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Role of ethylene signaling in the production of rice volatiles induced by the rice brown planthopper *Nilaparvata lugens*

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Abstract Ethylene signaling pathway plays an important role in induced plant direct defense against herbivores and pathogens; however, up to now, only few researches have focused on its role in induced plant indirect defense, i.e. the release of herbivore-induced volatiles, and the results are variable. Using a model system consisting of rice plants, the rice brown planthopper *Nilaparvata lugens* and its egg parasitoid *Anagrus nilaparvatae*, we examined the role of ethylene signaling in the production of rice volatiles induced by *N. lugens* by measuring both the timing of herbivore-induced ethylene levels and the relationships between ethylene, rice volatiles and attraction of the parasitoid. *N. lugens* infestation significantly enhanced the release of ethylene during 2—24 h after infestation. Plants treated with ethephon, a compound that breaks down to release ethylene at cytoplasmic pH, released volatiles profiles similar to those released by *N. lugens*-infested plants, and both of them showed an equal attraction of the parasitoid. Moreover, pretreatment with 1-MCP, an inhibitor of ethylene perception, reduced the release of most of rice volatiles whose amount was enhanced by *N. lugens* infestation and decreased the attractiveness to the parasitoid. These results demonstrate that ethylene signaling is required for the production of rice volatiles induced by *N. lugens*.

Keywords: ethylene signaling pathway, *Nilaparvata lugens, Anagrus nilaparvatae*, herbivore-induced rice volatiles, rice, tritrophic interaction.

In response to herbivory, plants release volatiles to attract the natural enemies of herbivores[1,2]. This phenomenon has been reported in more than 23 plant species[3—6], and several field studies have shown that these herbivore-induced volatiles enhance the parasitism or predation of herbivores[7—10]. Investigations into the mechanisms on the production of herbivore-induced plant volatiles have revealed a central role of signaling pathways. Jasmonic acid (JA) pathway in this regard is the best studied and its important role has been well elucidated with chemical, pharmacological and molecular approaches[11—15].

Ethylene, as a kind of plant hormone, has been proven to exert an active role in plant direct defense[16]. Ethylene signaling pathway, for instance, is involved in the activation of distinct sets of PR-genes in *Arabidopsis thaliana*[17,18], and has either synergistic[19—21] or antagonistic interactions[22,23] with JA signaling in the expression of plant defense responses to pathogens and herbivorous insects. Exogenous application of ethephon reduces the resistance of Arabidopsis to Egyptian cotton worm *Spodoptera littoralis* but not to diamondback moth *Plutella xylostella*[24]. Only few studies, however, have determined the role of ethylene signaling in volatile emission in plants and the results are variable. Horiuchi *et al*.[25] demonstrated that the amount of volatiles emitted from JA-treated detached leaves of lima bean is enhanced by exogenous applications of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. Similarly, in maize, ethylene greatly promotes the volatile emission induced by volicitin, JA or (Z)-3-hexen-ol but not mechanical damage alone[26,27]. In tobacco *Nicotiana attenuata*, however, ethylene neither elicits volatile release from unmanipulated plants nor alters volatile profiles emitted from methyl jasmonate-treated plants[28]. By contraries, in another tobacco species *N. tabacum*, Huang *et al*.[29] recently found that ethylene can regulate the magnitude and blend of induced volatiles during pathogen infection.

The purpose of this paper is to examine the role of ethylene signaling in the production of rice volatiles induced by *Nilaparvata lugens* (Stål), one of the most important insect pests in rice ecosystem. Previous work has shown that *N. lugens* feeding systemically alters the volatile profile of rice plants, which in turn attracts *Anagrus nilaparvatae* Pant et Wang[30,31], a major egg parasitoid of rice planthoppers, including *N. lugens*[32]. Moreover, the proteins in the saliva of *N. lugens* play an important role in the release of herbivore-induced

volatiles[33], and salicylate signaling in rice plants is involved in the production of the volatiles[34]. To investigate the role of ethylene signaling in the production of *N. lugens*-induced rice volatiles, we first examined whether the *N. lugens* attack increased the emission of ethylene. Then we compared the volatiles profile emitted from rice plants that were treated with ethephon and their attractiveness to the parasitoid with those from plants infested by *N. lugens*. Finally, 1-methyl-cyclopropene (1-MCP), a gaseous antagonist of ethylene receptors[35], was applied to the plants 4 h before they were attacked by female *N. lugens* adults to examine whether it inhibits the production of *N. lugens*-induced volatiles and whether it decreases the attractiveness to the parasitoid.

