Journal of Chemical Ecology, Vol. 31, No. 10, October 2005 (©2005)
DOI: 10.1007/s10886-005-7106-z

ATTRACTION OF THE PARASITOID *Anagrus nilaparvatae* TO RICE VOLATILES INDUCED BY THE RICE BROWN PLANTHOPPER *Nilaparvata lugens*

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(Received January 30, 2005; revised May 3, 2005; accepted June 3, 2005)

Abstract—*Anagrus nilaparvatae*, an egg parasitoid of the rice brown planthopper *Nilaparvata lugens*, was attracted to volatiles released from *N. lugens*-infested plants, whereas there was no attraction to volatiles from undamaged plants, artificially damaged plants, or volatiles from *N. lugens* nymphs, female adults, eggs, honeydew, and exuvia. There was no difference in attractiveness between plants infested by *N. lugens* nymphs or those infested by gravid females. Attraction was correlated with time after infestation and host density; attraction was only evident between 6 and 24 hr after infestation by 10 adult females per plant, but not before or after. Similarly, after 24 hr of infestation, wasps were attracted to plants with 10 to 20 female planthoppers, but not to plants with lower or higher numbers of female planthoppers. The attractive time periods and densities may be correlated with the survival chances of the wasps' offspring, which do not survive if the plants die before the wasps emerge. Wasps were also attracted to undamaged mature leaves of a rice plant when one of the other mature leaves had been infested by 10 *N. lugens* for 1 d, implying that the volatile cues involved in host location by the parasitoid are systemically released. Collection and analyses of volatiles revealed that 1 d of *N. lugens* infestation did not result in the emission of new compounds or an increase in the total amount of volatiles, but rather the proportions among the compounds in the blend were altered. The total amounts and proportions of the chemicals were also affected by infestation duration. These changes in volatile profiles might provide the wasps with specific information on host habitat quality and thus could explain the observed behavioral responses of the parasitoid.

Key Words—Host location, induced plant volatiles, rice, *Anagrus nilaparvatae*, *Nilaparvata lugens*, tritrophic interactions.

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INTRODUCTION

Herbivore-induced plant volatiles play a role in foraging behavior of predators and parasitoids of herbivores (Turlings et al., 1990; Takabayashi and Dicke, 1996; Sabelis et al., 1999; Dicke, 1999; Bertschy et al., 2001; Lou et al., 2002), and thus these volatiles are suggested to function as an indirect defense for plants (Vet and Dicke, 1992; Dicke, 1999; Sabelis et al., 1999; Turlings and Wäckers, 2004). So far, this phenomenon of indirect defense signaling has been reported in more than 23 plant species. It has been particularly well studied for corn, lima bean, cotton, and tobacco. In rice, a model monocot cereal species and one of the most important food crops in the world, however, few studies have examined the role of herbivore-induced volatiles in host or prey location by natural enemies of herbivores.

Nilaparvata lugens (Stål) is an important rice pest worldwide. It feeds on phloem sap, causing major physiological stress to the plant (Rubia-Sanchez et al., 1999; Watanabe and Kitagawa, 2000). Compared to chewing herbivores, sucking feeders that have been studied in the context of tritrophic interactions appear to cause only minor changes in volatile emissions, but these emissions are apparently used by natural enemies (Du et al., 1998; Turlings et al., 1998a; Birkett et al., 2003). Possible changes in volatiles profiles resulting from planthopper infestation have not yet been studied for any plant. In this study, we investigated the role of volatiles induced by the rice brown planthopper *N. lugens* in the host-searching behavior of *Anagrus nilaparvatae* Pang et Wang, an egg parasitoid of *N. lugens*. The parasitoid *A. nilaparvatae* is a major natural enemy of rice planthoppers, including *N. lugens* (Cheng and He, 1996). For host location, the wasp uses contact kairomones that are present in all developmental stages of the planthoppers as well as in their exuvia and honeydew (Lou and Cheng, 1994; Lou and Cheng, 2001), but volatiles released from hosts and by-products seem to have no attractiveness (Lou and Cheng, 1994). However, the volatiles emitted from planthopper-infested rice plants significantly attract the parasitoid (Lou and Cheng, 1996a). Nothing is known about the nature of these volatiles.

To elucidate the role of *N. lugens*-induced rice volatiles in the host-searching behavior of the parasitoid, we first measured the behavioral response of the parasitoid to the volatiles emitted from unmanipulated plants, artificially damaged plants, planthopper-infested plants, planthopper-damaged plants (planthoppers removed), as well as to volatiles from planthoppers alone or their by-products. Then, we checked the effect of infestation duration and planthopper density on attraction of the parasitoid. In an additional olfactometer experiment, it was shown that the volatiles are released systemically. Finally, we collected and identified the volatiles that were released from differently treated plants.

