研究论文

Screening and field evaluation of synthetic plant volatiles as attractants for *Anagrus nilaparvatae* Pang *et* Wang, an egg parasitoid of rice planthoppers

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Abstract Natural enemies of herbivores can effectively locate their hosts or prey by perceiving herbivore-induced plant volatiles. In order to develop attractants for *Anagrus nilaparvatae* Pang *et* Wang, an egg parasitoid of rice planthoppers, we tested the attractiveness of synthetic rice volatiles induced by the rice brown planthopper (BPH) *Nilaparvata lugens* (Stål) to the parasitoid in both laboratory and field. In the laboratory, using a Y-tube olfactometer, we found that five compounds (Z-3-hexenyl acetate, 1-penten-3-ol, Z-3-hexenal, linalool and MeSA) attract the parasitoid. Moreover, *A. nilapareatae* was strongly attracted by three mixtures, one of MeSA plus Z-3-hexenal, one containing Z-3-hexenal, Z-3-hexenyl acetate and linalool, and one containing MeSA, Z-3-hexenal, Z-3-hexenyl acetate and linalool. Field experiments demonstrated that the parasitism of BPH eggs by the parasitoid was significantly increased on plants that received a septa containing one of the 3 chemicals (Z-3-hexenal, Z-3-hexenyl acetate, linalool) or the mixture containing MeSA, Z-3-hexenal, Z-3-hexenyl acetate and linalool. The findings are of significance for improving the biological control of *N. lugens* in the future.

Key words *Nilaparvata lugens*, *Anagrus nilaparvatae*, herbivore-induced plant volatiles, attractant

1 Introduction

Ever since herbivore-induced plant volatiles (HIPVs) have been found to play an important role in host or prey location of the natural enemies of herbivores, these compounds have emerged as a potential tool for enhancing the efficiency of the natural enemies of herbivores. In recent years, many field studies have been published that exploited this aspect (James, 2003a, 2005; Yu *et al.*, 2008; Lee, 2010; Orre *et al.*, 2010). From these studies, it is clear that HIPVs increase the effectiveness of natural enemies on herbivores in some crops but not in others (James, 2003b; James and Price, 2004; Yu *et al.*, 2008; Lee, 2010; Orre *et al.*, 2010). Moreover, some compounds have been found to be general attractants for natural enemies, whereas some are specific for natural enemies. For example, methyl salicylate has been reported to attract many species of the natural enemies of herbivores, such as *Stethorus punctum picipes*, *Orius tristicolor*, *Erigonidium graminicolum*, *Orius similis*, *Chrysopa nigricornis*, *Deraeocoris brevis*, *Anagrus* spp. (James, 2003a; James and Grasswitz, 2005; Zhu and Park, 2005; Lee, 2010; Yu *et al.*, 2008; Orre *et al.*, 2010). Therefore, it is necessary to test the potential of HIPVs in increasing the effectiveness of natural enemies of herbivores in different crop types.

Rice planthoppers, including brown planthopper (BPH) *N. lugens* (Stål) and white-backed planthopper *Sogatella furcifera* (Hovarth) (Kajimura *et al.*, 1995; Rubia-Sanchez *et al.*, 1999), are the most important rice pests in China. They feed on the phloem and cause a decrease in leaf area, plant height, dry weight, leaf and stem nitrogen concentration, chlorophyll content and photosynthetic

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rate (Rubia-Sanchez *et al.*, 1999) that results in yield loss. Previous studies have shown that infestation by *N. lugens* or *S. furcifera* or treatment with jasmonic acid (JA) alters the volatile profiles of rice plants. These volatiles are strongly attractive to an egg parasitoid *A. nilaparvatae* (Lou *et al.*, 2005a, 2005b), a major natural enemy of the rice planthoppers (Lou *et al.*, 2002). Moreover, the parasitism of *N. lugens* eggs by *A. nilaparvatae* on plants that were surrounded by JA-treated plants is more than two-fold higher than on control plants in the greenhouse and field (Lou *et al.*, 2005a; Lou *et al.*, 2006), implying that augmenting the release of rice-produced attractants has the potential to enhance the effectiveness of the parasitoid in the control of *N. lugens*. However, which chemicals are attractive to the parasitoid and whether these active chemicals can be used to increase the efficiency of the parasitoid in the field remain unclear.

