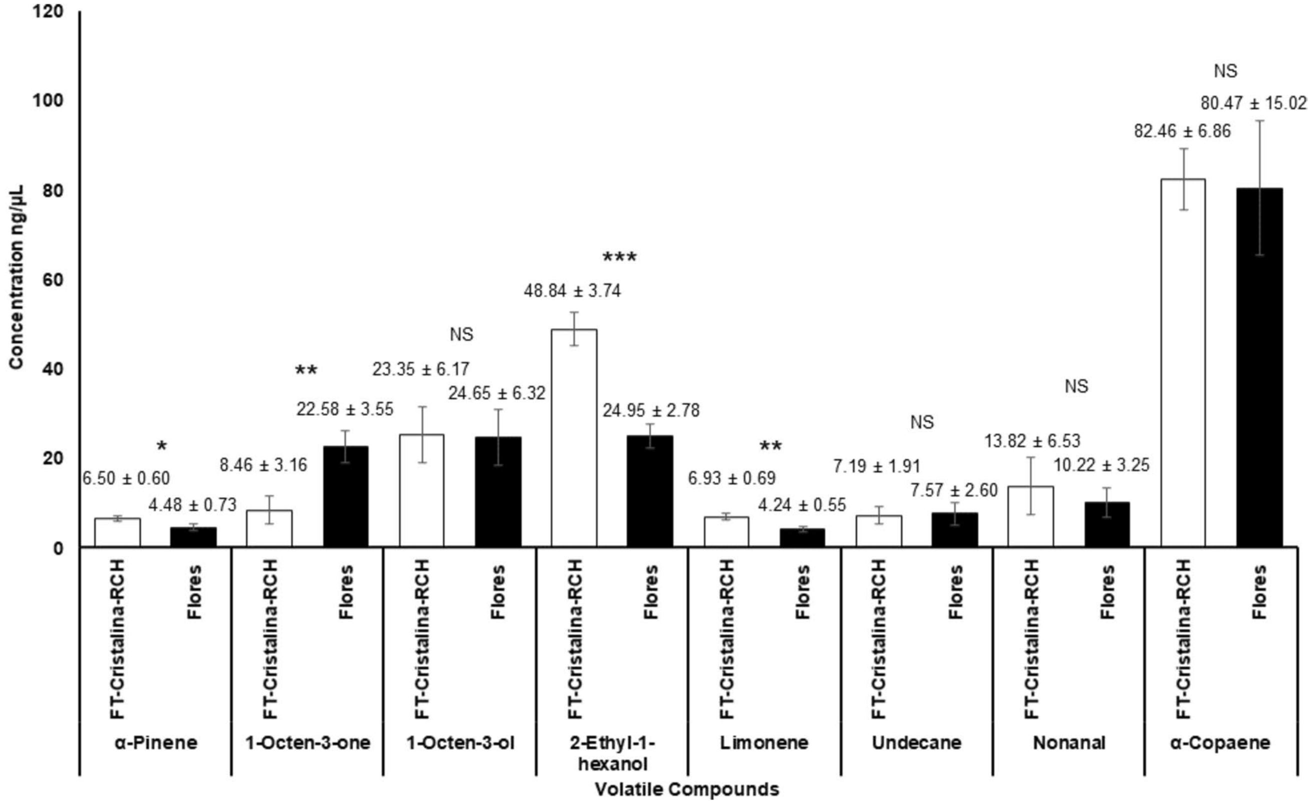

Fig. 4 Quantities (± SE) of the compounds emitted by flowers of the soybean varieties FT-Cristalina-RCH and Flores. Comparisons at $p < 0.05$ were significant (Kruskal–Wallis test). *NS* non-significant