METHODS AND MATERIALS

Plants. The rice variety used was Zhe 852, which is susceptible to *N. lugens* (Lou and Cheng, 2003). Pregerminated seeds were sown in a greenhouse, and after 20 d, seedlings were transplanted into clay pots (16 cm diam × 10 cm height), each with 10 plants. Plants were watered daily, and each pot was supplied with 0.10 g of urea 10, 20, and 30 d after transplanting, respectively. All plants were placed in a controlled climate room that was maintained at 28 ± 2°C, 70–80% r.h., and 12 hr photophase. Thirty to 35 d after transplanting, plants were used for experiments. Plantings were continued at regular intervals so that enough plants of suitable age were available for experiments.

Insects. The *N. lugens* culture was originally obtained from the China National Rice Research Institute (CNRRI), Fuyang, Zhejiang, and maintained on Zhe 852 rice plants in a greenhouse. Late instar nymphs of *N. lugens* were captured from the greenhouse and reared on potted Zhe 852 rice plants, which were confined in plastic cages (11 cm diam × 40 cm high). Caged rice plants were maintained in a controlled climate room at 28 ± 2°C, 12 hr photophase, and 70–80% r.h. Newly emerged adults of *N. lugens* were collected daily and fed on potted fresh Zhe 852 rice plants. Using this procedure, we obtained *N. lugens* adults of uniform age.

A laboratory colony of the egg parasitoid *A. nilaparvatae* was started from individuals trapped in rice fields in Hangzhou, using Zhe 852 rice plants with *N. lugens* eggs as bait. The colony was propagated on *N. lugens* eggs in rice shoots enclosed in glass tubes (2.5 cm diam × 20 cm high), which were kept in a controlled climate room at 26 ± 2°C, 12 hr photophase, and 70–80% r.h. Each day the newly emerged wasps were collected into clean glass tubes with access to both water and honey solution and held for at least 2 hr to ensure mating. From the second generation onwards, female parasitoids were used in experiments less than 24 hr after emergence.

Bioassays. Responses of *A. nilaparvatae* females to rice volatiles were measured in a Y-tube olfactometer, which was described in detail by Lou et al. (2005). Briefly, the olfactometer consisted of a Y-shaped glass tube of 1 cm diam. The base and the two arms of the Y tube were all 10 cm in length. Each arm was connected to an odor source container (a glass box, 10 cm long, 10 cm wide, 30 cm high). An airstream was generated and divided in two, and each secondary airstream was led through a flowmeter, a tube with activated charcoal, a humidifier bottle, and one of the odor containers. Subsequently, the two airstreams were led through the two arms of the Y-tube olfactometer at 150 ml/min. The Y-tube olfactometer was placed in a box painted white with an artificial light source consisting of a single 25-W lamp placed above the arms of the Y tube. All bioassays were conducted between 0900 and 1700 hours, and the temperature in the room was maintained at 25–28°C.

Mated female parasitoids were introduced individually into the base tube of the olfactometer and given 10 min to walk toward the end of one of the arms. Choice for an odor source was defined as a female crossing a line 7 cm after the division of the base tube and remaining there for at least 1 min. If a parasitoid did not make a choice within 10 min, this was recorded as no response. For each odor source pair, at least 32 females were tested.

Response of A. nilaparvatae Toward Plant–N. lugens Complexes and Their Individual Components. In this experiment, the following odor sources were prepared:

1. Plant–*N. lugens* gravid female complex (PF). The potted plants were washed with running water and trimmed to leave 10 plants per pot. Plants were then individually infested with 10 gravid *N. lugens* females that were attached to the upper and lower position of the plant stems by using two parafilm bags, each with five females. One d later, the 10 plants (cut off at soil level and the cut end wrapped with a piece of moist cotton) with 100 females were used as an odor source for bioassays.
2. Plant–*N. lugens* nymph complex (PN). This treatment was nearly the same as in treatment (1). The only difference was that we used 4th–5th instar nymphs of *N. lugens* instead of gravid females.
3. *N. lugens* gravid female-damaged plants (FDP). This treatment was the same as in treatment (1), but 1 day after infestation, we removed the females and used the 10 plants, which now carried eggs, as an odor source for bioassays.
4. *N. lugens* nymph-damaged plants (NDP). The treatment was the same as in treatment (2), but 1 d after infestation, nymphs were removed and 10 plants were used as an odor source.
5. Mechanically damaged plants (MP). Ten plants were individually damaged with a needle at the lower and upper position of the stems by pricking each 150 times at the start of the experiment. Twenty-four hr later, the plants were damaged again using the same method and then were cut off at soil level and used as one odor source.
6. Unmanipulated plants (CP). Ten plants were cut off at soil level and served as one odor source.
7. *N. lugens* exuvia and honeydew. Exuvia and honeydew were collected from five glass tubes (2.5 cm diam $\times$ 20 cm) in which, for 24 hr, 20 4th–5th instar nymphs had been feeding on two rice plants that had been hung upside down, with their roots wrapped in wet cotton. Honeydew in the glass tubes was collected with a syringe, and the exuvia with forceps. Honeydew or exuvia was placed on a small piece of filter paper (3 $\times$ 3 cm) as an odor source.

