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Attractiveness of tobacco volatiles induced by *Helicoverpa armigera* and *Helicoverpa assulta* to *Campoletis chlorideae*

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Abstract **The attraction of *Helicoverpa armigera*- and *Helicoverpa assulta*-induced and mechanical damage-induced tobacco volatiles to *Campoletis chlorideae* was investigated, and the induced volatiles were analyzed. In wind-tunnel, *C. chlorideae* was strongly attracted by herbivore-induced tobacco volatiles. Mechanically damaged tobacco leaves, whether treated with caterpillar regurgitant or water, were more attractive to the parasitoid than undamaged tobacco leaves. GC-MS analysis revealed that only 4 compounds were released from undamaged tobacco leaves, whereas 13 compounds were commonly emitted from herbivore-infested and mechanically damaged tobacco leaves. Compound β-pinene was specifically induced by the infestation of *H. armigera*, and (*Z*)-3-hexenal was only induced by the infestation of *H. armigera* and *H. assulta*, whereas hexyl acetate was only induced by mechanical damage. Tobacco leaves infested by *H. armigera* and *H. assulta* released larger amounts of volatiles than undamaged tobacco leaves did. Tobacco leaves treated with artificial damage plus caterpillars regurgitant or water emitted the same levels of volatiles, which were higher than that emitted by undamaged tobacco leaves. The emission amounts of single compounds were also different between differently treated plants. The differences were large between herbivore-induced and mechanical damage-induced compounds, and small between *H. armigera*- and *H. assulta*-induced compounds, and among compounds emitted from mechanically damaged plants treated with water or caterpillar regurgitant.**

Keywords: ***Helicoverpa armigera*** **(Hübner),** ***Helicoverpa assulta*** **(Guenée),** ***Campoletis chlorideae*** **Uchida, behavioral response, herbivore-induced tobacco volatiles.**

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Plants and insects have coexisted for as long as 350 million years, and have developed a series of relationships, of which the most common interaction involves insect predation of plants, and plant defenses against herbivorous insects[1]. Plant defenses against insects can be direct. For example, the synthesis and exudation of toxic secondary metabolites can poison attacking herbivores, or defensive proteins, such as protease inhibitors (PIs) or polyphenoloxidases (PPOs), which decrease nutrient availability and slow the growth of herbivores[2]. In addition, plants use indirect defenses that facilitate "top-down" control of herbivore populations by the herbivore's predators, parasitoids, and pathogens[3]. In the past two decades, tritrophic interactions between plants, herbivores, and natural enemies, have aroused intense interest among ecologists and show great potential for exploitation in the management of arthropod pests. Infochemicals play essential roles as mediators in tritrophic interactions, and herbivore-induced plant volatiles in particular have been shown to be key signals that guide parasitoids to their host herbivores.

Herbivory can cause both quantitative and qualitative changes of volatile emission[4−6]. The release of herbivore-induced plant volatiles is an active process, and some herbivore-induced terpenoids are synthesized de novo[7,8]. Generally, the induced emission of volatiles is not restricted to the site of damage but can occur systemically[9,10]. Consistent differences in volatile blends have been observed not only for different plant and herbivore species, but also for differences in plant age and the stage of the attacking herbivore[11−13].

Plant volatile emissions can be triggered by mere tissue damage. However, plant responses to herbivory may differ from generalized wound responses. Elicitors in oral secretions of the herbivores are at least partly responsible for the differences in response[5,14]. In the regurgitant of caterpillar *Pieris brassicae* the main elicitor was the enzyme β-glucosidase[15], whereas in the oral secretions of *Spodoptera exigua* volicitin, [N-(17-hydroxylinolenoyl)-L-glutamine], was isolated and identified as a potently non-protein elicitor of volatile biosynthesis[16]. Volicitin and its analogues are now known to occur in the oral secretions of numerous insect species[17−21], and of different instar larvae of *Spodoptera littoralis*[22].