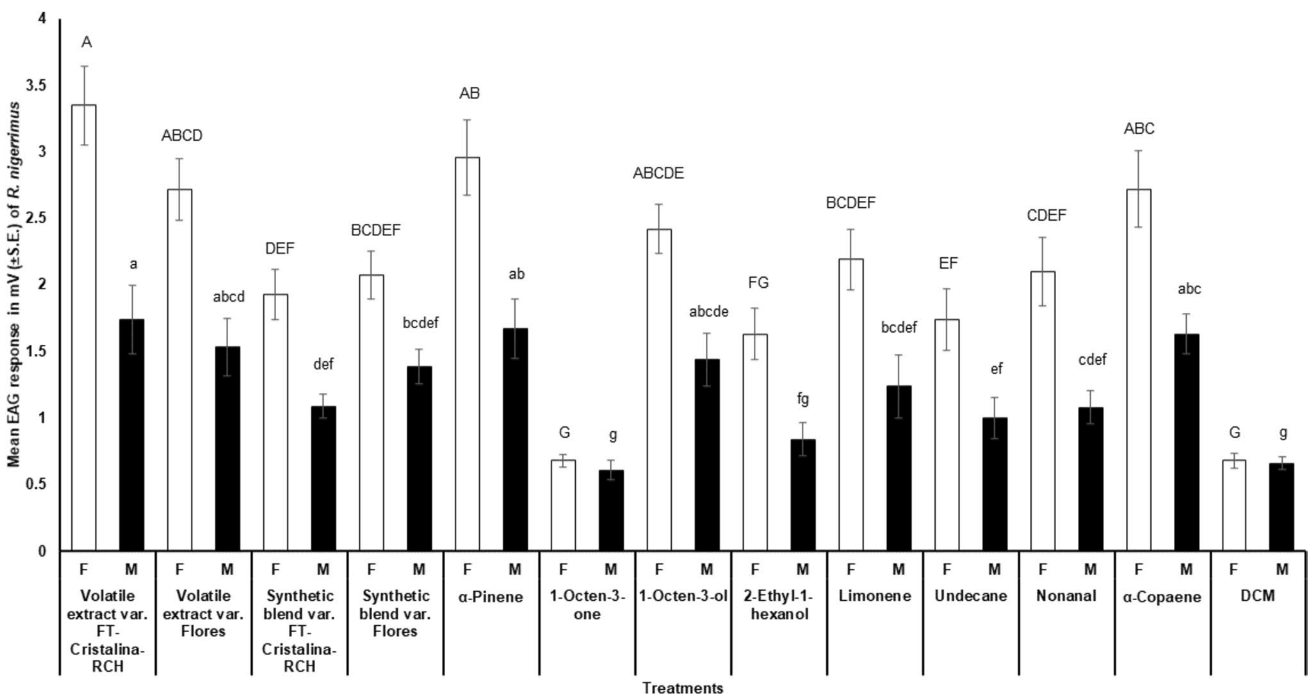

Fig. 5 Electroantennographic (± SE) response of *R. nigerrimus* males and females to volatile extracts, synthetic blends and individual compounds from soybean flowers. Capital letters indicate differences between treatments in females. Lowercase letters indicate differences between treatments in males ($p < 0.05$; ANOVA and Tukey test). *DCM* dichloromethane