To find the bioactive components in the rice volatiles and thus develop attractants for the parasitoid, in this study, we first determined the attractiveness of 18 individual volatile chemicals and 32 mixtures mixed with several active chemicals to the parasitoid in the lab. We then investigated the effectiveness of the active chemicals or mixtures in enhancing the parasitism and predation rates of BPH eggs in the field.

2 Materials and Methods

2.1 Insects

Colonies of BPH were originally obtained from rice fields in Hangzhou, China, and maintained on Xiushui 110 rice seedlings in a controlled climate room at (26 ± 2)℃, 12 h light phase and 80% relative humidity.

A laboratory colony of the egg parasitoid *A. nilaparvatae* was started from individuals trapped in rice fields in Hangzhou, using Xiushui 110 rice plants with *N. lugens* eggs as bait. The colony was propagated on *N. lugens* eggs in rice shoots enclosed in glass tubes (4 cm × 10 cm), which were kept in a controlled climate room at (26 ± 2)℃, 12 h light phase, and 80% relative humidity. Each day, newly emerged wasps were collected into clean glass tubes (2.5 cm × 20 cm) with access to both water and honey solution, and held for at least 2 h to ensure mating. From the second generation onwards, female parasitoids were used in experiments less than 24 h after emergence.

2.2 Chemicals

All the chemical standards of synthetic chemicals were at least 98% in purity and were bought from Sigma. Solvent used in all experiments is liquid olefin bought from Hangzhou Gaojing Chemical Industry Co., LTD, Hangzhou, China.

2.3 Behavioral response of *A. nilapareatae* to synthetic chemicals

Responses of *A. nilaparvata* females to rice volatiles were measured in a Y-tube olfactometer as described by Lou *et al.* (2005a). The two airstreams flowed 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 two 20 W fluorescent lamps placed in front of the box. All bioassays were conducted between 08:00 and 17:00. During experiments, the temperature in the room was maintained at 26-28℃.

A total of 18 single synthetic chemicals, each with two concentrations (5 and 50 ppm in liquid olefin) (Fig. 1), and 32 mixtures (each chemical with 50 ppm) (Table 1) were tested. When tested, 10 μL of one chemical solution in liquid olefin was applied to a filter paper (1 cm × 2 cm) and then the filter paper was placed into an odor source container. Another filter paper, after absorbing 10 μL of liquid olefin, was placed in the other odor source container as the control. Mated female parasitoids were introduced individually into the base tube of the Y-shaped olfactometer and given 10 min to make a choice. A 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." Forty females of *A. nilapareatae* were tested on each chemical. The data were subjected to a Chi-square test.

Table 1　List of 32 synthetic mixtures

No.	MeSA	Z-3-HAc	1-P-3-ol	Linalool	Z-3-H	E-2-H
1	+	+	−	−	−	−
2	+	−	−	−	+	−
3	+	−	−	+	−	−
4	+	−	+	−	−	−
5	−	+	−	+	−	−
6	−	+	−	−	+	−
7	−	+	+	−	−	−
8	−	−	−	+	+	−
9	−	−	+	−	+	−
10	−	−	+	+	−	−
11	+	+		+		
12	+	+			+	
13	+	−	+	−	+	−
14	+	+	+	−	−	−
15	+	−	+	+	−	−
16	+	−	−	+	+	−
17	+	+	−	−	−	+
18	−	+	−	+	+	−
19	−	−	−	+	+	+
20	−	−	+	+	+	−
21	−	−	+	+	−	+
22	−	−	+	−	+	+
23	+	+	−	+	+	−
24	+	+	−	−	+	−
25	+	+	−	−	−	+
26	+	−	+	−	−	+
27	+	−	+	+	−	+
28	+	−	+	+	+	−
29	+	−	+	+	−	−
30	+	+	+	−	+	−
31	−	+	+	+	+	−
32	+	+	+	+	+	−

Z-3-HAc, Z-3-hexenyl acetate; 1-P-3-ol, 1-penten-3-ol; Z-3-H, Z-3-hexenal; E-2-H, E-2-hexenal; β-CAL, β-caryophyllene.