Helicoverpa armigera and *Helicoverpa assulta* are sibling noctuid species. The two insects have similar morphological, biological and ecological characteristics, and can co-occur on tobacco plants as important pests. Differently, *H. armigera* is a typical polyphagous species, which feeds upon more than several hundreds of plants from 30 families, whereas *H. assulta* specializes on Solanaceae plants as oligophagous species. *Campoletis chlorideae* is a predominant parasitoid of *H. armigera* and *H. assulta*, and can parasitize many noctuid insects. *C. chlorideae* has been extensively studied as a potential biological control agent for *H. armigera* in China, Korea and India[23−25], but little is known about its host foraging behavior. In the present work, the attraction of volatiles emitted by tobacco leaves in response to damage caused by *H. armigera* and *H. assulta* to *C. chlorideae* was investigated, and the in-

duced volatiles were analyzed. The results not only revealed the relative importance of host-induced volatile emissions for host location by *C. chlorideae*, but also provided insight into how the parasitoid might deal with plant signals induced by different host herbivores.

1 Materials and methods

1.1 Insects and plant

H. armigera and *H. assulta* were obtained from Anyang and Xuchang of Henan Province, China and were reared on artificial diets at (26±1)℃, 75% RH, 16L/8D, as described in ref. [26]. A colony of the parasitoid *C. chlorideae* was started with cocoons collected from Zhengzhou in Henan Province. The colony was maintained on *H. armigera* larvae fed with artificial diet. Mated female wasps were allowed to once or twice sting host larvae at the late second or early third instar, and these parasitized host larvae were kept in an incubator under the same condition until cocoon formed. Fifteen cocoons were collected and kept in a glass tube (2 cm in diameter, 10 cm in length) plugged with cotton wool until adult emergence. Twenty adults were kept in a cage (10 cm in diameter, 20 cm in length) with a sex ratio of 1∶1. A honey solution (20%) was provided every day as food source.

Tobacco *Nicotiana tabacum* L. cultivar "Putongyan" obtained from Institute of Crop Breeding and Cultivation, the Chinese Academy of Agricultural Sciences (CAAS), was used in all experiments. Tobacco seeds were first germinated in a 60 cm (diameter) × 20 cm (deep) plastic basin filled with fertilized soil obtained from Institute of Vegetables and Flowers, CAAS. Tobacco seedlings were transplanted and cultivated individually in 16 cm (diameter) × 15 cm (deep) flowerpots, and were kept outdoors for growth under natural conditions with temperature of 24—33℃ from June to September, 2003. Seedlings were watered every day. A net cage (3 m in length, 3 m in width, 2 m in height) was used to prevent possible infestation by naturally occurring herbivores. Two to three months old tobacco plants with 5—6 leaves were used in all experiments.

1.2 Caterpillar regurgitant preparation

Regurgitant of third- and fourth-instar larvae of *H. armigera* and *H. assulta* feeding on artificial diet was collected using the methods in Turlings et al.[14]. About 5 μL of regurgitant from each caterpillar of *H. armigera* and *H. assulta* could be collected. All regurgitant was centrifuged for 10 min at 10000 *g* and the supernatant was filtered through a 0.22-μm sterile millipore filter to remove large particles and micro-organisms and subsequently stored at −20℃ prior to use.

1.3 Plant treatment

The third leaf counted from the bottom of the tobacco plant was cut with a razor blade at the petiole. A batch of three detached leaves was placed into a vial (100 mL) filled with water. Tobacco leaves were either left undamaged (control), or subjected to infestation by 12 third- instar *H. armigera* or *H. assulta* larvae, which have been starved for about 10 h (overnight). For artificially damaged treatments, each tobacco leaf was scratched with a razor blade over an area of c.a. 10 cm^2/leaf on the upper surface, and a 20 μL aliquot of distilled water or caterpillar regurgitant was subsequently applied onto the damaged area. In all cases, the plants were treated at 8:00—9:00 a.m.