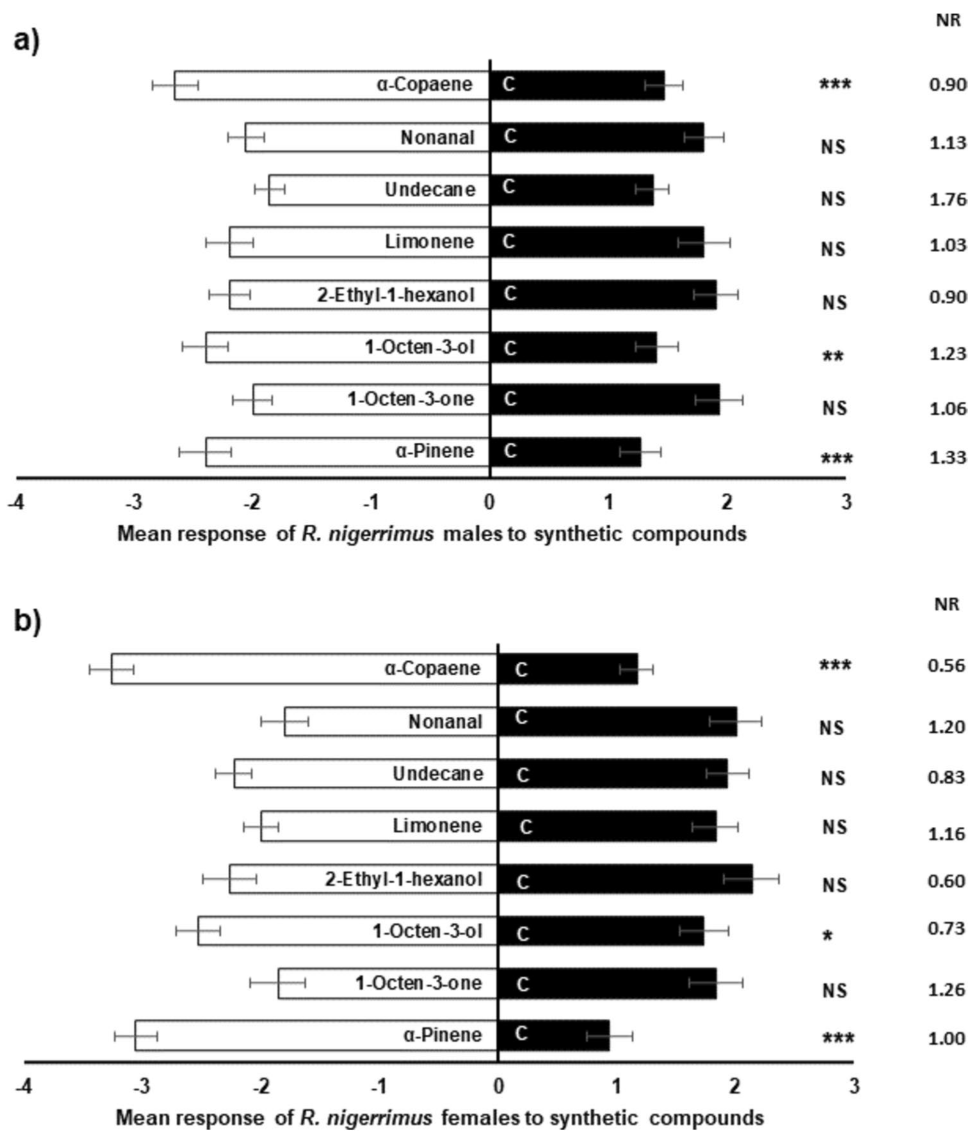

Fig. 6 Mean response (± SE) of *R. nigerrimus* to individual synthetic compounds, **a** males and **b** females. The asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) indicate significant differences between evaluated treatments (G test). *C* control, *NR* non-responding, *NS* non-significant

show preferences. This study must be complemented with additional experiments to corroborate this aspect.

The compounds 1-octen-3-ol, α-copaene and 2-ethyl-1-hexanol were identified as those present in higher proportion in the FT-Cristalina-RCH variety (24.40, 27.19 and 18.03%, respectively), while in the variety Flores the major compounds were 1-octen-3-ol, α-copaene and 1-octen-3-one (25.02, 28.00, 16.62%, respectively). These compounds were also reported by Espadas-Pinacho et al. (2021) in volatiles from soybean plants and pods, but at a lower proportion. Likewise, Boue et al. (2003) reported the presence of 1-octen-3-ol, 2-ethyl-1-hexanol and nonanal in soybean pods, while Cortés (2016) reported 1-octen-3-ol as one of the volatiles consistently released by soybean pods, leaves and seedlings. Nevertheless, one limitation of this work is that flowers compounds were collected using detached flowers and not with attached flowers, because the soybean flowers size and number in a plant make difficult to collect their volatiles using dynamic aeration in field condition.

Rhyssomatus nigerrimus responds electrophysiologically to flower volatiles. In this study, we found that *R. nigerrimus* females exhibit stronger antennal response than males to the different treatments. Likewise, Ceballos et al. (2015) and Adhikari et al. (2002) reported that female *Bruchus pisorum L.* and *Callosobruchus maculatus* (Fabricius) had a stronger electrophysiological response to the extracts from pea (*Pisum sativum L.*) and cowpea seeds (*Vigna unguiculata* L.), respectively. It is likely that this response obeys the female insect's need to find an appropriate host plant and to obtain the proteins necessary for oogenesis (Paukku and Kotiaho 2008).

