



Attraction of *Rhyssomatus nigerrimus* (Fahraeus) (Coleoptera: Curculionidae) to Odors from Alternate Host Plants

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The soybean weevil *Rhyssomatus nigerrimus* (Fahraeus), is an economically important pest that attacks soybean (*Glycine max* (L.) Merrill] crops in northern and southern Mexico. *Rhyssomatus nigerrimus* can be considered a specialist insect because it selects soybean plants for its reproduction and feeding. However, when the rainy season begins, new emerged adult weevils can feed and take refuge in other alternate host plants, such as *Rottboellia cochinchinensis* (Lour.) W.

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Clayton, *Hyparrhenia rufa* (Nees) Stapf, *Sorghum halepense* (L.) Persoon and *Ipomoea trifida* (Kunth) G. Don when soybean is not available. These host plants can potentially be used as attractant plants. The objectives of this study were a) to determine the attraction response of adult *R. nigerrimus* to four species of alternate host and their volatiles extracts, b) to identify the volatile compounds present in each alternate host plant species volatiles extracts, and c) to determine the antennal response (EAG) of female and male *R. nigerrimus* to volatiles extracts from the alternate host plants. The attraction bioassays were conducted in an arena, on which we evaluate the *R. nigerrimus* response to foliage and extract of the alternate host plants. The antennal response of *R. nigerrimus* females and males to volatiles from alternate host plants was determined by the electroantennogram (EAG) technique. Female and male *R. nigerrimus* were more attracted by the foliage and extracts from *I. trifida*, *R. cochinchinensis*, *H. rufa* and *S. halepense* than to the control. The strongest EAG response of female and male *R. nigerrimus* was observed with the volatiles from *S. halepense*, *H. rufa*, *I. trifida* and *R. cochinchinensis*. The control elicited the weakest antennal response. The GC-MS analysis of the volatile compounds from *I. trifida*, *S. halepense*, *H. rufa* and *R. cochinchinensis* revealed the presence of 17, 9, 9 and 9 compounds, respectively. Major volatiles compounds could be used as lures in traps to control *R. nigerrimus*.

Keywords: *The soybean weevil; volatiles; bioassays; EAG.*

1. INTRODUCTION

The soybean weevil, *Rhyssomatus nigerrimus* (Fahraeus) (Coleoptera: Curculionidae), is an economically important pest in soybean crops in Chiapas, Tamaulipas, San Luis Potosí and Veracruz, Mexico [1,2]. Although both male and female adults feed on seedlings, stems, petioles, buds, flowers, and pods causing serious lesions, the most severe damage is inflicted by females when they oviposit inside the developing pods where the larvae feed on the soybean seeds [1]. Damage of up to 70% of pods has been reported when no control measure is applied [2].

The soybean weevil is a specialist that selects only soybean plants (*Glycine max* L.; Fabaceae) as its main host for reproduction and feeding [3,2]. However, when soybean plants are not available, recently emerged adults can take refuge in and feed on foliage of some alternate host species of the Poaceae, Convolvulaceae, Asteraceae, Lorentaceae, Vervencaceae, Rubiaceae, Portulacaceae and Fabaceae families [1,3,2]. The same feeding habits on host weeds growing in the periphery of soybean fields have been reported in *Rhyssomatus subtilis* Fiedler (Coleoptera: Curculionidae) adult before soybeans are planted in Argentina [4]. For this reason, it is important to understand what chemical and physical factors are involved in selecting some alternate host plant species by the Curculionidae family [5-11].

Insects of the Curculionidae family are attracted to volatiles compounds emitted by its host plants and from its alternate host and non-hosts. For

example, *Chalcodermus aeneus* Boheman is attracted to odor of wild alternate hosts for feeding on leaves, stems and inflorescences [12]. *Diaprepes abbreviatus* (L.) and *Otiorhynchus sulcatus* F., are also attracted to volatiles from their alternate host plants [13,14]. Determining the behavioral and antennal response of adult *R. nigerrimus* to the main alternate host plant volatiles in soybean fields could help to establish monitoring and control programs for *R. nigerrimus* in soybean crops.

Objective of Study: The objectives of this study were a) to determine the attraction response of adult *R. nigerrimus* to four species of alternate host plants (*I. trifida*, *H. rufa*, *S. halepense* and *R. cochinchinensis*) and their extracts, b) to identify the volatile compounds present in each alternate host plant species extracts and c) to determine the antennal response (EAG) of female and male *R. nigerrimus* to volatiles from the alternate host plants extracts.

2. MATERIALS AND METHODS

2.1 Insects and Plants

We collected adult soybean weevils, *R. nigerrimus*, from August to November 2021 in alternate host plants on the soybean field boundaries which are located in the ejidos Francisco I. Madero (14° 51' 46" N; 92° 23' 16" W, 30 m) and Las Tinajas 2nd section (14°42' 49" N; 092° 20' 43" W, 16 m), in the municipality of Tapachula, Chiapas, Mexico. The collected adults were taken to the insectary of the Crop Protection Laboratory of INIFAP (Instituto Nacional de Investigaciones, Forestales,

Agrícolas y Pecuarias), Rosario Izapa Experimental Station, Tuxtla Chico, Chiapas, Mexico. The weevils were sexed under a stereoscopic microscope following the technique previously described [15]. Males and females were placed in separate 1 L plastic recipients. The insects were fed with pieces (0.5 mm X 0.5 mm) of sweet potato (*Ipomea batatas* L.) daily and kept in the insectarium of INIFAP at a temperature of 27 ± 1 °C, $70 \pm 5\%$ relative humidity, and a photoperiod of 12 h light:dark.

We collected the four alternate host plant species of adult *R. nigerrimus*: itchgrass [*Rottboellia cochinchinensis* (Lour.) W. Clayton], jaraguagrass [*Hyparrhenia rufa* (Nees) Stapf], Johnsongrass [*Sorghum halepense* (L.) Persoon (Poaceae)], and tie-vine morning glory [*Ipomoea trifida* (Kunth) G. Don (Convolvulaceae)] in the periphery of soybean fields in the ejidos Francisco I. Madero and Tinajas 2nd Sección, in the municipality of Tapachula, Chiapas. The plant material was stored separately in plastic bags that were labeled, sealed and transported in a cooler with refrigerating gel to the laboratory.