8. *N. lugens* eggs. Ten plants were individually infested with 10 gravid N. lugens females. One day later, plants were cut at soil level and dissected under a microscope. All eggs were then carefully collected with a pin and placed on a small piece of filter paper (3 × 3 cm) as an odor source.
9. *N. lugens* nymphs and female adults. One hundred 4th–5th instar nymphs and female adults were individually collected and then used as odor sources.

The behavioral response of the parasitoid to the following pairs of odors were tested in the olfactometer: (1) blank control (BK) vs. CP, MP, PF, PN, NDP, or FDP, respectively; 2) PF vs. CP, MP, or PN, respectively; 3) PN vs. CP, or MP, respectively; and 4) BK vs. *N. lugens* exuvia, *N. lugens* eggs, *N. lugens* nymphs, *N. lugens* gravid females, or *N. lugens* honeydew, respectively.

Effect of Host Density and Infestation Duration on Attraction of A. nilaparvatae. In this experiment, we determined whether the duration of infestation or planthopper density had an effect on parasitoid attraction, using plant–female complexes infested with 1, 5, 10, 20, 40, or 80 gravid females per plant for 1 d. Plants without *N. lugens* served as controls. We used 10 plants per treatment.

To assess the effect of the duration of infestation, olfactometer tests were performed at 1, 2, 6, 12, 24, 48, and 72 hr after the plants had been infested by *N. lugens*. Each plant was infested with 10 gravid females and 10 plants were used per treatment. Plants without *N. lugens* served as controls.

Response of A. nilaparvatae to Undamaged Leaves of Infested Plants. In this experiment, we determined whether the *N. lugens*-induced rice volatiles were produced systemically and whether the infested leaf position had an effect on the attraction of the parasitoid to uninfested leaves. Plants with four fully expanded leaves, which were respectively assigned first, second, third, and fourth leaf position from the top to the base, were chosen. The uninfested leaves including leaf sheaths were individually collected and used for bioassay in a Y-tube olfactometer 24 hr after one of the fully expanded leaves (the first, second, third, or fourth leaf position) of plants was infested with 10 gravid *N. lugens* females who were fixed on the middle veins of the leaves by using two parafilm bags, each with five females. For example, if the first leaves of 10 plants had been infested by *N. lugens*, then we measured the response of the parasitoid to the volatiles released from either the second, third, or fourth leaves of the 10 plants, respectively. The corresponding leaves from uninfested plants (with two empty parafilm bags per plant at the same positions as the infested plants) served as controls.

Collection and Identification of Rice Volatiles. Rice volatiles were collected for 2 hr by using solid-phase microextraction (SPME, Supelco Co., Bellefonte, PA, USA) with a silica fiber coated with a 100-μm-thick film of

polydimethylsiloxane. For each treatment, 10 plants were used. The plant was cut off at soil level, and the cut part of the stem was wrapped in a piece of moist cotton to avoid desiccation. Ten plants were placed in a glass cylinder (5 cm diam × 30 cm), which was covered by a glass lid (5 cm diam) with a hole (1 mm diam) through which the SPME fiber was inserted for odor collection. The volatiles emitted from the following treatments were collected: plants that had been individually infested by 10 gravid *N. lugens* females for 1, 2, or 3 d, respectively; plants that had been individually infested by 10 *N. lugens* nymphs for 1 d; mechanically damaged plants (MP); and unmanipulated plants. We also collected volatiles from a control, (the glass cylinder without plants) to confirm that the system was clean. Each treatment was replicated three times. The experiment was conducted in a room at 26–28°C. After collecting each sample, the cylinder was rinsed with an acidic washing solution ($K_2Cr_2O_7$ 60 g/98% H_2SO_4 460 ml/H_2O 300 ml Caution! Highly corrosive!) followed by distilled water and then heated at 200°C for 1 hr. The SPME fiber was desorbed in a GC injector at 250°C for 15 min before use.