1 Materials and methods

1.1 Plants

Rice varieties used in this study are Xiushui 11 and Xianyou 63 that are moderately resistant and susceptible to *N. lugens*, respectively[36,37]. Pre-germinated seeds were sown in a greenhouse, and after 20—25 d, the seedlings were transplanted into clay pots (16 cm diameter×14 cm height), 10 or 15 plants each. The plants were watered daily, and 0.125 g urea per pot was applied 15 and 25 d after transplanting, respectively. Plants were used for experiments 30—40 d after transplanting.

1.2 Insects

The *N. lugens* colony was originally obtained from the China National Rice Research Institute (CNRRI), Fuyang, Zhejiang, and maintained on Xianyou 63 rice plants using the same method as described in ref. [15]. A laboratory colony of the egg parasitoid *A. nilaparvatae* was started from individuals trapped in rice fields in Hangzhou, using Xianyou 63 rice plants with *N. lugens* eggs as bait. The colony was then propagated on *N. lugens* eggs in rice shoots enclosed in glass tubes (2.5 cm diameter×20 cm height) as described in ref. [15].

1.3 Plant treatments

(i) Ethephon treatment. The potted plants were washed with running water, and trimmed to leave 10 plants per pot. Each pot of plants was covered with a sealed plastic cage (12 cm diameter×36 cm height), and then a 50 mL glass beaker with 20 mL of either 0.1, 0.25, 0.5, 1 or 2 mmol/L ethephon (Sigma-Aldrich company, USA) in distilled water (pH=7.2) was placed into the cage. The control plants were similarly treated with 20 mL of distilled water. The cages with plants were then moved into a controlled climate room at (28±2)℃, 12 h photophase and 70%—80% r.h., and used for bioassay and chemical analysis 6, 12 or 24 h after the start of the treatment.

(ii) 1-MCP treatment. One cleaned potted plants (10 plants) was introduced into a sealed chamber (55 cm×60 cm×58 cm), and then a 50-mL glass beaker with 6 mL of AnsiP® (0.07% 1-MCP, Lytone Enterprise, Inc, Taiwan) solution (containing 492, 246 or 123 mg AnsiP®, which corresponded to 0.344, 0.172 or 0.086 mg 1-MCP respectively, in 6 mL distilled water) was placed into the chamber. The plants that were similarly treated with 6 ml of distilled water served as controls. The chambers were then transferred into a controlled climate room at (25±2)℃, 12 h photophase and 70%—80% r.h. 4 h after treatment, the rice plants were taken out for herbivore treatment or other experiments.

(iii) Herbivore treatment. Potted plants (10 plants per pot) were individually infested with 10 gravid *N. lugens* females that were fixed at the upper and lower position of the plant stems using two parafilm bags (6 cm×5 cm, with 60 small hole made by a needle) each with 5 females. Control plants were similarly treated with two empty parafilm bags (uninfested plants). The plants were placed into a controlled climate room at (28±2)℃, 12 h photophase and 70%—80% r.h., and used for assays at 12 h after treatment.

1.4 Bioassay

Responses of *A. nilaparvatae* females to rice volatiles were measured in a Y-tube olfactometer, which has been described in detail by Lou *et al*.[15]. Briefly, the olfactometer consisted of a Y-shaped glass tube 1 cm in diameter. The base and the two arms of the Y tube were both 10 cm in length. Each arm was connected to an odor source container (a glass cylinder, 46 cm in length, and 6 cm in diameter). An air stream was generated and divided in two, and each secondary air stream was led through a flowmeter, a tube with active charcoal, a humidifier bottle and one of the odor containers. Subsequently, the two airstreams were let to pass through the two arms of the Y-tube olfactometer at 150 mL/min. The Y-tube olfactometer was placed in a box (45 cm × 50 cm × 30 cm) painted white with an artificial light source consisting of two 20-W fluorescent lamps placed

in the front of the box. All bioassays were conducted during 09:00 and 17:00 h, and the temperature in the room was maintained at 25—28℃.