Generally, α-copaene and α-pinene are dominant terpenoids in the mixtures of floral volatiles. They are common compounds emitted by plants and have preponderant roles

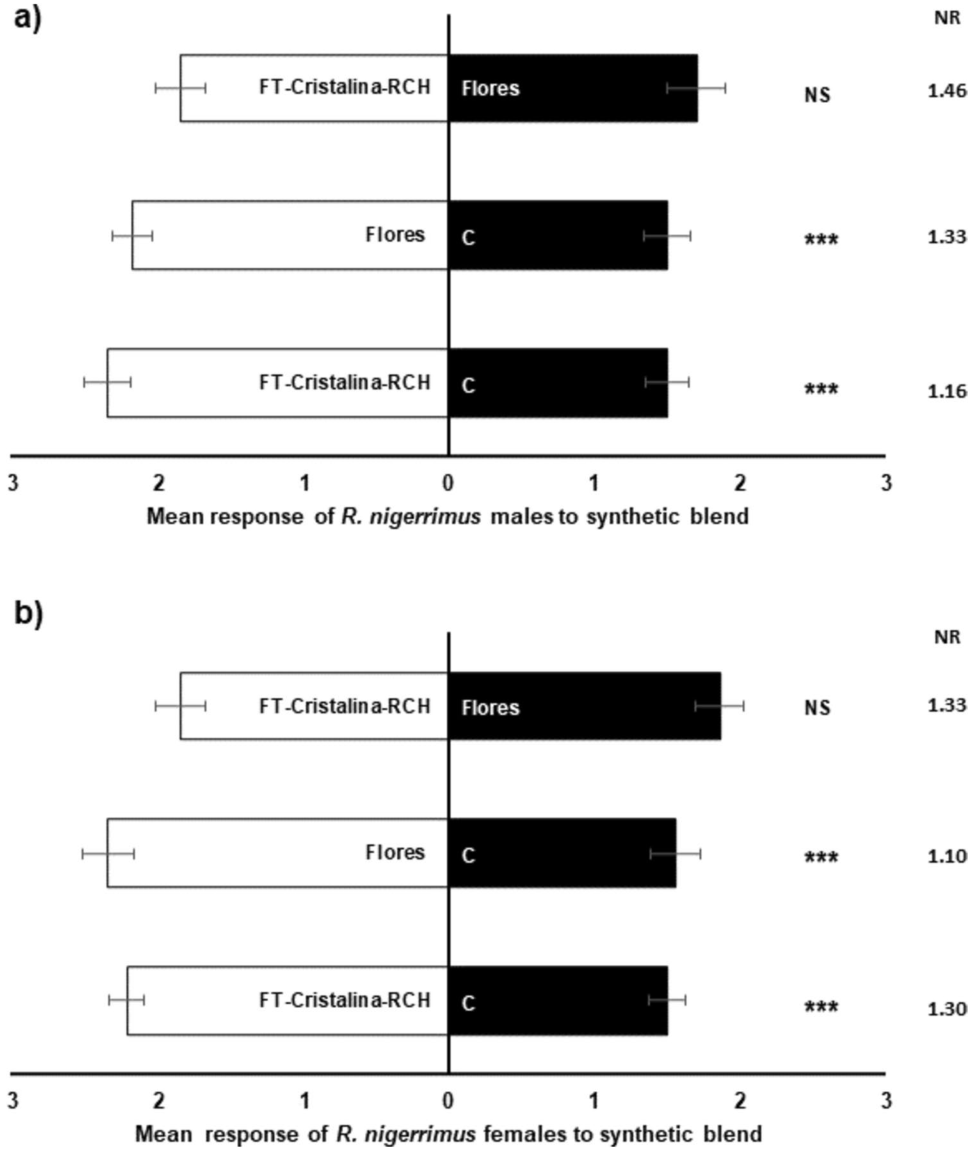

Fig. 7 Mean response (±SE) of *R. nigerrimus* **a** males and **b** females to the eight compounds blend that simulate the odor of varieties of soybean flowers (*G. max* L.): FT-Cristalina-RCH, Flores and both flower varieties, in tests conducted in a Y-tube olfactometer. The asterisks (*$p<0.05$; **$p<0.01$; ***$p<0.001$) indicate significant differences between evaluated treatments (G test). *C* control, *NR* non-responding, *NS* non-significant