2.4　Field experiment

Based on the bioassay in laboratory, we evaluated five individual chemicals -MeSA, Z-3-hexenal, linalool, 1-penten-3-ol and Z-3-hexenyl acetate, and one mixture (MeSA + Z-3-hexenal + linalool + Z-3-hexenyl acetate) -for their role in enhancing the effectiveness of natural enemies on BPH eggs in the field. The field experiment was carried out from 4 September to 20 September 2010 in a rice field in Yangzhou, China. The field consisted of 36 blocks (3 m × 3 m), and each block was surrounded by a one-meter-wide area of rice plants. For each chemical or mixture, 6 blocks were arranged: 3 blocks for chemical treatment and 3 blocks for control (Fig. 1). Silicon rubber septa were used as odor dispensers: each septum was filled with

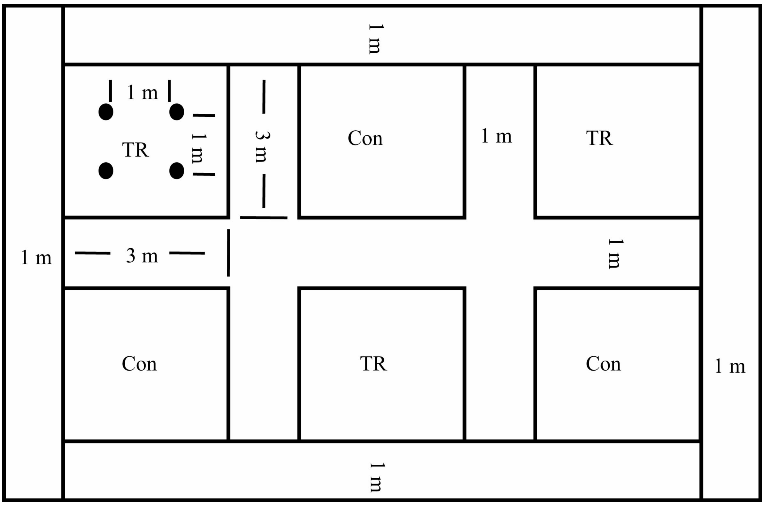

Fig. 1 Design of the field experiment

20 μL of one of the candidate chemicals (50 ppm in liquid olefin), the mixture (the concentration of every chemical is 50 ppm) or the solvent (liquid olefin); after airing for 2 h, the septa were stored at −20℃ until they were used for the experiment. For each chemical treatment block, 4 septa that were filled with one of chemicals were individually hung on a hill of rice plants by a string, 20 cm above the ground and 1 meter apart from one another (Fig. 1). Each control block received 4 septa filled only with liquid olefin. Seven days after the septa were hung out, 4 hills of rice plants (per block) that received the septa were harvested and the BPH eggs, including intact eggs, parasitized eggs, and predated eggs, on the rice plants were counted. Parasitism and predation rates of BPH eggs were calculated based on the data from the above records.

2.5 Statistical analysis of data

Differences in the behavioral responses of the parasitoid to synthetic chemicals and the solvent were determined by the χ^2 test. To test the differences in parasitism and predation rates between synthetic chemical treatments and controls, we used student's *t*- test. Data were analyzed with Statistica (SAS, Institute Inc., Cary, NC, USA).

3 Results

3.1 Behavioral response of *A. nilapareatae* to synthetic individual chemicals

Among the 18 chemicals tested, five chemicals (Z-3-hexenyl acetate, 1-penten-3-ol, Z-3-hexenal, linalool and MeSA) were attractive to the parasitoid (Fig. 2). All five active compounds showed their bioactivity only in 50 ppm except, for MeSA, which was attractive to the parasitoid in both 5 and 50 ppm (Fig. 2). Compared to the control, 75% of the tested female adults were attracted by Z-3-hexenyl acetate or linalool, 70% were attracted by MeSA, and 60%-65% were attracted by 1-penten-3-ol or Z-3-hexenal. Interestingly, two chemicals, (+)-limonene and 2-heptanol, in both 5 and 50 ppm, were repellent to the parasitoid (Fig. 2).