Analyses were done with a Hewlett-Packard (HP) 6890A gas chromatograph (GC) equipped with an HP-5 (30 m × 0.25 mm i.d., 0.25 μm film thickness) column. Volatile compounds collected on the SPME fiber were directly desorbed in the GC injector at 250°C for 1 min, in splitless mode. Following injection, the column was programmed from 40°C/2 min, 6°C/min to 250°C, hold for 2 min. Helium (1 ml/min) was used as the carrier gas. All compounds were analyzed by an HP 5970B mass spectrometer in the electron impact ionization mode. Compounds were identified by comparison of retention times and mass spectra with those of authentic standards. The authentic standard chemicals (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, limonene, cyclohexanol, linalool, β-cyclocitral, 1-undecene, *n*-tetradecane, *n*-hexadecane, dodecanal, *n*-heptadecane, *n*-octadecane, *n*-nonadecane, *n*-eicosane, and *n*-henicosane were purchased from Sigma Chemical (St. Louis, MO, USA); tetradecanal was purchased from ChemExper, Belgium. The relative proportions of the compounds in the blend were calculated according to their peak areas.

Statistical Analysis. Differences in behavioral responses of the parasitoid to pairs of odors were determined by χ^2 tests. Volatiles data were analyzed by ANOVA. If the ANOVA analysis was significant ($P < 0.05$), Fisher LSD *post hoc* tests to detect significant differences between groups were conducted. Data were analyzed with Statistica (SAS Institute Inc., Cary, NC, USA).

RESULTS

Response of A. nilaparvatae Toward Plant–N. lugens Complexes and Their Individual Components. Volatiles released from unmanipulated plants and

mechanically damaged plants were no more attractive to the parasitoid than blank controls, whereas the volatiles from plant–*N. lugens* complexes (PF, PN), and *N. lugens*-damaged plants (FDP, NDP) were attractive (Figure 1). The plant–*N. lugens* complexes were also more attractive than unmanipulated plants or mechanically damaged plants. No difference was observed in attraction of the parasitoid to volatiles emitted from plant–nymph complex and those from plant–gravid female complex (Figure 1).

The parasitoid was not attracted to the volatiles released from *N. lugens* materials, including *N. lugens* nymphs, female adults, eggs, honeydew, or exuvia (Figure 2).

Effect of Host Density and Infestation Duration on Attraction of A. nilaparvatae. N. lugens density had a significant effect on attraction of the parasitoid (Figure 3B). Plants that had been infested with 10 or 20 gravid females per plant for 1 d were more attractive than unmanipulated plants, whereas plants that were infested with 1, 5, 40, or 80 gravid females per plant were no different from uninfested plants (Figure 3B).

There was a similar effect of duration of infestation on the attraction of the parasitoid (Figure 3A). Plants that had been infested with 10 gravid *N. lugens* females for 6–24 hr were more attractive to the parasitoid than unmanipulated

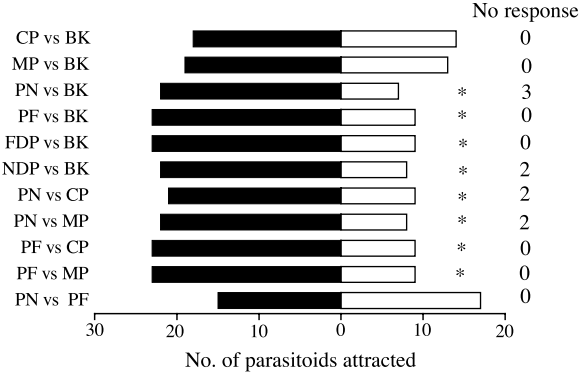

FIG. 1. Numbers of *A. nilaparvatae* adult females attracted by volatiles released from pairs of odors: Unmanipulated plants (CP) vs. blank control (BK); mechanically damaged plants (MP) vs. BK; *N. lugens* nymph–plant complex (PN) (infested for 1 d) vs. BK; *N. lugens* gravid female–plant complex (PF) (infested for 1 d) vs. BK; *N. lugens* gravid female-damaged plants (FDP) (females removed 1 d after infestation) vs. BK; *N. lugens* nymph-damaged plants (NDP) (nymphs removed 1 d after infestation) vs. BK; PN vs. CP; PN vs. MP; PF vs. CP; PF vs. MP; and PN vs. PF. For explanation of treatments and methodology see "Methods and Materials." Asterisks indicate significant differences between members of a pair ($P < 0.05$, χ^2 test).

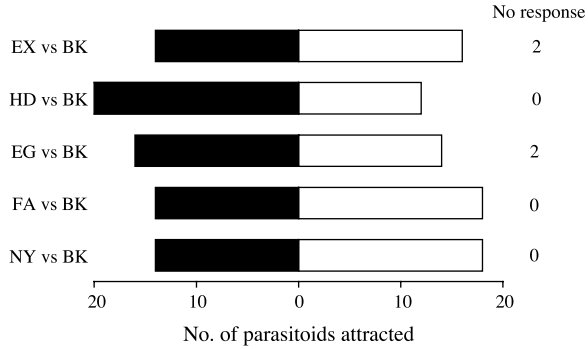

FIG. 2. Number of *A. nilaparvatae* adult females attracted by volatiles released from pairs of odors: *N. lugens* exuvia (EX) vs. blank control (BK); *N. lugens* honeydew (HD) vs. BK; *N. lugens* eggs (EG) vs. BK; *N. lugens* female adults (FA) vs. BK; and *N. lugens* nymphs (NY) vs. BK.