in flower-insect and plant–insect interactions (Ramya et al. 2020). However, in our case, the only compound found in larger quantity was α-copaene, while *α*-pinene had interesting results during the bioassays with individual synthetic compounds; we observed that male and female *R. nigerrimus* were attracted to both compounds. Regarding 1-octen-3-ol, there is evidence that it is an attractant for several insect species and is of paramount importance in plant–insect and plant-plant interactions (Chen et al 2019; Ramoni et al. 2001). This was confirmed during the bioassays in which both sexes of *R. nigerrimus* were attracted. Espadas-Pinacho et al. (2021) found volatiles induced by the damage caused by *R. nigerrimus* when it feeds on the soybean plant, this could be similar with respect to the weevil-soybean flower interaction, where the flowers probably emit induced volatiles when the weevil feeds of them, however this hypothesis needs to be tested.

In conclusion, we have demonstrated, through bioassays, that the volatiles emitted by flowers of two soybean varieties attract *R. nigerrimus*. We have also demonstrated that *R. nigerrimus* uses the sense of smell by means of its antennae to detect these volatiles. A blend of eight compounds attracted *R. nigerrimus* under laboratory conditions. These results provide the basis for obtaining an attractant that can be used in the field for management of *R. nigerrimus*.

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Author contributions MG-D conducted experiments, analyzed data, and wrote the first draft of the paper. LC-L and GL-G designed and supervised the development of the project and contributed critical comments to the manuscript and provided biological material from the field. All the authors read and approved the final manuscript.

Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

References

Aceves-Chong L, Cruz-López L, Sánchez-Guillén D, Grajales-Conesa J (2018) Differences in volatile composition and sexual morphs in rambutan (*Nephelium lappaceum* L.) flowers and their effect in the *Apis mellifera* L. (Hymenoptera, Apidae) attraction. Rev Bras Entomol 62:66–70. https://doi.org/10.1016/j.rbe.2017.09.002

Adhikari A, Paranagama P, Abeywickrama K, Premarathne K (2002) Behavioral studies of cowpea seed bruchid, *Callosobruchus maculatus* (F.) against volatile leaf extracts of lemongrass, neem and curry leaf. Trop Agric Res 14:138–147

Baldelomar M, Lacerda M, Jane F (2018) El rol de los compuestos orgánicos volátiles florales en las interacciones planta-insecto. Oecol Aust 22:348–361. https://doi.org/10.4257/oeco.2018.2204.02. (**in Spanish with English abstract**)

Bautista-Lozada A, Bravo-Monzón A, Espinosa-García F (2012) Importancia ecológica de la emisión de compuestos volátiles vegetales. In: Rojas JC, Malo EA (eds) Temas selectos en ecología química de insectos. El Colegio de la Frontera Sur, México, p 446 (**in Spanish**)

Boue SM, Shih BY, Carter-Wientjes C, Cleveland TE (2003) Identification of volatile compounds in soybean at various developmental stages using solid phase microextraction. J Agric Food Chem 51:4873–4876. https://doi.org/10.1021/jf030051q

Bouwmeester H, Schuurink RC, Bleeker PM, Schiestl F (2019) The role of volatiles in plant communication. Plant J 100:892–907. https://doi.org/10.1111/tpj.14496

Campbell DR, Sosenski P, Raguso RA (2019) Phenotypic plasticity of floral volatiles in response to increasing drought stress. Ann Bot 123:601–610. https://doi.org/10.1093/aob/mcy193