3.2 Behavioral response of *A. nilapareatae* to synthetic mixtures

Among the 32 mixtures tested, *A. nilapareatae* were strongly attracted by three mixtures, namely MeSA (50 ppm) plus Z-3-hexenal (50 ppm); a mixture containing Z-3-hexenal (50 ppm), Z-3-hexenyl acetate (50 ppm) and linalool (50 ppm); and a mixture containing MeSA (50 ppm), Z-3-hexenal (50 ppm), Z-3-hexenyl acetate (50 ppm) and linalool (50 ppm), respectively (Fig. 3).

3.3 Field experiment

The five single candidate chemicals and one blend containing MeSA (50 ppm), Z-3-hexenal (50 ppm), Z-3-hexenyl acetate (50 ppm) and linalool

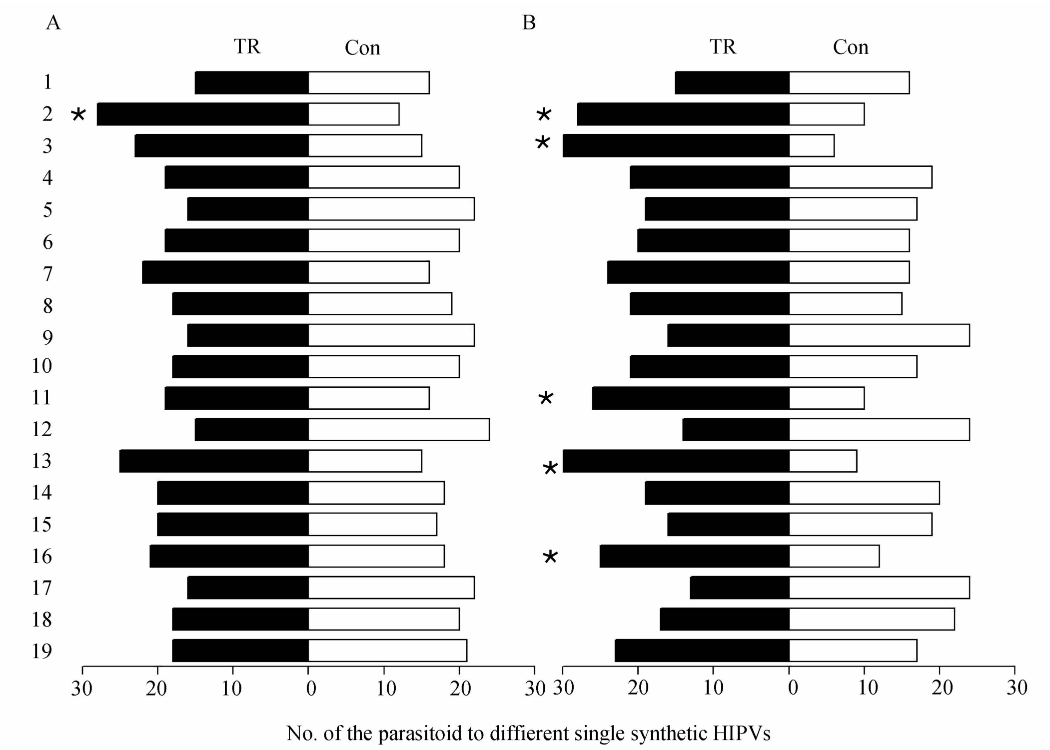

Fig. 2　Number of *Anagrus nilaparvatae* female adults attracted by single synthetic chemicals with 5 ppm (A) and 50 ppm (B) in liquid olefin

Asterisks indicate significant difference between members of a pair ($P < 0.05$, χ^2 test).
1. liquid olefin; 2. MeSA; 3. Z-3-hexenyl acetate; 4. Z-2-hexenal; 5. β-ocimene; 6. farnesene; 7. benzaldehyde; 8. Z-3-hexenyl butyl acetate; 9. methyl benzoate; 10. β-caryophyllene; 11. 1-penten-3-ol; 12. (+)-limonene; 13. linalool; 14. octadecene; 15. cis-nerolidol; 16. Z-3-hexenal; 17. 2-heptanol; 18. 2-heptanone; 19. linalool oxide.