2.2 *Rhysomatus nigerrimus* Attraction Response to Foliage and Volatiles from Alternate Hosts

We conducted bioassays in a rectangular plastic arena (36 x 26 cm, with a 14-cm-tall wall), which contained a layer of 8 cm of sterilized soil, similar to the arena described by [16]. At one extreme end of the arena, we placed 16 g of foliage of any the alternate host plant (*I. trifida*, *H. rufa*, *S. halepense* or *R. cochinchinensis*) on a 5 cm diameter circle of Whatman #2 filter paper. At the opposite extreme end of the arena, we placed only a filter paper circle 5 cm in diameter, which was used as the control. For the bioassays with alternate host plant extracts, 100 µL of one extract was applied in one of the piece of filter paper, respectively, and 100 µL dichloromethane as the control in the other piece of filter paper. After application of each extracts or solvent, the circles were left for 20 s to evaporate the solvents. The methodology to collect the volatiles is described in the section of volatile capture from alternate hosts. At the center of the arena, we released three female or male *R. nigerrimus* weevils per replication and treatment (foliage or dichloromethane extracts of alternate plant hosts), respectively. The weevils that were used in the bioassays had fasted for 24 h. Insect response was observed for 5 min, and the number of insects attracted to the treatment or

control was recorded. The bioassays were conducted in a dark room at a temperature of 25 ± 2 °C between 9:00 and 18:00 h, the period of the most activity of *R. nigerrimus* in natural conditions [17]. A total of 50 replications per sex of weevil were conducted for each treatment and the control.

2.3 Volatile Capture from Alternate Host Plants

The volatile extracts from alternate plant hosts of *R. nigerrimus* that were used in the behavioral bioassays were collected using the dynamic aeration method described in detail by [18]. The complete plant (approximately 500 mg) was placed in a glass chamber (1 L). On top of the chamber, we installed a collector with two entries, we placed an activated carbon filter in one of the entry and in the other one we placed the adsorbent Super Q (5 mg) (Alltech Associates, Deerfield, IL) where the volatiles emitted by the plant were trapped. The volatiles contained in the collection chamber were suctioned with a vacuum pump (air flow at 1 L/min) connected to a flowmeter (Gilmont Instruments Cole Parmer, USA), which in turn was connected to the collector. The collection process lasted 24 hours. The volatiles adsorbed by the Super Q were extracted with 400 µL HPLC grade dichloromethane and stored at up to -20°C in capped 2 mL glass vials. During all collections of volatiles, illumination was provided by four fluorescent lamps of 39 watts in a room without windows, with room temperature at 25 ± 2 °C and 50-60% RH.

2.4 Chemical Identification of Compounds from Alternate Host Plants

The volatile compounds from the extracts were identified in a Gas Chromatograph Shimadzu GC-2010 Plus (Tokyo, Japan) coupled to a mass spectrometer plus triple quadrupole TQ8040 (Colombia, Maryland, USA). A 5% phenyl-methyl-silicone capillary column (DB5-MS) 30 m x 0.25 mm internal diameter and 0.25 µm film thickness was used. The temperature schedule was initiated at 5 °C and maintained for 2 min, and then was elevated 15 °C/min up to 280 °C and finally maintained for 10 min. The injector temperature was 250 °C, and ionization was carried out by electron impact at 70 eV and 250 °C. The analyzed aliquot was 1 µL per extract of each alternate host plant extract obtained by dynamic aeration.

Volatile compounds were identified by comparing the retention index, the mass spectrum, and the retention times of synthetic standards. Other compounds were tentatively identified based on comparison with the spectra of the library of the National Institute of Standards and Technology (NIST, version 2.0). The relative proportions of each of the compounds were quantified with the peak areas of the chromatographs.

2.5 Electrophysiological Response of *R. nigerrimus* to Volatiles from their Alternate Host Plants

The antennal response of *R. nigerrimus* females and males to volatiles from the *H. rufa*, *S. halepense*, *R. cochinchinensis* and *I. trifida* extracts was determined by the electroantennogram (EAG) technique. The thorax of *R. nigerrimus* adult was gently cut, and a reference electrode was inserted into its base using a glass capillary filled with physiological Ringer solution (NaCl 0.35 g, CaCl₂ 0.21 g, KCl 0.35 g, and NaHCO₃ 0.2 g dissolved in 1 L water). A section of the antenna scape was carefully filed to insert the glass capillary of the recording electrode [19]. The signals generated by the antenna passed through a high impedance amplifier (NL 1200; Syntech, Hilversum, The Netherlands) and were visualized in a monitor using Syntech software to process the EAG signals. We used a stimulus flow regulator (CS-05; Syntech, Hilversum, The Netherlands) at 1-min intervals. A constant current of purified and humidified air was directed over the antenna (0.7 L/min) through a glass tube 10 mm in diameter in which the sample was placed for its analysis. The samples were placed separately inside a Pasteur pipette 150 mm long. 1 µl of the alternate host plant extract was applied on a small piece of filter paper and then it was introduced into each pipette. Purified air was used as the control. To present the stimulus, the tip of the pipette that contained the sample was inserted through a lateral orifice located at mid-point of a glass tube through which pure humidified air passed at 0.5 L/min. Duration of the stimulus was 1 s. 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 samples were evaluated in random order. The treatments were replicated for each insect; we evaluated the

volatiles of four alternate hosts on 10 male and 10 female *R. nigerrimus* adults.

2.6 Statistical Analysis

The data on adult female and male *R. nigerrimus* behavioral responses to foliage and volatiles extracts from alternate host plants were analyzed with *G* tests with Williams' correction with $\alpha = 0.05$ [20]. Data on antennal responses of male and female *R. nigerrimus* to volatiles from the different species of alternate hosts were analyzed with an analysis of variance (ANOVA) and Tukey comparison of means ($\alpha = 0.05$). The analyses were carried out using R [21].

3. RESULTS

3.1 Attraction Response of *R. nigerrimus* to Foliage of their Alternate Hosts

Adult female and male *R. nigerrimus* were more attracted to the foliage of *R. cochinchinensis* (females: $G = 81.38$, $df = 1$, $P = 0.00$; males: $G = 87.96$, $df = 1$, $P = 0.00$) than to the control (Fig. 1).

Similarly, adult female and male *R. nigerrimus* were more attracted to the foliage of *H. rufa* (females: $G = 41.4$, $df = 1$, $P = 0.00$; males: $G = 45.3$, $df = 1$, $P = 0.00$) than to the control (Fig. 2).

Both adult female and male *R. nigerrimus* responded significantly to the foliage of *S. halepense* relative to the response to the control (females: $G = 54.9$, $df = 1$, $P = 0.00$; males: $G = 63.3$, $df = 1$, $P = 0.00$) (Fig. 3).