1.4 Wind tunnel bioassay

To prevent the caterpillars from escaping, after having received insects or artificial damage, plants were caged with a fine net bag. As control, undamaged plants were also caged with the net bag. The bag and caterpillars were removed after 6 h of initial treatments, and the plants were subsequently used for bioassay. Attraction of tobacco leaves to the parasitoid *C. chlorideae* was tested in a Plexiglass wind tunnel (interior measurements of 90×30×30 cm). A fan and a set of screen upwind produced an airflow 50 cm/s. The wind tunnel was lit by two white fluorescent lamps (40 W each), and indirect light was reflected from a white board, which together result in an intensity of 1500 lux inside the wind tunnel. Temperature was maintained at (25±1)℃ during the test. A batch of three tobacco leaves that had undergone the same treatment was placed in one vial. Two vials each with undamaged or differently treated plants were placed 15 cm apart at the upwind end of the tunnel. Two to three days old naive (no experience with hosts and plants) female wasp was released individually from a release device (2 mL vial) placed onto a take-off platform, 80 cm downwind from the plants with its top at mid height in wind tunnel. Behavioral responses were categorized as follows: (i) "choosing", wasps flew upwind from taking-off and landed on or arrived at plants within five minutes; (ii) "no choosing", wasps flew upwind but ended by landing on the wind tunnel wall or inner-top and did not arrive at plants within 5 min, or stayed on the platform for more than 5 minutes. The position of the plants was exchanged after testing 2—3 wasps to eliminate the asymmetric effects. The wind tunnel and the take-off platform were cleaned with alcohol after each test. Each wasp was used only once. Each combination was tested 3—4 times with fresh batches of plants on different days. 10—15 wasps were tested each time. Totally, more than 40 wasps were tested for each of the 5 combinations.

1.5 Volatiles collection and analysis

Tobacco leaves were immediately placed into a glass jar (12 cm in diameter, 21 cm in height) after treatments.

Volatiles were collected using a push-pull technique (compressed air and vacuum). Clean air was led to pass through a water bubbler for humidification, and a flowmeter for measuring and regulating the air flow, and a charcoal filter for purification. The moist and pure air then entered the jar at 300 mL/min from the lower part of the jar, passed over the plant materials, and then passed through an outlet at the top of the jar. The blend of volatiles was trapped in a glass tube (10 cm long, 6 mm in diameter) that contained 25 mg of 80/100 mesh Super Q adsorbent (Altech Assoc., USA). The trap was connected through Teflon tube to the outlet of the jar at one end, and via another flowmeter at the other end to a vacuum pump. During the collection, the temperature in the jar was kept at (25±2)℃. Two fluorescent lamps (each 40 W) producing a light intensity of about 2000 lux were suspended over the jar to illuminate the plants during the collection. Two collection systems were used in parallel every time and the collection was run for 12 h. Each treatment was repeated 5 times with fresh batches of plants.

After collection, the trap was rinsed with 200 μL redistilled hexane. Two internal standards (800 ng of *n*-decane and benzyl acetate in 10 μL hexane) were added. Identification and quantification of volatiles were carried out by coupled gas chromatography-mass spectrometry (GC-MS) on a Hewlett-Packard 6890 GC-5973 MSD. The GC was equipped with a DB-WAX column (Polyethylene Glycol 20000, 60 m × 0.25 mm ID; film thickness 0.15 μm). Helium was used as carrier gas with a constant flow of 26 cm/s. A 2 μL of aliquot volatile samples was injected, and then immediately split with a purge flow of 30 mL/min. The injector temperature was 250℃ and the GC-MS transfer line temperature was 280℃, source 230℃, quadrupole 150℃, ionization potential 70 eV, and scan range 30—300 m/z. Following injection, the column temperature was increased from 55℃ to 200℃ at 8℃/min, and held at 200℃ for 20 min. Compounds were identified by comparing mass spectra with NIST library spectra (Agilent Technologies, USA), and some of them were confirmed with authentic reference compounds. Compounds were quantified by their total ion abundances relative to that of the internal standards.