(50 ppm) were used to evaluate their attractiveness to *A. nilapareatae* and predators in the field. The results showed that all of the tested chemicals increased the parasitism of BPH eggs by the parasitoid, especially the chemicals Z-3-hexenal, Z-3-hexenyl acetate and linalool, and the mixture for which the parasitism of BPH eggs was significantly enhanced (Fig. 4). The parasitism rates of BPH eggs by the parasitoid on plants that received a septa containing the mixture (containing MeSA, Z-3-hexenal, Z-3-hexenyl acetate and linalool), linalool, Z-3-hexenal and Z-3-hexenyl acetate was 5.94-, 4.95-, 3.32- and 2.01- fold higher than that on the control plants, respectively. The densities of BPH eggs in chemical-treated blocks were lower than or similar to those in control blocks (Table 2).

The plants receiving septa containing either MeSA, Z-3-hexenyl acetate, 1-penten-3-ol, linalool or the mixture also increased the predation rates of BPH eggs, but there were no significant difference compared to the rates for the corresponding control (Fig. 5).

Table 2　Mean number (±SE) of eggs per plant in different treatments in field experiments

	Treatment	Control	*t* test
MeSA	103.69 ± 10.96	80.59 ± 4.72	n. s.
Z-3-hexenal	77.10 ± 7.03	82.50 ± 1.00	n. s.
Z-3-hexenyl acetate	45.38 ± 15.08	80.01 ± 3.77	n. s.
1-penten-3-ol	57.87 ± 9.42	81.73 ± 1.77	*
Linalool	72.49 ± 8.00	79.39 ± 1.87	n. s.
Mixture	79.75 ± 5.02	73.95 ± 20.10	n. s.

Asterisk indicates significant difference, n. s. indicates no significant difference.

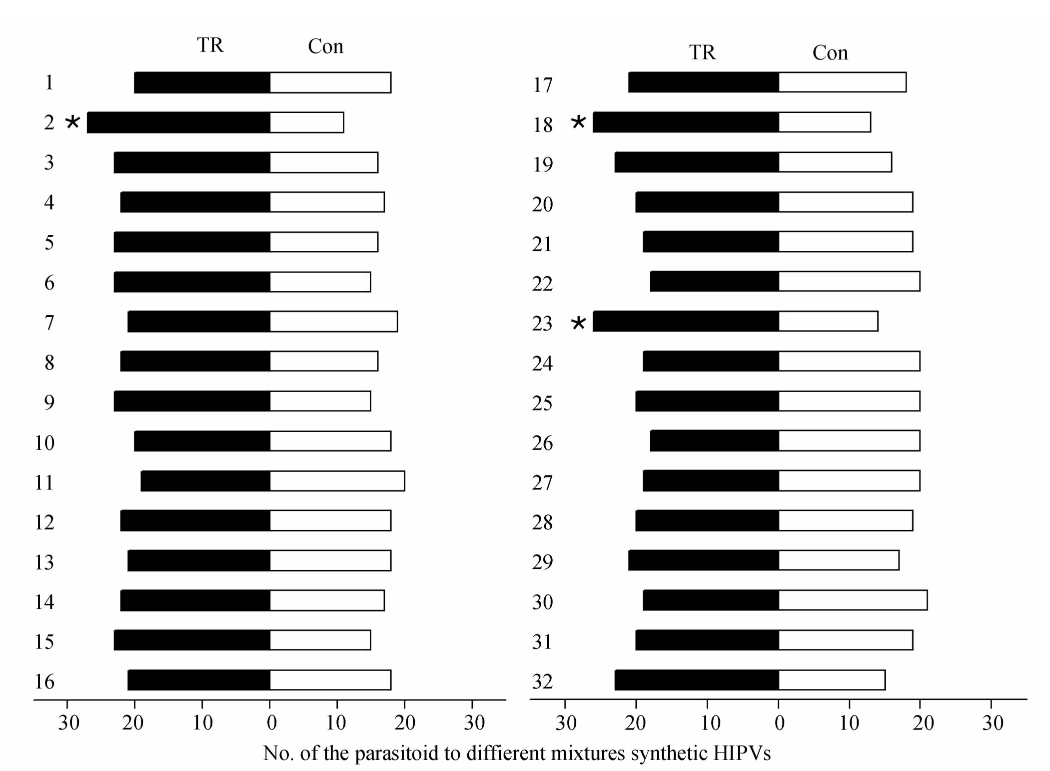

Fig. 3 Number of *Anagrus nilaparvatae* female adults attracted by different blends

Asterisks indicate significant difference between members of a pair ($P < 0.05$, χ^2 test).