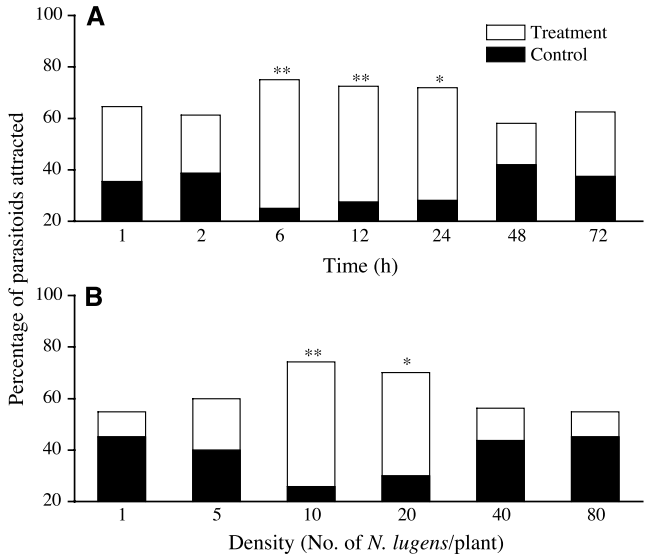

FIG. 3. Percentage of *A. nilaparvatae* adult females attracted by volatiles released from pairs of odors: unmanipulated plants (Control) vs. (A) plants that were individually infested by 10 *N. lugens* gravid females for 1, 2, 6, 12, 24, 48, or 78 hr (Treatment); (B) plants that were individually infested with 1, 5, 10, 20, 40, or 80 *N. lugens* gravid females for 1 d (Treatment). Asterisks indicate significant differences between members of a pair (*$P < 0.05$, **$P < 0.01$, χ^2 test).

plants, but plants that had been infested with 10 gravid *N. lugens* gravid females for a shorter (1–2 hr) or longer time (48–72 hr) were no different than uninfested plants (Figure 3A).

Response of A. nilaparvatae to Undamaged Leaves of Infested Plants. Infestation of one of the fully expanded leaves by *N. lugens* resulted in an increase in attractiveness of the other three undamaged leaves, and the leaf position that was infested by *N. lugens* had no effect on this attractiveness (Figure 4).

Volatiles Profile Comparisons. More than 20 compounds were collected from the headspace of rice plants by SPME, 16 of which were identified, including 8 aliphatic hydrocarbons, 3 aldehydes, 3 terpenoids, and 2 alcohols (Table 1). Of these compounds, aliphatic hydrocarbons, specifically 7 aliphatic hydrocarbons, dominated the blends, comprising more than 48% of the total peak areas of compounds emitted in every assay (Table 1). In addition, two green leafy volatiles, (*E*)-2-hexenal and (*E*)-2-hexen-1-ol, as well as tetradecanal and unknowns 1, 3, and 4 were also relatively abundant in the headspace of the rice plants (Table 1).

Compared to unmanipulated plants, *N. lugens* infestation did not induce rice plants to release new compounds, nor did the total amount of volatiles

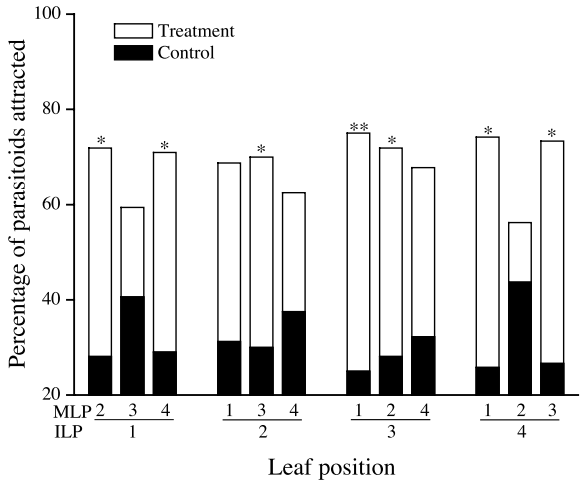

FIG. 4. Percentage of *A. nilaparvatae* female adults attracted by volatiles released from pairs of leaves: undamaged individual leaves, growing at positions 1–4, of 10 damaged plants on which a single leaf, at position 1–4, was infested with 10 *N. lugens* gravid females for 24 hr (Treatment) vs. the same leaves of unmanipulated plants (Control). MLP, measured leaf position; ILP, infested leaf positions. Asterisks indicate significant differences between members of a pair (*$P < 0.05$, **$P < 0.01$, χ^2 test).