Behavioral response of the parasitoid to the following pairs of odor were observed: plants treated with either 0.1 (12 h), 0.25 (12 or 24 h), 0.5 (12 or 24 h), 1 (6 or 12 h) or 2 mmol/L (6 or 12 h) ethephon vs. plants treated with distilled water; gravid *N. lugen* female-infested plants (infested for 12 h) vs. plants treated with either 0.5 (24 h), 1 (12 h) or 2 mmol/L (6 or 12 h) ethephon; plants treated with either 0.344, 0.172 or 0.086 mg 1-MCP followed by female adult infestation for 12 h vs. plants treated with distilled water followed by female adult infestation for 12 h. To test for a possible effect of the treatment solutions *per se* we added experiments without plants, but with the solutions applied to 1.5 mL vials (500 μL of 1 mmol/L ethephon vs. 500 μL of distilled water; 500 μL of 0.172 mg 1-MCP vs. 500 μL of distilled water). For each treatment, 10 plants were used, and the odor sources were replaced by a new set of 10 plants after testing 8 wasps and for each odor source combination at least 32 females were tested.

1.5 Collection and identification of the rice volatiles

Rice volatiles were collected for 3 h using a solid phase microextraction (SPME, Suplco Co., Bellefonte, PA), which was the same as in ref. [31]. The volatiles emitted from the following treatments were collected: plants treated with either 0.5 (24 h) or 2 mmol/L (6 or 12 h) ethephon; plants treated with distilled water; gravid *N. lugen* female-infested plants (infested for 12 h); uninfested plants; plants treated with 1-MCP (0.344, 0.172 or 0.086 mg) followed by female adult infestation for 12 h; plants treated with distilled water followed by female adult infestation for 12 h and plants treated with distilled water or 0.172 mg 1-MCP for 4 h. We also collected the volatiles from a blank, only the glass cylinder without plants, to check if the system is clean. For each treatment, ten plants were used, and each treatment was replicated 4 times. The experiment was conducted in a room at a temperature of 26—28℃.

Analyses were done with a Hewlett Packard HP 6890A gas chromatograph (GC) on an HP-5 MS (30 m×0.25 mm i.d., 0.25 μm film thickness) capillary fused-silica column. Volatile compounds collected on the silica fiber of SPME were directly heat-desorbed in the GC injector at 250℃ for 1 min following a splitless model. Following injection, the column temperature was maintained at 40℃ for 2 min, increased to 250℃ at 6℃/min and held at 250℃ for 2 min. Helium (1 mL/min) was used as the carrier gas. All compounds were analyzed by an HP 5970B mass spectrometer operated in the electron impact ionization mode. Compounds were identified by comparison of retention times and mass spectra with those of authentic standards. The compounds were expressed as percentage of peak areas relative to the external standard (ES), octane (200 μL/L, 1 μL), per 3 h of trapping 10 plants.

1.6 Effect of 1-MCP on N. lugens feeding

Damage levels by *N. lugens* influence the volatile profile of rice plants and the attraction of the parasitoid[16]. Thus, the possible effect of 1-MCP on *N. lugens* feeding was measured. Five newly emerging macroteprous female *N. lugens* adults were placed into a small parafilm bag (6 cm×5 cm, with 60 small hole made by a needle), which was then fixed on the stems of plants which had been pretreated with 0.172 mg 1-MCP or distilled water for 4 h. All plants were placed in the controlled climate room at (28±2)℃, 12 L: 12 D, and 80% r.h. The amount of honeydew excreted by 5 female adults was weighed (to 0.1 mg) 24 h after the start of the experiment. The experiment was replicated 6 times.

1.7 Ethylene analysis

Cleaned potted plants (15 plants per pot) were individually infested with 15 gravid *N. lugens* females that were fixed at the upper, middle and lower positions of the plant stems using three parafilm bags (6 cm×5 cm, with 60 small hole made by a needle) each with 5 females. Control plants were similarly treated with empty parafilm bags. Each pot was confined with a sealed plastic cage (40.5 cm high×13 cm internal diameter). Ethylene production was determined by taking 5 mL of headspace using a syringe from the cage at 2, 4, 6, 8, 10, 12, 22 and 24 h since the start of the treatment at 13:00. Each treatment was replicated 3 times. The ethylene samples were analyzed by gas chromatography (GC) on a HP6890 with a Hayesep Q (80/100 mesh) stainless-steel column (1.8 m long×2.1 mm internal diameter, 3.7 mm external diameter) (Supelco Company, USA). The temperatures of the injector, oven and frame ionization detector were 110℃, 75℃, and 250℃, respectively. Nitrogen (30 mL/min) was used as carrier gas. The production of ethylene was quantified by 5 mL injections of known ethylene standards (20.5 μL/L, Beijing

AP Beifen Gases Industry Limited Company).