1.6 Statistical analysis

Ducan's new multiple range test after ANOVA was made to determine statistical differences (p= 0.05) of the average amounts of single compounds and the total amounts of the headspace volatiles emitted from undamaged, herbivore-infested, and mechanically damaged tobacco leaves. Chi-square analysis was performed to test differences between numbers of wasp that chose undamaged and differently treated plants they were offered, and wasps defined as "no choosing" were not included in statistical analysis. All the above analyses were carried out with SPSS 10.0 for Windows.

2 Results

2.1 Behavioral responses of *C. chlorideae*

Plants infested by *H. armigera* and *H. assulta* were more attractive to *C. chlorideae* than undamaged plants (Fig. 1). The percentage choices made by *C. chlorideae* to *H. armigera*- and *H. assulta*-infested plants were 75% and 80% respectively. Mechanically damaged plants, whether they were treated with caterpillar regurgitant or water, attracted more parasitoids than undamaged plants did (Fig. 1), and the percentage choices for *H. armigera* regrugitant, *H. assulta* regurgitant and water treated plants were 78%, 79% and 74%, respectively.

2.2 Tobacco volatiles

Fig. 2 shows characteristic total ion current chromatograms and average total amounts of the headspace volatiles emitted from undamaged, herbivore-infested, and mechanically damaged tobacco leaves. The average amounts of single compounds are listed in Table 1. Only four compounds, (*Z*)-3-hexenyl acetate, 1,4-dichlorobenzene, nicotine and one unknown compound were emitted from undamaged tobacco leaves (Fig. 2 and Table 1).

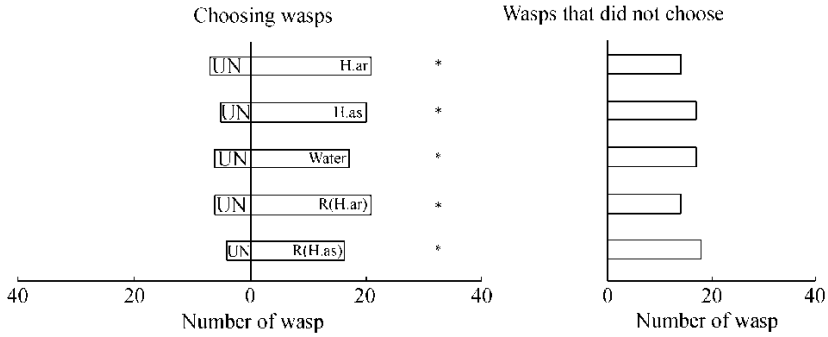

Fig. 1. Number of choosing and no choosing wasps in wind tunnel bioassay with undamaged and differently treated tobacco leaves. UN, undamaged; H. ar, infested by *H. armigera*; H. as, infested by *H.assulta*; Water, mechanically damaged and treated with water; R(H. ar), mechanically damaged and treated with regurgitant of *H. armigera*; R(H. as), mechanically damaged and treated with regurgitant of *H. assulta*. Asterisks indicate a significant difference within a choice test (χ^2 test, p<0.05).