Numbers (1-32) indicate chemicals stated as in Table 1.

4 DISCUSSION

In this study, we tested the olfactory response of *A. nilapareatae* to 18 single synthetic chemicals in a Y-tube olfactometer under laboratory conditions and found that five chemicals (Z-3-hexenal, Z-3-hexenyl acetate, 1-penten-3-ol, linalool and MeSA) were attractive to the parasitoid. Most of the five active chemicals were also found to play an important role in manipulating tritrophic interactions in other systems involving plant herbivores and the natural enemies of the herbivores. Engelberth *et al.* (2004), for example, found that Z-3-hexenal and (Z)-3-hexenyl acetate have a priming effect on maize plants. Martín *et al.* (2007) demonstrated that the parasitoid (*Aphidius funebris*) of a specialist aphid (*Uroleucon jaceae*) on *Centaurea nigra* was attracted by (Z)-3-hexenyl acetate. Parasitism of *Lygus lineolaris* eggs by the parasitoid (*Anaphes iole*) in a cotton field was greater when the eggs were associated with (Z)-3-hexenyl acetate than parasitism in the control field (Livy *et al.*, 2008). Takeshi (2010) demonstrated that linalool and MeSA emitted from lima bean leaves infested by two-spotted spider mites (*Tetranychus urticae*) strongly attract the predatory mites *Neoseiulus californicus*. James (2005) found eleven insect species or families were attracted by 13 synthetic HIPVs, including (Z)-3-hexenyl acetate and MeSA. Zhu and Park (2005) found that the seven-spotted lady beetle (*Coccinella septempunctata*), a predator of soybean aphid (*Aphis glycines*), was highly attracted by MeSA in the field. This suggests that *A. nilaparvatae* also uses the general plant volatiles to locate its hosts.

By testing the behavioral responses of the parasitoid to 32 mixtures mixed with two to five of seven chemicals which include the five active chemicals (Table 2), we found that only three blends that were mixed with active chemicals were attractive (Fig. 3). This demonstrates that the