1.8 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), Duncan's multiple range tests to detect significant differences between groups were conducted. Differences in ethylene production were determined by Student t test. Data were analyzed with Statistica (Statistica, SAS, Institute Inc., Cary, NC, USA).

2 Results

2.1 N. lugens infestation increases the release of ethylene

Compared to the control plants, *N. lugens* infestation rapidly and significantly increased the release of ethylene from plants: a detectable significant difference between the *N. lugens*-infested plants and the control plants started 2 h after the start of the treatment and the difference was kept during the whole measurement duration (24 h) (Fig. 1).

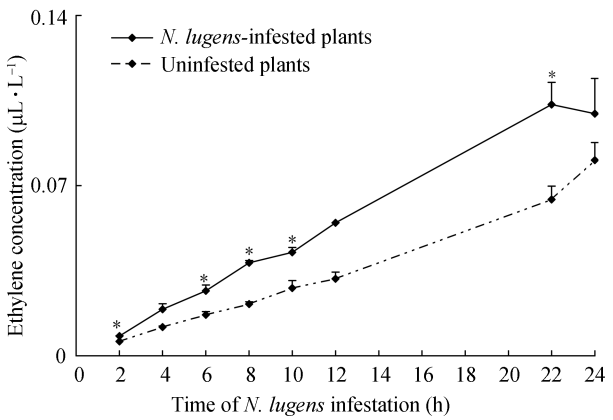

Fig. 1. Comparison of ethylene concentrations (μL/L) in cages that was released from uninfested plants or plants that were individually infested with 15 gravid *N. lugens* females. Asterisks indicate significant differences between members of a pair (*, P<0.05, t test).

2.2 Exogenous application of ethephon enhances rice volatiles release and attraction of the parasitoid

When tested against the volatiles from plants that were treated with distilled water, the volatiles released from the plants that were treated with either 0.1 (12 h), 0.25 (12, 24 h), 0.5 (12 h) or 1 mmol/L (6 h) ethephon showed no attractiveness to the parasitoids, whereas the volatiles from the plants that were treated with either 0.5 (24 h), 1 (12 h) or 2 mmol/L (6, 12 h) ethephon were attractive (Fig. 2) and all of these plants showed no significant differences in attractiveness to the parasitoids compared to *N. lugens*-infested plants (Fig. 3). Ethylene released from 2 mmol/L ethephon solution itself did not attract the parasitoids (the number of the parasitoid attracted by 2 mmol/L ethephon solution and distilled water was 16 and 16, respectively).

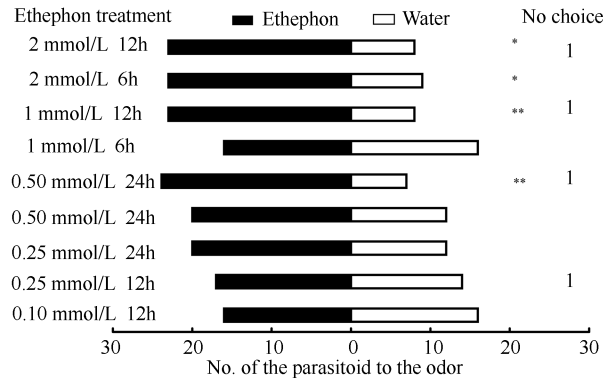

Fig. 2. Behavioral responses of *Anagrus nilaparvatae* to volatiles released from plants that were treated with either 0.1 (12 h), 0.25 (12 or 24 h), 0.5 (12 or 24 h), 1 mmol/L (6 or 12 h) or 2 mmol/L (6 or 12 h) ethephon (Ethephon) vs. those from plants that were treated with distilled water (Water). Asterisks indicate level of significant differences between members of a pair (*, P<0.05; **, P<0.01, χ^2 test).