Finally, adult female and male *R. nigerrimus* were more attracted to the foliage of *I. trifida* (females: $G = 72.5$, $df = 1$, $P = 0.00$; males: $G = 75.8$, $df = 1$, $P = 0.00$) than to the control (Fig. 4).

3.2 Attraction Response of *R. nigerrimus* to Volatiles from their Alternate Hosts

Adult male and female *R. nigerrimus* response to the volatiles extracts from *R. cochinchinensis* was significantly stronger than to the control (females: $G = 93.86$, $df = 1$, $P = 0.00$; males: $G = 106.92$, $df = 1$, $P = 0.00$) (Fig. 5).

Similarly, both male and female *R. nigerrimus* responded more to the volatiles extracts from *H. rufa* than to the control (females: $G = 115.1$, $df = 1$, $P = 0.00$; males: $G = 130.8$, $df = 1$, $P = 0.00$) (Fig. 6).

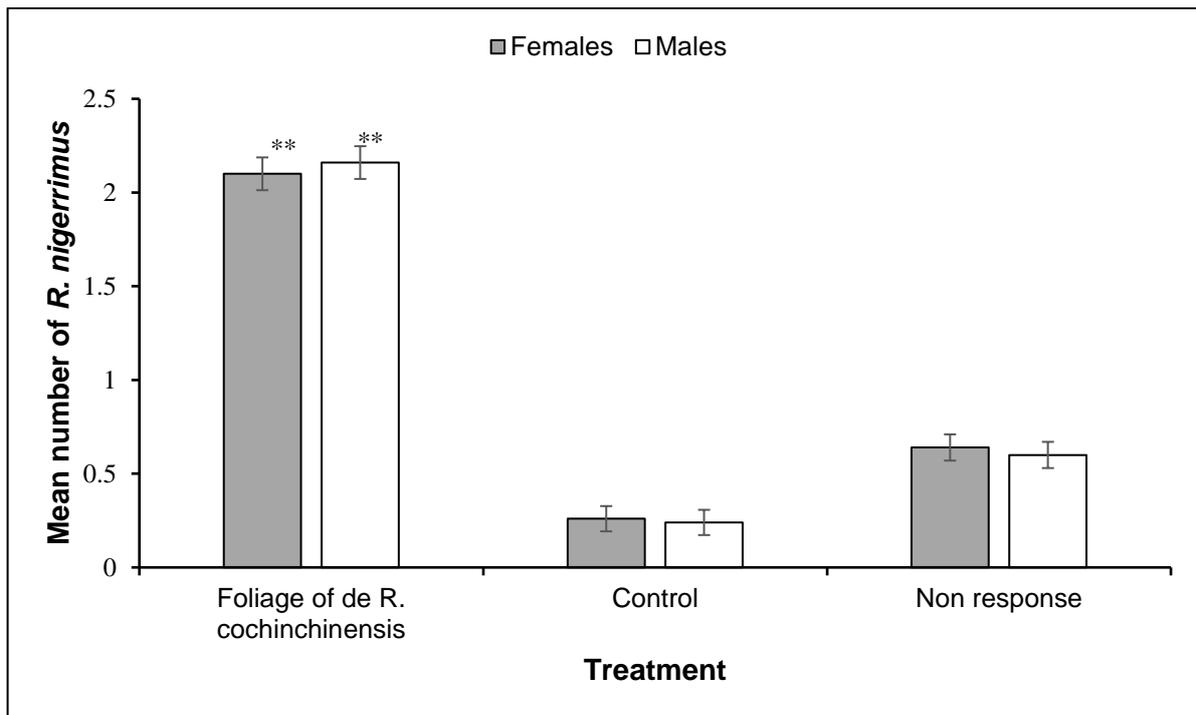


Fig. 1. Average response (means \pm S.E.) of adult male and female *R. nigerrimus* to *R. cochinchinensis* foliage. Bars marked with ** indicate significant differences with respect to the bar of the same color (G tests with Williams' correction, P = 0.00).

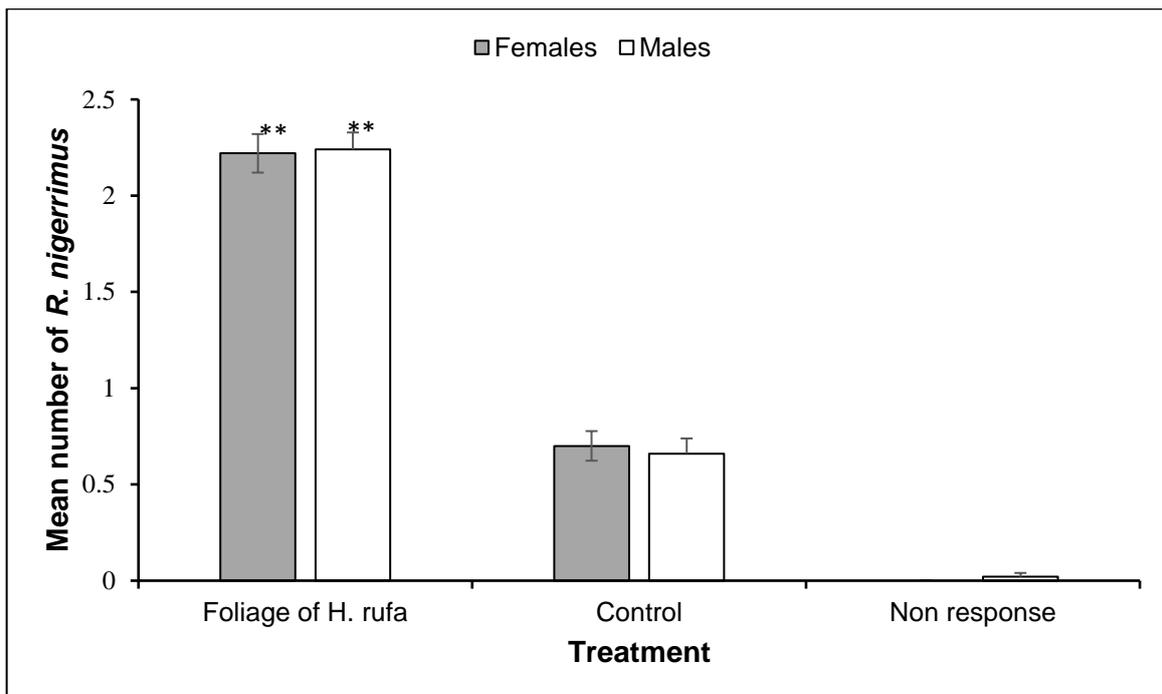


Fig. 2. Average response (means \pm S.E.) of adult female *R. nigerrimus* to *H. rufa* foliage. Bars marked with ** indicate significant differences with respect to the bar of the same color (G tests with Williams' correction, P = 0.00).