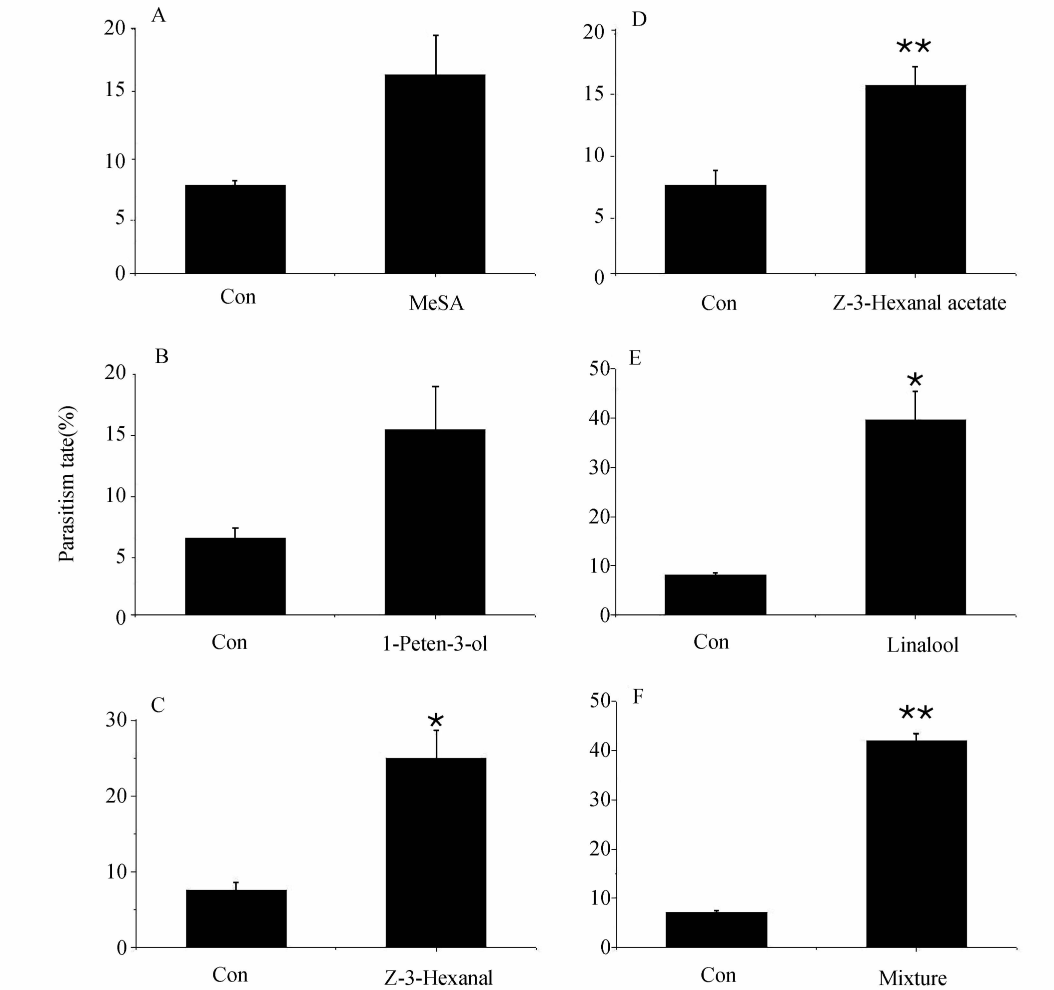

Fig. 4 Mean (± SE) parasitism rates (%) of *Nilaparvata lugens* eggs by *Anagrus nilaparvatae* on plants receiving septa containing different chemicals in the field

A. MeSA; B. 1-penten-3-ol; C. Z-3-hexenal; D. Z-3-hexenyl acetate; E. linalool; F. mixture (MeSA + Z-3-hexenal + linalool + Z-3-hexenyl acetate). Con, control.

composition and ratio of the chemicals in the mixture determined whether or not the mixture was perceived by the parasitoid, a phenomenon that has been reported in many plant-herbivore-parasitoid system (Dicke, 1999; Gouinguené *et al.*, 2001).

Based on the laboratory results, we chose five chemicals and one blend that are attractive to the parasitoid in the olfactometer and tested their effect on the parasitism and predation rates of BPH eggs by *A. nilapareatae* and predators in the field. The results showed that three of the tested single chemicals, Z-3-hexenal, Z-3-hexenyl acetate and linalool, and one blend (MeSA, Z-3-hexenal, Z-3-hexenyl acetate and linalool) significantly increased the parasitism of BPH eggs; predation rates also increased (Fig. 4 and Fig. 5). Since the densities of BPH eggs, which positively influence the parasitism or predation rates of BPH eggs by parasitoids or predators (Lou and Cheng, 1996) in chemical-treated blocks were mostly lower than or similar to those in control blocks (Table 2), it could be concluded that the chemicals used in this study enhance the effectiveness of natural enemies on BPH eggs. Recently, quite a lot of studies have reported that the application of synthetic blends can increase the efficiency of the natural enemies of herbivores.