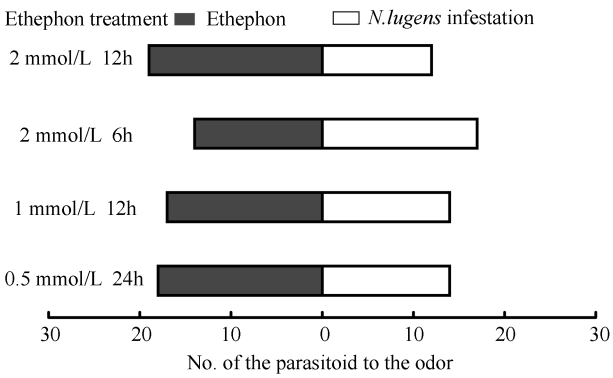

Fig. 3. Behavioral responses of *Anagrus nilaparvatae* to volatiles released from gravid *N. lugen* female-infested plants (infested for 12 h) (*N. lugens*) vs. those from plants that were treated with either 0.5 (24 h), 1 (12 h) or 2 mmol/L (6 or 12 h) ethephon (Ethephon).

Seventeen major compounds were collected from the headspace of rice plants by SPME, among which 11 chemicals were identified, including 7 aliphatic hydrocarbons, 3 terpenoids and one aldehyde (Tables 1 and 2). Compared to uninfested plants, *N. lugens* infestation increased the amount of the total rice volatiles and chemicals tretradecane, pentadecane, unknown 3 and 4, and induced linalool emission (Table 1). Exogenous

Table 1 Comparison of volatile compounds released from differently treated rice plants[a)]

Compounds	Uninfested plants	*N. lugens*-infested plants	Treated with water	Treated with ethephon		
				0.5 mmol/L (24 h)	2 mmol/L (6 h)	2 mmol/L (12 h)
Limonene	0.09±0.03 a	0.23±0.01 a	0.09±0.02 a	0.07±0.02 a	0.24±0.02 a	0.79±0.23 a
Linalool	–	0.52±0.02 a	–	0.05±0.00 a	0.19±0.02 a	0.61±0.01 a
Cyclocitral	0.15±0.01 a	0.09±0.01 a	0.06±0.01 a	0.07±0.00 a	0.15±0.01a	0.64±0.35 a
Unknown1	0.57±0.06 bc	0.58±0.03 bc	0.44±0.09 c	0.28±0.02 c	0.59±0.12 c	2.61±0.86 a
n-tretradecane	0.74±0.10 c	2.39±0.08 ab	0.86±0.11 c	1.46±0.21 bc	1.52±0.54 bc	2.84±0.14 a
Unkonwn2	1.53±0.03 bc	2.59±0.04 ab	2.24±0.08 abc	1.37±0.18 bc	1.26±0.32 c	3.07±0.02 a
Unknown3	1.80±0.07 b	3.24±0.10 a	1.27±0.24 b	3.30±0.07 a	3.22±0.15 a	1.93±0.12 a
n-pentadecane	3.09±0.16 b	6.13±0.41 a	2.70±0.33 b	5.92±0.21 a	6.78±0.82 a	6.44±0.62 a
Unknown4	2.21±0.29 b	4.49±0.11 a	1.79±0.08 bc	2.61±0.08 b	2.12±0.14 b	0.83±0.02 c
Dodecenal	2.80±0.16 ab	3.65±0.17 a	2.05±0.28 bc	2.40±0.31 b	2.00±0.01 bc	0.80±0.06 d
n-hexadecane	4.80±0.11 ab	5.45±0.28 a	4.73±0.30 ab	3.21±0.10 c	3.77±0.24 bc	4.62±0.77 ab
Unknown5	1.04±0.17 bc	1.46±0.08 ab	1.40±0.12 abc	1.21±0.19 bc	0.26±0.03 c	2.37±0.41 ab
n-heptadecane	1.55±0.18 b	1.57±0.22 bc	1.16±0.20 bc	2.31±0.30 ab	2.92±0.15 a	0.91±0.27 c
Unknown6	1.22±0.08 b	1.32±0.18 b	1.59±0.11 b	1.64±0.41ab	0.95±0.22 b	2.58±0.24 a
n-octadecane	0.49±0.05 b	0.55±0.06 b	0.54±0.08 b	1.40±0.08 ab	1.19±0.53 ab	2.13±0.36 a
n-nonadecane	0.56±0.03 a	0.29±0.07 a	0.24±.05 a	0.36±0.01 a	0.48±0.05 a	0.65±0.13 a
n-eicosane	0.31±0.03 a	0.26±0.01 a	–	0.21±0.01 a	0.43±0.05 a	0.27±0.03 a
Total	22.45±0.65 c	33.75±1.04 a	20.25±1.10 c	21.43±1.10 c	28.42±1.66 b	32.87±2.59 b

a) Data in the table are mean peak area (% of ES) ± Se (n=3); letters in a same row indicate significant differences among treatments (P<0.05, Duncan's multiple range test).

application of ethephon also resulted in some similar changes in rice volatiles compared to its corresponding control plants: increased the levels of the total volatiles and chemicals tretradecane, pentadecane and unknown 3, and induced the release of linalool (Table 1). However, there were some differences between ethephon treatment and *N. lugens* infestation: ethephon treatment did not increase the release of the unknown 4, and had lower levels of the total volatiles and dodecenal but higher levels of unknown 5 and n-octadecane (Table 1).