TABLE 1. PROPORTIONS (% OF TOTAL PEAK AREA) OF VOLATILE COMPOUNDS EMITTED FROM UNMANIPULATED PLANTS (CP), MECHANICALLY DAMAGED PLANTS (MP), AND *Nilaparvata lugens* GRAVID FEMALE INFESTED PLANTS FOR 1 (PF1), 2 (PF2), OR 3 (PF3) DAYS, AND *N. lugens*-NYMPH INFESTED PLANTS FOR 1 DAY (PN1)[a]

Chemical	CP	MP	PF1	PF2	PF3	PN1
1. (*E*)-2-Hexenal	5.69 ± 1.26 bc	6.73 ± 0.40 ab	5.26 ± 0.75 bc	4.93 ± 0.18 bc	4.04 ± 0.59 c	8.32 ± 1.07 a
2. Unknown 1	7.63 ± 0.20 1ab	7.92 ± 1.32 a	5.55 ± 0.90 bc	5.29 ± 0.40 c	3.67 ± 0.54 c	5.25 ± 0.41 c
3. (*E*)-2-Hexen-1-ol	6.67 ± 0.89 ab	8.73 ± 1.19 a	3.75 ± 0.21 cd	2.95 ± 0.72 d	2.03 ± 0.05 d	5.76 ± 1.36 bc
4. Unknown 2	0.23 ± 0.03 a	0.24 ± 0.03 a	0.28 ± 0.06 a	0.18 ± 0.02 a	0.32 ± 0.04 a	0.23 ± 0.03 a
5. Limonene	0.21 ± 0.06 a	0.24 ± 0.05 a	0.25 ± 0.08 a	0.20 ± 0.03 a	0.31 ± 0.08 a	0.23 ± 0.03 a
6. Cyclohexanol	1.09 ± 0.19 a	1.19 ± 0.15 a	0.39 ± 0.08 bc	0.22 ± 0.03 bc	0.21 ± 0.03 c	0.57 ± 0.11 b
7. Linalool	0.17 ± 0.03 bc	0.19 ± 0.05 bc	0.24 ± 0.03 bc	0.34 ± 0.07 b	1.25 ± 0.11 a	0.13 ± 0.03 c
8. β-Cyclocitral	0.59 ± 0.06 a	0.90 ± 0.15 a	0.94 ± 0.18 a	0.84 ± 0.19 a	1.10 ± 0.14 a	0.90 ± 0.25 a
9. 1-Undecene	0.98 ± 0.07 bc	1.48 ± 0.28 ab	1.26 ± 0.31 abc	1.77 ± 0.31 a	1.97 ± 0.07 a	0.74 ± 0.19 c
10. Unknown 3	3.76 ± 0.20 c	4.09 ± 0.67 bc	4.31 ± 0.69 bc	4.04 ± 0.63 bc	5.75 ± 0.81 ab	6.28 ± 0.59 a
11. *n*-Tetradecane	4.24 ± 0.56 bc	3.95 ± 0.21 bc	5.24 ± 0.52 b	7.21 ± 0.74 a	4.18 ± 0.82 bc	3.00 ± 0.52 c
12. *n*-Hexadecane	2.40 ± 0.35 a	2.71 ± 0.51 a	2.48 ± 0.24 a	2.73 ± 0.55 a	1.75 ± 0.46 a	1.85 ± 0.61 a
13. Dodecanal	0.23 ± 0.03 b	0.50 ± 0.19 b	0.35 ± 0.04 b	0.27 ± 0.11 b	1.16 ± 0.11 a	0.41 ± 0.06 b
14. *n*-Heptadecane	29.37 ± 0.60 e	30.30 ± 2.16 de	41.72 ± 3.12 ab	44.64 ± 1.33 a	38.31 ± 0.47 bc	34.84 ± 0.77 cd
15. Tetradecanal	2.39 ± 0.18 a	3.90 ± 2.54 a	3.08 ± 0.37 a	2.98 ± 0.48 a	5.86 ± 0.93 a	3.88 ± 0.44 a
16. *n*-Octadecane	3.65 ± 0.19 a	2.85 ± 0.44 b	1.45 ± 0.23 c	0.86 ± 0.20 c	0.69 ± 0.17 c	1.44 ± 0.23 c
17. Unknown 4	3.36 ± 0.04 c	2.94 ± 0.27 c	3.52 ± 0.70 c	10.20 ± 1.10 b	14.18 ± 0.97 a	4.03 ± 0.42 c
18. *n*-Nonadecane	8.48 ± 0.27 a	5.97 ± 0.96 b	5.36 ± 0.42 b	3.24 ± 0.08 c	3.16 ± 0.15 c	5.64 ± 0.48 b
19. *n*-Eicosane	6.52 ± 0.18 a	3.96 ± 0.53 b	3.49 ± 0.34 b	1.00 ± 0.17 c	0.38 ± 0.09 c	2.92 ± 0.47 b
20. *n*-Henicosane	2.98 ± 0.27 a	1.76 ± 0.27 b	2.30 ± 0.36 ab	0.79 ± 0.14 c	0.49 ± 0.15 c	2.05 ± 0.50 ab
Total peak area ($\times 10^8$)	9.79 ± 1.10 ab	11.43 ± 1.28 a	8.52 ± 0.98 ab	7.78 ± 0.83 b	7.02 ± 1.20 b	9.15 ± 1.30 ab