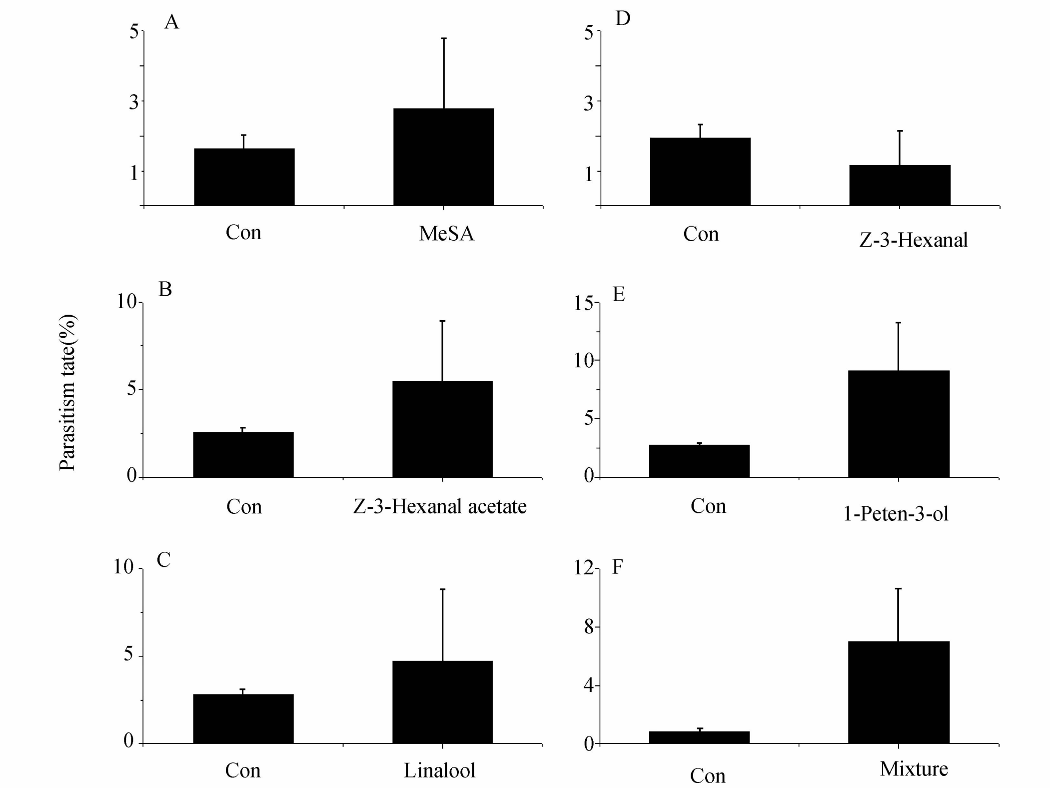

Fig. 5 Mean (±SE) predation rates (%) of *Nilaparvata lugens* eggs by predators on plants receiving septa containing different chemicals in the field

A. MeSA; B. Z-3-hexenyl acetate; C. linalool; D. Z-3-hexenal; E. 1-penten-3-ol; F. mixture (MeSA + Z-3-hexenal + linalool + Z-3-hexenyl acetate). Con, control.

For instance, Kessler and Baldwin (2001) found that applying synthetic HIPVs in a field of *Nicotiana attenuata* increased the predation rates of *Manduca sexta* eggs. Yu *et al*. (2008) tested several synthetic HIPVs for their attractiveness to beneficial insects in an open cotton field and found that the mixture of nonanal and (*Z*)-3-hexen-1-ol is attractive to the predatory bug (*Orius similis*) and the syrphid fly (*Paragus quadrifasciatus*). In a field trapping test, the numbers of common green lacewings caught (*Chrysoperla carnea sensu lato*) increased when acetic acid was added to lures with phenylacetaldehyde and methyl salicylate (Toth *et al*., 2009). This suggests that it is possible to enhance the effectiveness of natural enemies of herbivores, including *A. nilapareatae*, by applying synthetic HIPVs. Further studies should confirm the effects of these chemicals in large fields and determine the best application method, including concentration, application time and application area.

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稻飞虱卵期寄生蜂稻虱缨小蜂引诱剂的筛选与田间试验

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摘　要　植食性昆虫的天敌能够利用虫害诱导的挥发物进行有效的寄主或猎物定位。为了开发稻飞虱卵期天敌稻虱缨小蜂 *Anagrus nilaparvatae* Pang *et* Wang 的引诱剂，分别在室内和室外检测了多种褐飞虱诱导的水稻挥发物组分对褐飞虱卵期天敌稻虱缨小蜂的引诱作用。Y 型嗅觉仪实验结果表明，5 种单一化合物，Z-3-己烯乙酸酯，1-戊烯基-3-醇，Z-3-己烯醛，芳樟醇和水杨酸甲酯，以及 3 种混合物，水杨酸甲酯 + Z-3-己烯醛，Z-3-己烯醛 + Z-3-己烯乙酸酯 + 芳樟醇，水杨酸甲酯 + Z-3-己烯醛 + Z-3-己烯乙酸酯 + 芳樟醇，对稻虱缨小蜂具有明显引诱作用。田间试验表明，3 种单一化合物，Z-3-己烯乙酸酯，Z-3-己烯醛和芳樟醇，以及一种混合物，水杨酸甲酯 + Z-3-己烯醛 + Z-3-己烯乙酸酯 + 芳樟醇，能明显提高稻虱缨小蜂对褐飞虱卵的寄生作用。这些结果对于改善褐飞虱治理具有重要的意义。

关键词　褐飞虱，稻虱缨小蜂，虫害诱导挥发物，引诱剂