2.3 Pretreatment with 1-MCP reduces N. lugens-induced volatiles and attraction of the parasitoid

The attractiveness of *N. lugens*-infested plants to the parasitoid decreased when the plants had been pretreated with 1-MCP for 4 h, and the inhibition efficiency was positively correlated with the concentrations of 1-MCP (Fig. 4). 1-MCP itself did not repel the parasitoid (the number of the parasitoid attracted by 1-MCP solution and distilled water was 14 and 17, respectively; one female was no choice) and did not influence *N. lugens* feeding: the amount of honeydew excreted by 5 gravid *N. lugens* females on 1-MCP-treated plants was (30.45±4.80) mg, but was (37.35±6.62) mg on distilled water-treated plants.

Volatiles analysis revealed that pretreatment with 1-MCP for *N. lugens*-infested plants resulted in a decrease in the amount of the total volatiles and chemicals

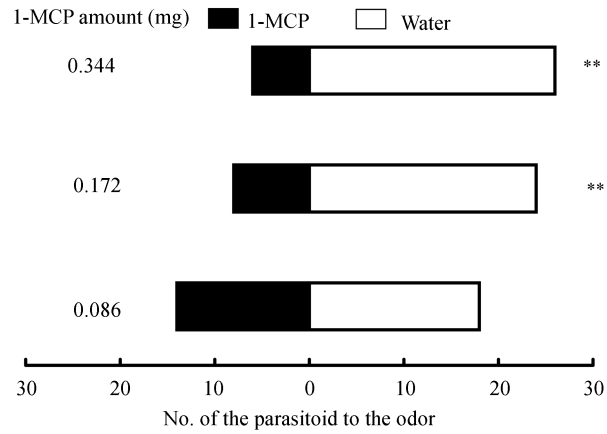

Fig. 4. Behavioral responses of *Anagrus nilaparvatae* to volatiles released from plants that were treated with either 0.344, 0.172 or 0.086 mg 1-MCP followed by 12 h of *N. lugens* infestation (1-MCP) vs. those from plants that were treated with distilled water followed by 12 h of *N. lugens* infestation (Water). Asterisks indicate level of significant differences between members of a pair (*, P<0.05; **, P<0.01, χ^2 test).

tretradecane, dodecenal, *n*-hexadecane, *n*-eicosane, linalool and unknown 2—4 (Table 2). These chemicals included all of compounds whose levels were increased by *N. lugens* infestation except for n-pentadecane that was not inhibited by 1-MCP (Tables 1 and 2). *N. lugens*-infested plants that had been pretreated with high concentrations of 1-MCP increased the level of n-heptadecane (Table 2). Compared to control plants, treatment with 1-MCP alone only resulted in a slight

Table 2 Effect of 1-MCP on the production of rice volatiles induced by *N. lugens*[a)]