[a]For explanation of treatments and methodology see "Methods and Materials." Data represent the mean of three replications. Different letters in the same row indicate significant differences among treatments ($P < 0.05$, Fisher LSD *post hoc* tests).

change with duration of infestation (Table 1). However, *N. lugens* infestation altered the proportions of chemicals in the blend (Table 1). Compared to unmanipulated plants, *N. lugens*-infested plants (infested for 1 d) had a higher proportion of *n*-heptadecane and lower proportions of (*E*)-2-hexen-1-ol, cyclohexanol, *n*-octadecane, *n*-nonadecane, and *n*-eicosane. Moreover, 1 d of nymph infestation increased the proportions of (*E*)-2-hexenal and unknown 3 (Table 1). With duration of *N. lugens* infestation, the proportions of dodecanal, linalool, and unknown 4 increased, whereas the proportions of *n*-nonadecane, *n*-eicosane, and *n*-henicosane decreased (Table 1). Between the volatiles emitted from *N. lugens* nymph-infested plants and those from gravid female-infested plants, there was no significant difference except for the chemicals (*E*)-2-hexenal and unknown 3, both of which were released in higher proportions by nymph-infested plants (Table 1).

Mechanical damage resulted in no increase in the total volatiles compared to unmanipulated plants. However, the total volatiles released from mechanically damaged plants were higher than those from plants infested by *N. lugens* for 2–3 d (Table 1). The proportions of chemicals that decreased after *N. lugens* infestation did not decrease after artificial damage, except for the chemicals *n*-octadecane, *n*-nonadecane, *n*-eicosane, and *n*-henicosane (Table 1).

DISCUSSION

A. nilaparvatae was attracted to volatiles released from *N. lugens* plant complexes (damaged for 1 d), but not to those from unmanipulated plants or artificially damaged plants. Wasps also were not attracted to volatiles from *N. lugens* nymphs, female adults, eggs, honeydew, or exuvia. Moreover, no obvious difference was observed in attraction between *N. lugens* nymph–plant complex and gravid female–plant complex. These results were similar to those observed in a tritrophic system consisting of the phloem feeder *Phenacoccus herreni*, cassava, and an encyrtid parasitoid (Bertschy et al., 2001). This suggests that volatiles induced by *N. lugens* feeding on rice are used in host location by *A. nilaparvatae*, as has been reported for many parasitoids of insect larvae (Dicke and Vet, 1999; Turlings and Wäckers, 2004). *Nilaparvata lugens* is an insect pest with completely overlapping generations; all developmental stages can co-occur in the same plant and field. Therefore, the indirect association of *N. lugens* feeding with egg presence is reliable, suggesting that the response of the parasitoid to *N. lugens* feeding-induced volatiles is adaptive.

Anagrus nilaparvatae females were not attracted to volatiles from undamaged rice plants. It is commonly reported that healthy plants are unattractive to parasitoids (Turlings et al., 1990; Guerrieri et al., 1993; Potting

et al., 1995; Finidori-Logli et al., 1996; Du et al., 1996; Steidle and Schöller, 1997), which may be attributed to the minor amounts of volatiles released by healthy plants or the low reliability of such volatiles in informing the parasitoid of host presence (Vet et al., 1991; Vet and Dicke, 1992). Volatiles released by artificially damaged plants, such as green leaf volatiles, are often attractive to parasitoids (Turlings et al., 1990; Steinberg et al., 1993; Geervliet et al., 1994; Mattiacci et al., 1994; Potting et al., 1999; Hoballah and Turlings, 2005). Here, however, artificially damaged rice plants were not attractive to *A. nilaparvatae* females possibly because the limited amount of damage resulted in only minor changes to the green leafy volatiles (*E*)-2-hexen-1-ol and (*E*)-2-hexenal.