Cantúa-Ayala JA, Flores-Olivas A, Valenzuela-Soto JH (2019) Compuestos orgánicos volátiles de plantas inducidos por insectos: situación actual en México. Revista Mexicana De Ciencias Agrícolas 10:729–742 (**in Spanish**)

Ceballos R, Fernández N, Zuñiga S, Zapata N (2015) Electrophysiological and behavioral responses of pea weevil *Bruchus pisorum* L. (Coleoptera: Bruchidae) to volatiles collected from its host *Pisum sativum* L. Chil J Agric Res 75:202–209. https://doi.org/10.4067/S0718-58392015000200009

Chen H, Yang R, Chen J, Luo Q, Cui X, Yan X, Gerwick WH (2019) 1-Octen-3-ol, a self-stimulating oxylipin messenger, can prime and induce defense of marine alga. BMC Plant Biol 19:37. https://doi.org/10.1186/s12870-019-1642-0

Collatz J, Dorn S (2013) A host-plant-derived volatile blend to attract the apple blossom weevil *Anthonomus pomorum*—the essential volatiles include a repellent constituent. Pest Manag Sci 69:1092–1098. https://doi.org/10.1002/ps.3477

Cortés F (2016) Atracción del picudo negro de la soya (*Rhyssomatus nigerrimus*) a los volátiles de la planta de soya. Instituto Tecnológico de Tapachula, México, 35 p. Tesis de licenciatura (**in Spanish**)

Delle-Vedove R, Schatz B, Dufay M (2017) Understanding intraspecific variation of floral scent in light of evolutionary ecology. Ann Bot 120:1–20. https://doi.org/10.1093/aob/mcx055

Dötterl S, Wolfe LM, Jürgens A (2005) Qualitative and quantitative analyses of flower scent in *Silene latifolia*. Phytochemistry 66:203–213. https://doi.org/10.1016/j.phytochem.2004.12.002

Dudareva N, Klempien A, Muhlemann JK, Kaplan I (2013) Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol 198:16–32. https://doi.org/10.1111/nph.12145

Espadas-Pinacho K, López-Guillén G, Gómez-Ruiz J, Cruz-López L (2021) Induced volatiles in the interaction between soybean (*Glycine max*) and the Mexican soybean weevil (*Rhyssomatus nigerrimus*). Braz J Biol 81:611–620. https://doi.org/10.1590/1519-6984.227271

Farré-Armengol G, Filella I, Llusiá J, Peñuelas J (2015) Pollination mode determines floral scent. Biochem Syst Ecol 61:44–53. https://doi.org/10.1016/j.bse.2015.05.007

Fox J, Bouchet-Valat M (2022) Rcmdr: R Commander. R Package version 2.8-0

Hetherington-Rauth MC, Ramírez SR (2016) Evolution and diversity of floral scent chemistry in the euglossine bee-pollinated orchid genus *Gongora*. Ann Bot 118:135–148. https://doi.org/10.1093/aob/mcw072

Hu L, Zhang K, Wu Z, Xu J, Erb M (2021) Plant volatiles as regulators of plant defense and herbivore immunity: molecular mechanisms and unanswered questions. Curr Opin Insect Sci 44:82–88. https://doi.org/10.1016/j.cois.2021.03.010

Junker RR, Höcherl N, Blüthgen N (2010) Responses to olfactory signals reflect network structure of flower-visitor interactions. J Anim Ecol 79:818–823. https://doi.org/10.1111/j.1365-2656.2010.01698.x

Karmakar A, Mitra P, Koner A, Das S, Barik A (2020) Fruit volatiles of creeping cucumber (*Solena amplexicaulis*) attract a generalist insect herbivore. J Chem Ecol 46:275–287. https://doi.org/10.1007/s10886-020-01154-w

López-Guillén G, Gómez-Ruiz J, Cruz-López L, Terán-Vargas AP (2012a) El picudo mexicano de la soya (*Rhyssomatus nigerrimus*) una plaga nueva del trópico. Agroproductividad 5:9–14 (**in Spanish**)