Compound	Water-treated plants followed by *N. lugens* infestation	1-MCP treated plants followed by *N. lugens* infestation		
		0.086 mg	0.712 mg	0.344 mg
Limonene	0.19 ±0.01 a	0.45±0.12 a	0.25±0.01 a	0.23±0.01 a
Linalool	0.50±0.02	–	–	–
β-cyclocitral	0.09±0.01 a	0.13±0.01 a	0.13±0.01 a	0.12±0.01 a
Unknown1	0.54±0.02 a	0.18±0.03 a	0.24±0.01 a	0.21±0.00 a
n-tretradecane	1.19±0.05 a	1.51±0.26 ab	1.24±0.24 b	1.36±0.17 b
Unknown2	2.58±0.04 a	2.18±0.10 b	1.87±0.15 bc	1.75±0.14 c
Unknown3	3.23±0.10 a	1.74±0.06 b	2.55±0.14 ab	2.07±0.43 b
n-pentadecane	5.67±0.42 b	8.59±0.28 a	5.04±0.17 b	4.65±0.18 b
Unknown4	3.92±0.13 a	2.24±0.19 b	2.18±0.25 b	2.24±0.11 b
Dodecenal	3.44±0.11 a	1.31±0.09 b	1.23±0.07 b	1.15±0.07 b
n-hexadecane	4.65±0.27 a	3.47±0.22 b	2.31±0.06 c	2.15±0.06 c
Unknown5	3.21±0.13 a	1.99±0.14 a	2.81±0.12 a	2.47±0.14 a
n-heptadecane	1.51±0.06 b	1.92±0.24 ab	2.51±0.15 a	2.50±0.12 a
Unkmown6	1.32±0.18 a	1.20±0.23 a	1.44±0.09 a	1.40±0.07 a
n-octadecane	0.49±0.06 b	0.93±0.21 a	0.63±0.12 ab	0.59±0.12 ab
n-nonadecane	0.27±0.05 a	0.17±0.04 a	0.19±0.02 a	0.17±0.02 a
n-eicosane	0.24±0.00 a	–	–	–
Total	31.45±0.58 a	26.69±1.36 b	22.01±0.81 c	20.78±0.52 b

a) Data in the table are mean peak area (% of ES) ± Se (n=3); letters in a same row indicate significant differences among treatments (P<0.05, Duncan's multiple range test).

change in volatiles: a decrease in β-cyclocitral and an increase in n-hexadecane (Fig. 5).

3 Discussion

The results of ethylene analysis showed that *N. lugens* infestation obviously enhanced the release of ethylene starting at 2 h after infestation (Fig. 1), prior to the emission of *N. lugens*-induced volatiles that is at 6 h after the plants were individually infested with 10 gravid females[31]. This indicates that *N. lugens* infestation activates the ethylene signaling in rice plants, which might be involved in the production of rice volatiles induced by *N. lugens*. Plants that were treated with enough concentrations of ethephon for some time released similar volatile profiles to that of *N. lugens*-infested plants (Table 1), and both of them showed an equal attraction of the parasitoid (Figs. 2 and 3); the time required for the production of rice volatiles induced by ethephon is comparable with that *N. lugens* infestation needs[31] if the concentration of ethephon is high enough, less than 6 h after elicitation, for example, with 2 mmol/L ethephon (Fig. 2). Moreover, pretreatment with 1-MCP, which binds ethylene receptors and then breaks down the ethylene signaling in plants, resulted in a decrease in the amount of volatiles, including most of *N. lugens*-induced chemicals, from the plants in response to *N. lugens* infestation (Table 2) and in attractiveness to the parasitoid (Fig. 4). This was

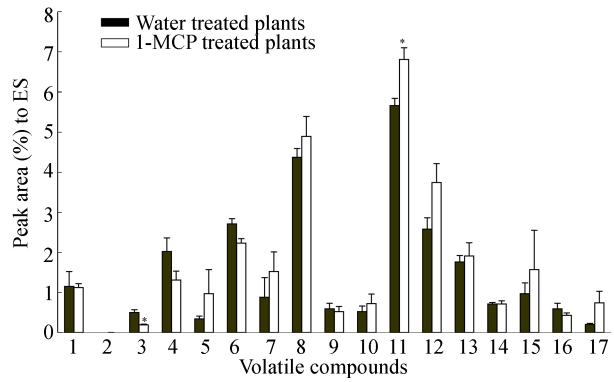

Fig. 5. Comparison of volatile compounds released from rice plants that were treated by distilled water (Water) or 0.172 mg 1-MCP (1-MCP) for 4 h. Data in the figure are mean (+ Se, n = 3) peak area (% of ES). 1, Limonene; 2, linalool; 3, β-cyclocitral; 4, unknown 1; 5, *n*-tretradecane; 6, unknown 2; 7, unknown 3; 8, *n*-pentadecane; 9, unknown 4; 10, dodecenal; 11, *n*-hexadecane; 12, *n*-heptadecane; 13, unknown 5; 14, *n*-octadecane; 15, unknown 6; 16, *n*-nonadecane; 17, *n*-eicosane. Asterisks indicate significant differences between members of a pair(*, P<0.05; **, P<0.01, χ^2 test).

neither due to an effect of 1-MCP itself on plant volatiles (Fig. 5), nor an effect on *N. lugens* feeding. These results clearly demonstrate that the ethylene signaling pathway plays an important role in the production of rice volatiles induced by *N. lugens*.