Nilaparvata lugens infestation for 1 d did not result in detectable production of new compounds, nor was there an increase in the total amount of volatiles compared to controls. However, infestation did alter the relative proportions among the volatiles compared to undamaged and artificially damaged plants. Thus, *N. lugens* infestation appears to alter the plant volatiles profile only slightly, which may be common for phloem feeders and is in contrast to the substantial induction caused by tissue feeders. For instance, Turlings et al. (1998a) found that the aphid *Rhopalosiphum maidis* induced no measurable emissions of volatiles in maize, even after heavy infestation, whereas feeding by caterpillars caused major increases in the volatile emissions. Similarly, infestation by the silverleaf whitefly *Bemisia tabaci* did not appear to induce volatiles emissions in cotton (Rodriguez-Saona et al., 2003). Phloem-sucking insects, such as rice planthoppers and aphids, inflict only minor physical damage to host plants, which is likely to reduce the chances of triggering a physiological reaction in the plant (Turlings et al., 1998a). Quantitative (amount) (e.g., apple fruit, Hern and Dorn, 2001) and/or qualitative changes (production of new compounds or changes in proportions) (e.g., maize, Turlings et al., 1990) in volatiles from plants in response to herbivory have been reported in many plant–herbivore systems, providing exploitable cues for parasitoids (Dicke, 1999; Gouinguené et al., 2001). The attraction of *A. nilaparvatae* females to *N. lugens*-infested plants is best explained by a change in the proportions among the volatiles, a qualitative change.

Systemic release of herbivore-induced volatiles in plants has been reported in many plant species (Turlings and Tumlinson, 1992; Dicke et al., 1993; Cortesero et al., 1997; Guerrieri et al., 1999; Halitschke et al., 2000; Neveu et al., 2002; Röse and Tumlinson, 2004). We too found an attraction of the parasitoid to the undamaged leaves of plants in which one of the mature leaves had been infested by *N. lugens* for 1 d. Wasps were similarly attracted to undamaged, mature leaves of plants that had been damaged by *N. lugens* on another leaf. Although the mature leaves of plants mainly export their photoassimilates to roots and younger leaves (sinks) via the phloem, a solute

exchange does occur between the phloem and the xylem (Fisher, 2000). Thus, the wound signal from infested mature rice leaves may reach other undamaged mature leaves via the xylem, eliciting the undamaged leaves to release volatiles.

We also studied the effect of host density and infestation duration on attraction of *A. nilaparvatae*, and both had a significant effect. The degree of attraction of the parasitoid and the *N. lugens* density or the infestation duration did not fit a dose related positive relationship as reported in other studies (Turlings et al., 1990; Gols et al., 2003), but rather reached a maximum at intermediate densities, with no attraction at low or very high infestations. A similar effect was found for the duration of infestation; wasps were not attracted early on, but attraction was strong after 6–24 hr of infestation, and then declined after 48 hr. The time lag for the induction of volatiles attractive to parasitoids has also been observed in other studies (Turlings et al., 1998b; Guerrieri et al., 1999), suggesting the involvement of active physiological and biochemical processes that result in changes in volatile profiles. A threshold for damage level has also been reported for the attraction of an aphid parasitoid to broad bean plants infested with varying aphid densities (Powell et al., 1998; Guerrieri et al., 1999), as well as for induced direct resistance in plants (Karban and Baldwin, 1997; Lou and Cheng, 1997). These results suggest that an adequately strong elicitation signal is needed for the induction of direct and indirect resistance.

The decline in attraction of the parasitoid to plants that had been infested by either 10 *N. lugens* females for 2–3 d or 30–40 females for 1 d may be related to the survival chances of the parasitoid offspring. *Nilaparvata lugens* feeds and oviposits on the same plant even if plants are heavily damaged. Heavy damage in the field by 50–90 female adults per hill (about 20 plants) causes susceptible plants to wilt and eventually die after ~10 d (Li et al., 1996). The parasitoid spends 9–10 d in the host egg before it emerges as an adult at appropriate temperatures of 25–28°C (Lou and Cheng, 1996b), and developing wasps may die due to desiccation if the plant dies before parasitoid emergence. This may explain the declining response with increasing levels of plant damage. Discrimination between heavily and lightly damaged plants may be possible due to the differences in volatile profiles. For example, volatiles released from plants that were infested by 10 females for 1 d showed lower proportions of unknown 4 and higher proportions of *n*-nonadecane, *n*-eicosane, and *n*-henicosane compared to plants that were infested by 10 females for 2–3 d. These differences could provide important cues on the profitability status of the plants for the parasitoid. To our knowledge, this is the first study to report a decrease in attractiveness in tritrophic interactions after heavy herbivore infestation. The possible adaptive value of the lack of response to heavily infested plants may warrant further investigation.

Acknowledgments—We thank Menghao Du, Guoxin Zhou, Xia Wang, and Bailu Liao for assistance with laboratory work and Ted Turlings for revising the manuscript. The study was sponsored by the Ministry of Science and Technology of China (973) (G2000016208) and the National Natural Science Foundation of China (30270233).

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