López-Guillén G, Terán-Varga AP, Gómez Ruiz J, San-Juan Lara J, Rosado-Neto GH, O'Brien CW, Cruz-López L, Rodríguez-del-Bosque LA, Alatorre-Rosas R (2012b) First record of *Rhyssomatus nigerrimus* (Curculionidae: Molytinae: Cleogonini) infestations in soybeans in Mexico. Fla Entomol 95:524–528

López-Guillén G, Valdez-Carrasco J, Gómez-Ruiz J, Martínez-Zarate C, Cruz-López L (2016) Dimorfismo y proporción sexual de poblaciones naturales de adultos de *Rhyssomatus nigerrimus*. Southwest Entomol 41:837–844. https://doi.org/10.3958/059.041.0325. (**in Spanish with English abstract**)

Marín-Loaiza JC, Céspedes CL (2007) Compuestos volátiles de plantas. Origen, emisión, efectos, análisis y aplicaciones al agro. Rev Fitotec Mex 30:327–351 (**in Spanish with English abstract**)

Mozūraitis R, Hall D, Trandem N, Ralle B, Tunström K et al (2020) Composition of strawberry floral volatiles and their effects on behavior of strawberry blossom weevil, *Anthonomus rubi*. J Chem Ecol 46:1069–1081. https://doi.org/10.1007/s10886-020-01221-2

Pan H, Lu Y, Xiu C, Geng H, Cai X et al (2015) Volatile fragrances associated with flowers mediate host plant alternation of a polyphagous mirid bug. Sci Rep 5:14805. https://doi.org/10.1038/srep14805

Paukku S, Kotiaho JS (2008) Female oviposition decisions and their impact on progeny life-history traits. J Insect Behav 21:505–520. https://doi.org/10.1007/s10905-008-9146-z

Ramoni R, Vincent F, Grolli S, Conti V, Malosse C, Boyer FD, Nagnan-Le Meillour P, Spinelli S, Cambillau C, Tegoni M (2001)

The insect attractant 1-octen-3-ol is the natural ligand of bovine odorant-binding protein. J Biol Chem 276:7150–7155. https://doi.org/10.1074/jbc.M010368200
Ramya M, Jang S, An HR, Lee SY, Park PM, Park PH (2020) Volatile organic compounds from orchids: from synthesis and function to gene regulation. Int J Mol Sci 21:1160. https://doi.org/10.3390/ijms21031160
Schiestl FP (2015) Ecology and evolution of floral volatile-mediated information transfer in plants. New Phytol 206:571–577. https://doi.org/10.1111/nph.13243
Song J, Lee G, Jung J, Moon JK, Kim SG (2022) Effect of soybean volatiles on the behavior of the bean bug, *Riptortus pedestris*. J Chem Ecol 48:207–208. https://doi.org/10.1007/s10886-021-01343-1
Szendrei Z, Malo E, Stelinski L, Rodriguez-Saona C (2009) Response of cramberry weevil (Coleoptera: Curculionidae) to host plant volatiles. Environ Entomol 38:861–869. https://doi.org/10.1603/022.038.0340
Terán-Vargas AP, López Guillén G (2014) El picudo de la soya *Rhyssomatus nigerrimus* Fahraeus 1837 (Coleoptera: Curculionidae). Folleto técnico No. MX-0-310304-47-03-14-09-38. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, pp 1–25 (**in Spanish**)
Wang H, Zheng P, Aoki D, Miyake T, Yagami S, Matsushita Y, Fukushima K, Nakagawa M (2018) Sexual and temporal variations in floral scent in the subdioecious shrub *Eurya japonica* Thunb. Ecol Evol 8:8266–8272. https://doi.org/10.1002/ece3.4378
Wang TN, Clifford MR, Martínez-Gómez J, Johnson JC, Riffell JA, Di Stilio JS (2019) Scent matters: differential contribution of scent to insect response in flowers with insect vs. wind pollination traits. Ann Bot 123:289–301. https://doi.org/10.1093/aob/mcy131
Zhang FP, Yang QY, Wang G, Zhang SB (2016) Multiple functions of volatiles in flowers and leaves of *Elsholtzia rugulosa* (Lamiaceae) from southwestern China. Sci Rep 6:27616. https://doi.org/10.1038/srep27616