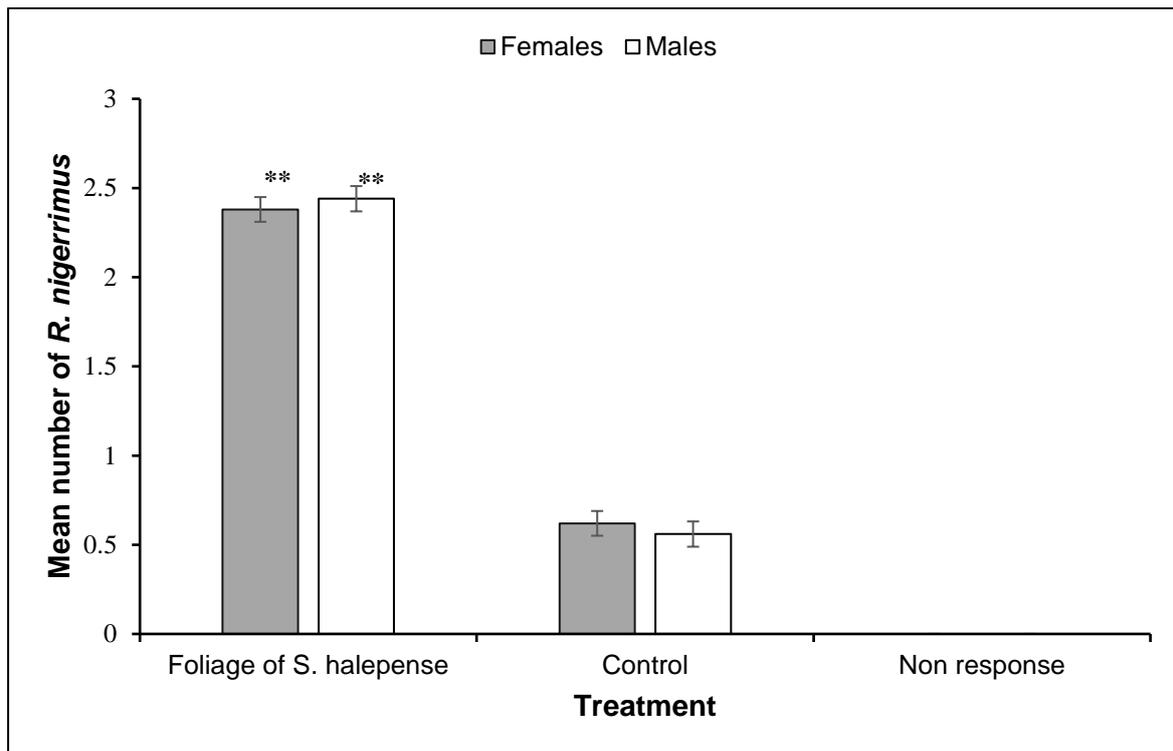


Fig. 3. Average response (means \pm S.E.) of adult female and male *R. nigerrimus* to *S. halepense* tissue. Bars marked with ** indicate significant differences with respect to the bar of the same color (G tests with Williams' correction, P = 0.00).

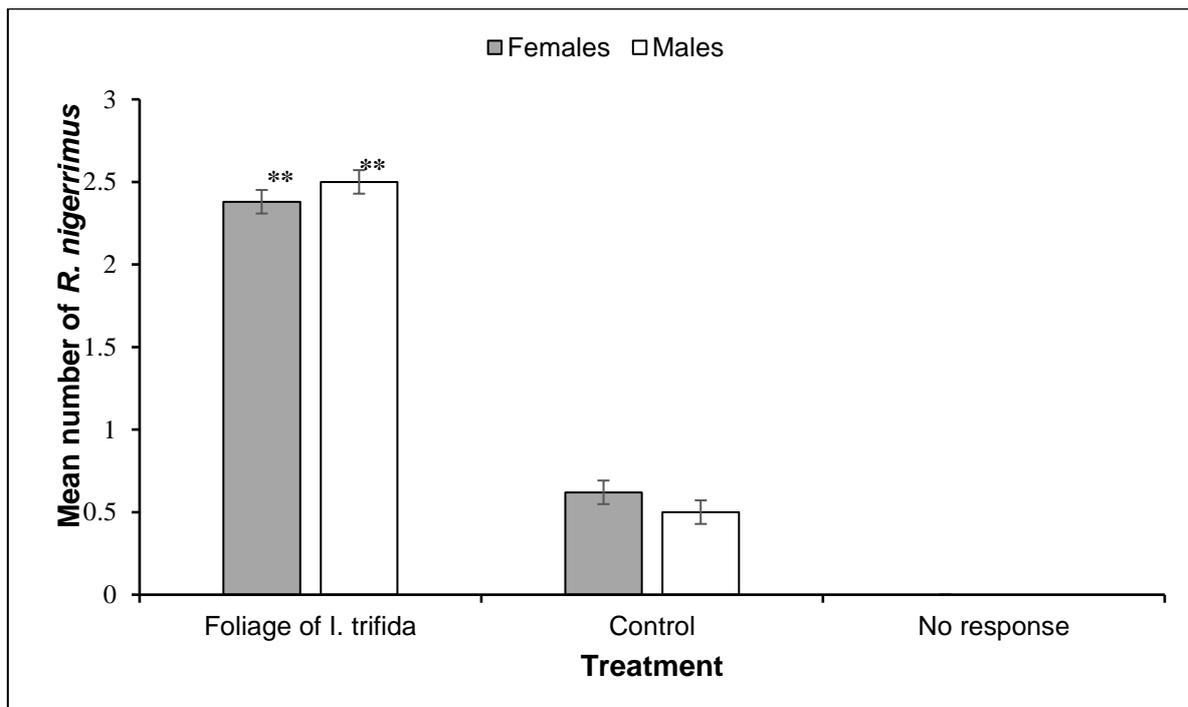


Fig. 4. Average response (means \pm S.E.) of adult female and male *R. nigerrimus* to *I. trifida* foliage. Bars marked with ** indicate significant differences with respect to the bar of the same color (G tests with Williams' correction, P = 0.00).