That herbivore infestation increases ethylene production has been reported in several plants[25−27,38,39]. As is clear in this work, the ethylene production of plants in response to herbivory and its role in plant

volatile production vary in different plant-herbivore systems. In maize, for instance, ethylene production in response to attack by *Spodoptera exigua* is slow (later than the release of induced volatiles) and dependent on photoperiod[39]. Moreover, ethylene promotes the volatile emission induced by volicitin and JA but not mechanical damage alone[26]. In contrast, ethylene in tobacco *Nicotiana attenuata* is rapidly increased after herbivore attack, and its release appears to be independent of photoperiod and it does not influence the volatiles emission[27]. In our experiment, we found that *N. lugens* infestation rapidly increased the ethylene production of rice plants (Fig. 1) as in the case with *N. attenuate*[28]. We also demonstrated an important role of the ethylene signaling in the production of rice volatiles induced by *N. lugens* (Figs. 2 and 3; Tables 1 and 2), which was different from those reported in maize[26,39], tobacco[27] and lima bean[25], suggesting a specific role of ethylene signaling in volatiles release of different plant-herbivore systems.

To determine if exogenous application of ethephon to rice plants elicited volatiles release, the effects of a series of concentrations and elicitation durations of ethephon were evaluated (Fig. 2). The result showed that ethephon treatments, 0.5 mmol/L for 24 h, 1 mmol/L for 12 h, and 2 mmol/L for 6 or 12 h induced the release of volatiles attractive to the parasitoid (Fig. 1 and Table 1). The question, however, is whether the ethylene concentrations we applied in the study are near the physiological levels of ethylene in *N. lugens*-infested plants. Ethephon is stable in aqueous solution below pH 4, above which it decomposes into ethylene, phosphate and chloride ion[40]. The half-life of ethephon depends on pH value and the temperature of the solution[41]. Its half-lives, for example, at pH 7 at 15℃ and 35℃ are 100 and 4 h, respectively[42]. In our experiments, pH of ethephon solution was 7.2 and the temperature was 28℃. If there are linear relationships between the half-life of ethephon and the temperature and between the time and the amount of ethylene released, the concentration of ethylene in the container at 6 h, when 2 mmol/L ephephon solution is applied, will be about 17.4 μL/L (6 h/38 h×110 μL/L; the half-life of ethephon in our experiment (at 28℃) is about 38 h and at that time the concentration of ethylene is about 110 μL/L). It is about 170-fold higher than the highest concentration of ethylene in *N. lugens*-infested plants (at 22 h after herbivory). Since the concentrations of ethylene we detected were those in the container released from plants, which should be far lower than the concentrations of ethylene around the leaf boundary layer (about 5 mm thick) of the plant[43] or within the plant, the ethylene concentration we applied in the study is comparable with the physiological concentrations in *N. lugens*-infested plants.

Different from our previous results that *N. lugens* infestation did not significantly increase the amount of volatiles[31], here we found that the amount of the total rice volatiles and some individual chemicals were slightly increased following attack by the herbivore (Table 1). This difference is likely due to the different rice varieties we used: Xiushui 11, a moderately resistant variety to *N. lugens* in this study, whereas a susceptible variety Zhe 852 was used before. It has been well documented that plant genotypes obviously influence the herbivore-induced volatiles in rice[33,44] and other plant species[45−48].

Chemical analysis revealed that exogenous application of ethephon to rice plants elicited the release of some chemicals as *N. lugens* infestation did; however, there were still differences between them (Table 1). Similarly, 1-MCP treatment did not inhibit all chemicals whose amount was enhanced by *N. lugens* infestation (Table 2). These results suggest that *N. lugens* infestation may also activate some other signaling pathways in addition to ethylene signaling. Indeed, we have found that *N. lugens* infestation activates SA signaling and exogenous application of SA, within its physiological concentration in *N. lugens*-infested plants, gives rise to volatiles attractive to the parasitoid[34]. The role of SA in the production of plant volatiles induced by herbivores has also been reported in lima bean[49]. Therefore, the production of *N. lugens*-induced rice volatiles might be a result of crosstalk among several signaling pathways, at least including ethylene and SA signaling. The proposed role of crosstalk of SA and ethylene signaling in the production of *N. lugens*-induced volatiles requires further investigation.

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