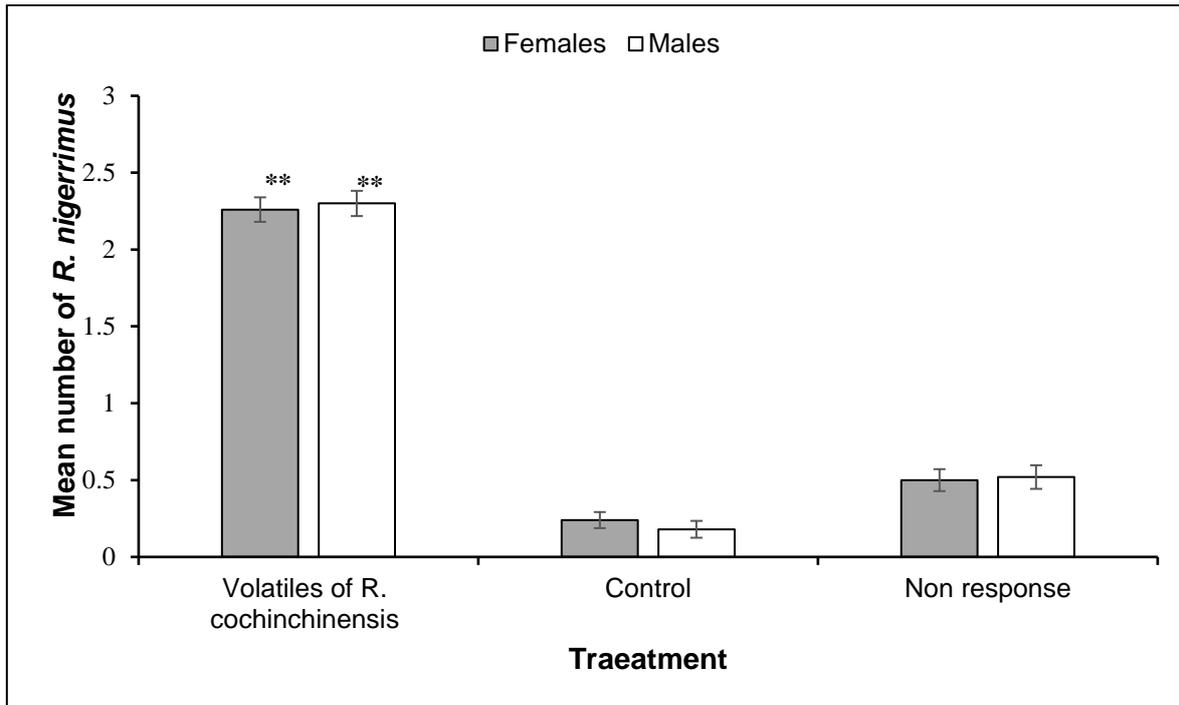


Fig. 5. Average response (means \pm S.E.) of adult female and male de *R. nigerrimus* to *R. cochinchinensis* volatiles. Bars marked with ** indicate significant differences with respect to the bar of the same color (G tests with Williams' correction, P = 0.00).

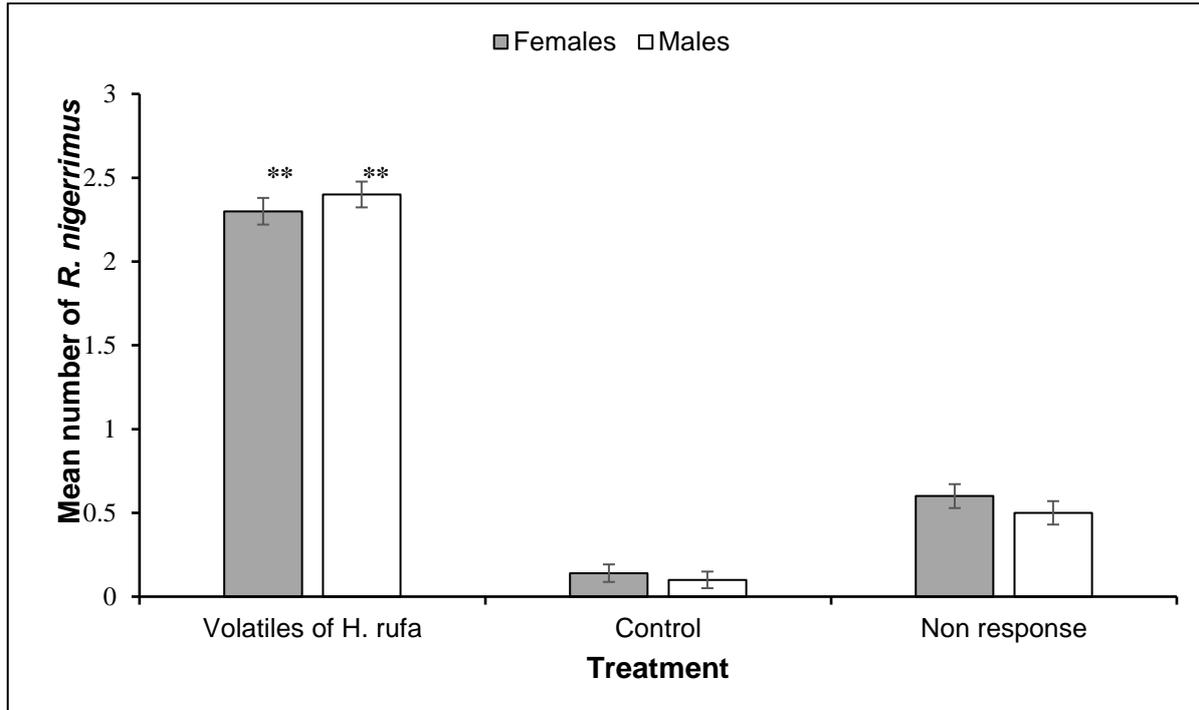


Fig. 6. Average response (means \pm S.E.) of adult female and male *R. nigerrimus* to *H. rufa* volatiles. Bars marked with ** indicate significant differences with respect to the bar of the same color (G tests with Williams' correction, P = 0.00).

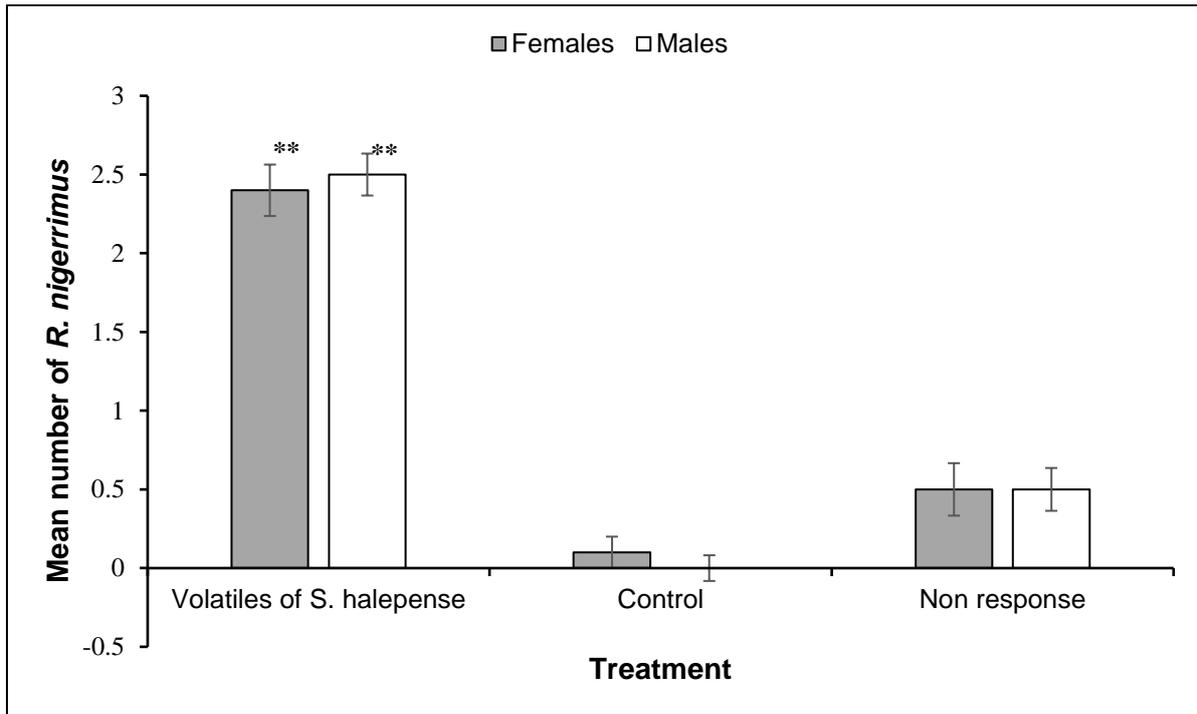


Fig. 7. Average response (means \pm S.E.) of adult female and male *R. nigerrimus* to *S. halepense* volatiles. Bars marked with ** indicate significant differences with respect to the bar of the same color (*G* tests with Williams' correction, $P = 0.00$).

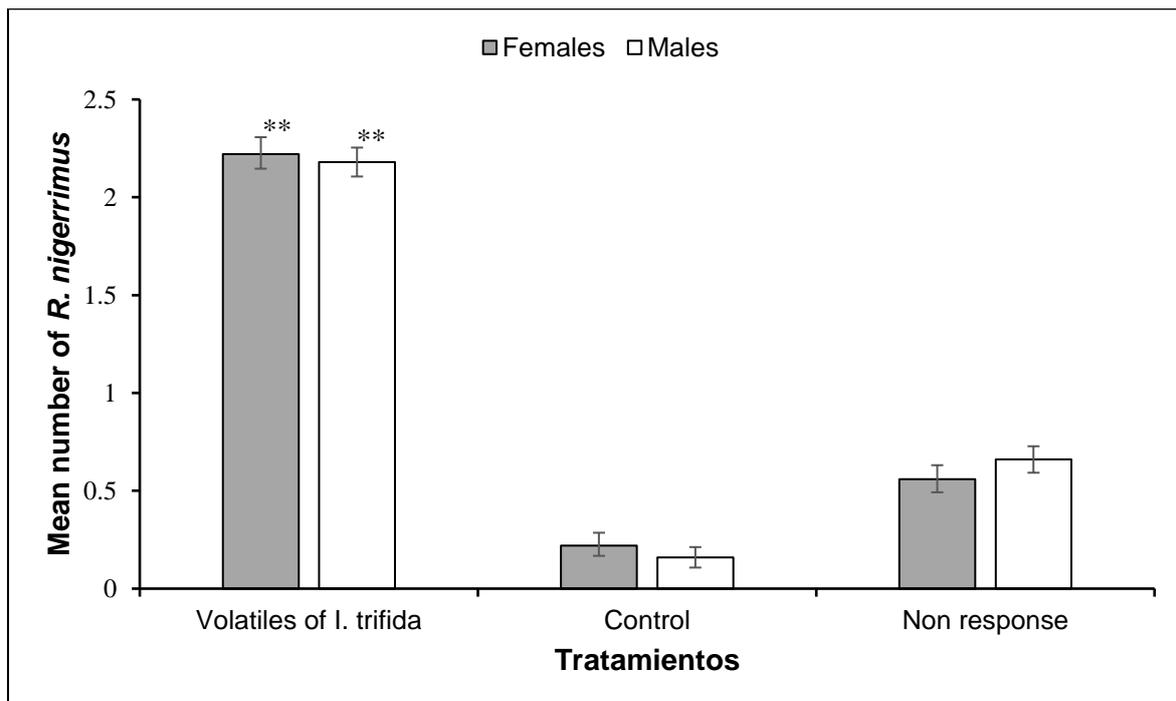


Fig. 8. Average response (means \pm S.E.) of adult female and adult *R. nigerrimus* to *I. trifida* volatiles. Bars marked with ** indicate significant differences with respect to the bar of the same color (*G* tests with Williams' correction, $P = 0.00$).

Table 1. Relative abundance of the compounds identified in *I. trifida*, *S. halepense*, *H. rufa* and *R. cochinchinensis*

#	RT (min)	RI	Compound	Area (%)			
				<i>I. trifida</i> ^a	<i>S. halepense</i> ^b	<i>H. rufa</i> ^c	<i>R. cochinchinensis</i> ^d
1	3.71	868	<i>cis</i> -3-Hexenol	1.36	37.44		35.49
2	4.76	870	Anisole		4.13		1.94
3	4.98	937	α -Pinene		2.08	20.62	2.24
4	5.83	948	β -Pinene	0.42			7.13
5	6.04	964	<i>cis</i> -3-Hexenyl acetate	1.01	23.81	0.29	22.62
6	6.15	972	3-Carene	0.55		39.96	
7	6.28	982	β -Cymene	4.93			
8	6.45	994	2-Ethyl-1-hexanol		15.22		
9	6.58	1004	β -Ocimene	1.33			
10	6.71	1017	Benzeneacetaldehyde				5.69
11	6.78	1022	γ -Terpinene	1.04			
12	7.18	1057	Terpinolene			1.95	
13	7.45	1079	(<i>E</i>)-4,8-Dimethylnona-1,3,7-triene			3.23	
14	7.47	1082	<i>D</i> -Verbenone	51.26			
15	7.68	1098	Phenylethyl alcohol		8.53		14.37
16	8.13	1141	2-Ethyl-1-hexanol acetate		1.79		4.66
17	8.87	1209	β -Cyclocitral		1.39		5.86
18	10.11	1337	α -Cubebene	0.98			
19	10.44	1372	α -Copaene	1.37			
20	10.77	1407	Cedr-8-ene			19.41	
21	10.98	1433	β -Caryophyllene	14.13	5.61	6.0	
22	11.29	1468	Humulene	2.47		1.55	
23	11.37	1478	β -Guaiene	4.29			
24	11.47	1489	β -copaene	1.55			
25	11.60	1503	β -Humulene	12.46		6.99	
26	11.81	1530	<i>trans</i> -Calamenene	0.27			
27	12.03	1557	Nerolidol	0.58			

retention index (RI), retention time (RT), superindices.

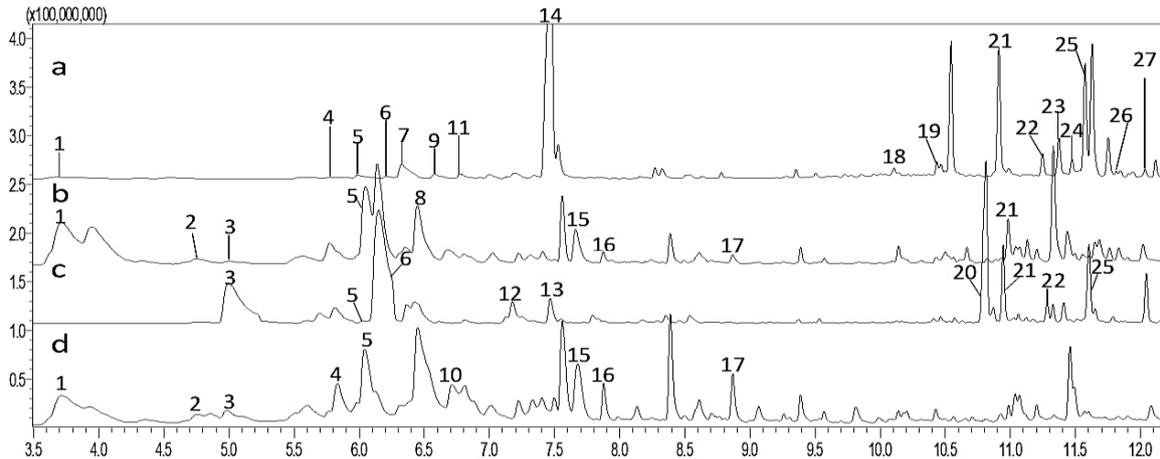


Fig. 9. Chromatograms of total ions of *I. trifida*, *S. halepense*, *H. rufa* and *R. cochinchinensis* volatiles analyzed by GC-MS.

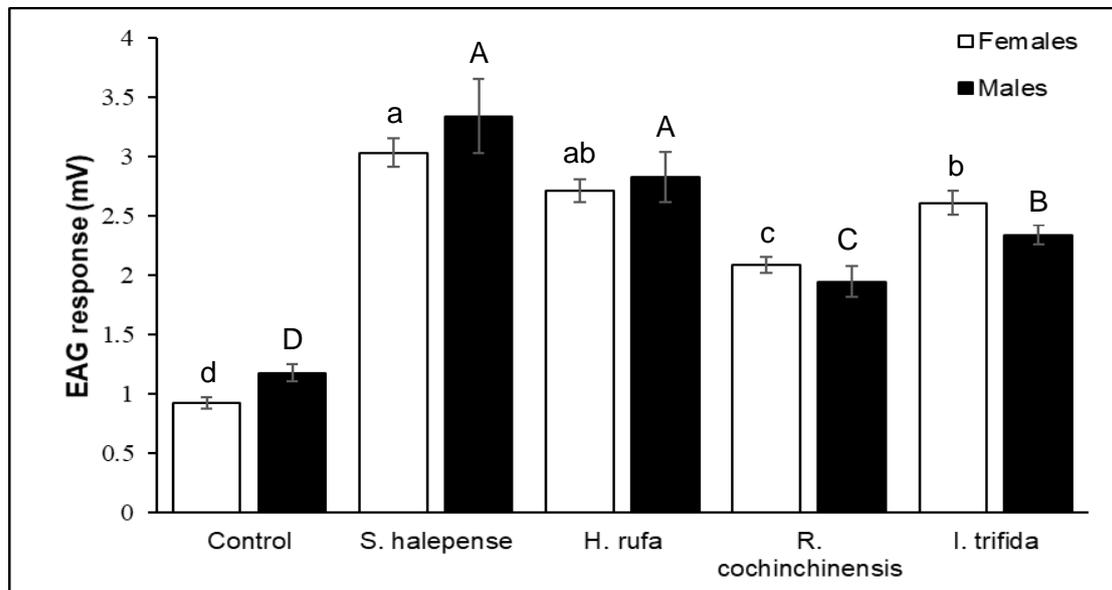


Fig. 10. EAG responses (means \pm S.E.) of *R. nigerrimus* to volatiles from alternate hosts. Means followed by different letters (lower case for female and upper case for males) are significantly different (HSD Tukey, $P = 0.05$).

Response of both adult male and female *R. nigerrimus* were stronger to the volatiles extracts from *S. halepense* (females: $G = 25.7$, $df = 1$, $P = 0.00$; males: $G = 44.2$, $df = 1$, $P = 0.00$) than to the control (Fig. 7), as well as to the volatiles from *I. trifida* than the control (females: $G = 94.83$, $df = 1$, $P = 0.00$; males: $G = 103.39$, $df = 1$, $P = 0.00$) (Fig. 8).

3.3 Chemical Composition of Volatiles from Alternate Hosts

The GC-MS analysis of the volatile extracts from the grasses showed that in *I. trifida* extract

contains *D*-verbenone (51.26%), β -caryophyllene (14.13%) and β -humulene (12.46%) as mayor components. For *S. halepense*, the most abundant compounds were *cis*-3-hexenol (37.44%), *cis*-3-hexenyl acetate (23.81%) and 2-ethyl-1-hexanol (15.22%). For *I. rufa*, the most abundant compounds were 3-carene (39.9%), α -pinene (20.6) and cedr-8-ene (19.4%). Finally, in *R. cochinchinensis*, the compounds found in greater relative abundance were *cis*-3-hexenol (35.49%), *cis*-3-hexenyl acetate (22.62%) and phenylethyl alcohol (14.37%) (Fig. 9, Table 1).

3.4 Electroantennographic (EAG) Response of *R. nigerrimus* to Weed Volatiles

The level of antennal response emitted for both *R. nigerrimus* females and males was significantly different between the evaluated treatments (females: $F= 83.49$; $df= 4,45$; $P = 0.05$; males: $F=20.2$; $df= 4,45$; $P = 0.05$). The strongest antennal response of female *R. nigerrimus* was observed with volatiles extracts from *S. halepense* and *H. rufa*, followed by their response to volatiles from *I. trifida*, which was similar to the response from *R. cochinchinensis*. The weakest antennal response was to the control (Fig. 10). The antennae of male *R. nigerrimus* showed more EAG activity with the volatiles extract from *S. halepense* and *H. rufa*. The antennal response to *R. cochinchinensis* and *I. trifida* volatiles was intermediate between the response to the volatiles from *S. halepense* and *H. rufa* and the response to the control. The weakest antennal response was to the control (Fig. 10).

4. DISCUSSION

The results of our study show that adult female and male *R. nigerrimus* were attracted to plant foliage and volatiles extracts of each of the extracts of the alternate host plant species evaluated. The chemical stimuli produced by *R. nigerrimus* alternate plant hosts can be considered responsible for the attraction, in the absence of visual and other types of stimuli. Previous work has reported that some weevil species are also attracted by volatiles produced by alternate plant hosts. For example, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) is attracted by odors from *Solanum lycopersicum* L. and *Solanum melongena* L. [22]; *Otyorhynchus sulcatus* (F.) is attracted by volatiles from *Euonymus fortunei* (Turcz.) Hand.-Mazz [14]; *Cosmopolites sordidus* (Germar) is attracted by odors from *Xanthosoma sagittifolium* (L.) Schott and *Dioscorea rotundata* Poir. [23]; *Rynchophorus ferrugineus* L. is attracted by odors from *Areca catechu* L. (Puwak) and *A. concinna* Thw. Enum. (Lentheti) [24]; *Chalcodermus aeneus* Boheman (Coleoptera: Curculionidae) feeds on leaves, stems and inflorescences of wild alternate hosts, which are sources of nutrition for adults that come out of winter diapause and cannot yet find cowpea plants (*Vigna unguiculata* L.) [20]. These weevil species were more attracted by odors from their alternate plant hosts, compared to

odors from non-host plants. This demonstrates that certain volatile compounds from species of alternate hosts serve to attract the weevils.

The positive responses of adult *R. nigerrimus* to foliage and volatile extracts of the four alternate host species is associated with the high specificity of the weevils to the released volatiles. In field conditions, when adult *R. nigerrimus* populations emerge and soybean crops have not yet been established, weevil populations have been observed feeding on *I. trifida*, *S. halepense*, *H. rufa* and *R. cochinchinensis*, but not on other plant species [3]. This feeding habit has also been reported in *R. subtilis* populations before soybean planting in Argentina but in other alternative host plant species [4]. *Rhyssomatus subtilis* feeds only on alternate host plant species such as *Coryza bonariensis* (L.) Cronquist, *Brassica campestris* (L.), Metzg. and *Sphaeralcea bonariensis* (Cav.) Griseb. The attraction response of *R. nigerrimus* to volatiles released by alternate host plants suggests that the weevils receive specific signals from host plant species that serve to recognize them as suitable sources of food for their survival. This has been demonstrated with other insect species and their alternate host plants [25,26,27].

In our study, we found that the grass *I. trifida* had the largest number of volatile compounds followed by *S. halepense* and *H. rufa* and *R. cochinchinensis*. The compounds α -pinene and *cis*-3-hexenyl acetate were common to the three grass species, while the compound *cis*-3-hexenol is the major compound in *S. halepense* and *R. cochinchinensis*. The compound α -pinene was found to be the second most abundant compound in *H. rufa* but it was lower in the other two grass species. In *H. rufa*, the major compound was 3-carene, which was also identified as a component of *H. rufa* essential oils [28]. Previously, *cis*-3-hexanol was reported as the major compound in *R. cochinchinensis* and *Cynodon nlemfuensis*, species considered alternate hosts of the spittle bugs, *Aeneolamia* spp. and *Prosapia* spp. (Hemiptera: Cercopidae) [29]. The compound *cis*-3-hexenyl acetate was the only compound identified in the volatiles of four alternate host species of *R. nigerrimus* studied. This compound was found in a low proportion in *I. trifida* and *H. rufa*, and in high proportion in *S. halepense* and *R. cochinchinensis*. *Cis*-3-hexenyl acetate had not been reported previously in volatiles from the plant species evaluated in this study. However, in previous work with insects of the Curculionidae

family, we reported antennal activity with some of the volatile compounds identified in *R. nigerrimus* alternate hosts. Among the compounds with biological activity involved in host location are found *cis*-3-hexenyl acetate, *cis*-3-hexenol, α -pinene, 3-carene and β -pinene [30,31]. To demonstrate which compounds are responsible for attracting adult *R. nigerrimus* to their alternate host plants it is necessary to conduct tests with GC-EAD. Then, the compounds that show biological activity should be evaluated using their synthetic counterparts in olfactometer tests.

We found through the EAG tests that the antennae of *R. nigerrimus* had a stronger response to the volatiles from alternate plant hosts than to the control. Likewise, it has been reported that the antennae of *Conotrachelus nenuphar* (Herbst) females and males responded more to the volatiles from two host plant species, *Prunus domestica* L. and *Malus domestica*, than to the control [7]. We also found that the amplitude of *R. nigerrimus* male and female antennal response to the four host plant species was similar. Similar results have been reported for amplitude of antennal response of female and male *Anthonomus musculus* and *C. nenuphar* to the odors of their host plants [7,32]. *Rhyssomatus nigerrimus* antennal response of greatest amplitude was found with *S. halepense* and *H. rufa*, likely because they have unique compounds or specific proportions of compounds that elicit better antennal response, as was suggested with *C. nenuphar* antennae [33].

5. CONCLUSION

In the present study we have demonstrated that *R. nigerrimus* adults were attracted by the odors of the four alternate host species through olfactometer trials and that the weevils are able to detect antennally the odors through electrophysiological tests. Hence, we suggest future work to evaluate traps lured with alternate host plants extracts and major volatiles compounds as 3-hexenol and *cis*-3-hexenyl acetate, when the weevil begin to emerge from the soil and before soybean planting to estimate the *R. nigerrimus* attraction capacity to extracts in field conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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