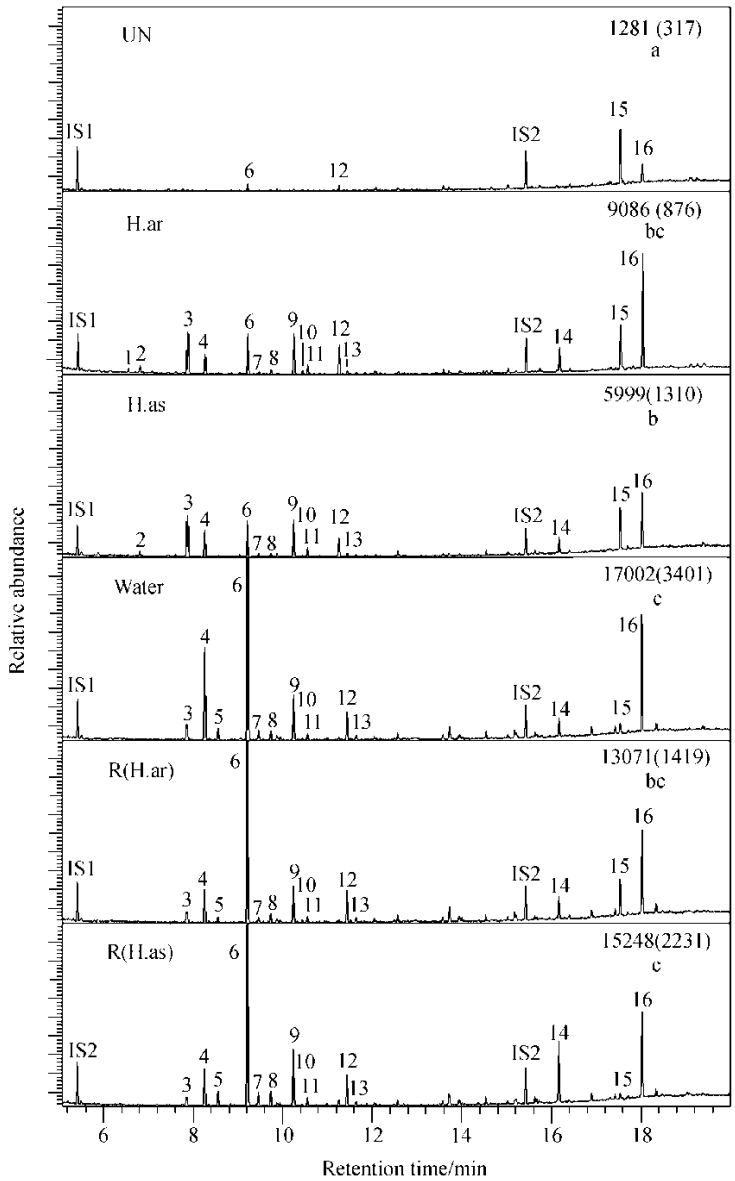

Fig. 2. Representative total ion current chromatograms of the headspace volatiles from tobacco leaves with different treatments: undamaged (UN), infested by *H. armigera* (H. ar) or *H. assulta* (H. as), mechanically damaged and treated with water (Water), or regurgitant of *H. armigera* (R(H. ar)) or *H. assulta* (R(H. as)). Peak numbers correspond with numbers in Table 1. IS1 and IS2 are the internal standards *n*-decane and benzyl acetate. Values in the right-hand corners are mean (n = 5) total amounts (ng·3 leaves^{-1}·12 h^{-1}) of tobacco volatiles and their standard errors. Means with different letters are significantly different (Ducan's new multiple range test after ANOVA, p<0.05).

Plants infested by *H. armigera* or *H. assulta*, or damaged artificially and treated with water or caterpillar regurgitant released largely the same compounds, including (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, 1-hexanol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, (*Z*)-3-hexenyl butyrate, *n*-nonanal, γ-terpinene, 1,4-dichlorobenzene, methyl salicylate, nicotine and the unknown compounds (Fig. 2 and Table 1). However, β-pinene was specifically induced by the infestation of *H. armigera*, and (*Z*)-3-hexenal was only induced by the infestation of *H. armigera* or *H. assulta*, whereas hexyl acetate was only induced by mechanical damage (Fig. 2 and Table 1).

Tobacco leaves infested by *H. armigera* and *H. assulta* released larger amounts of volatiles than undamaged tobacco leaves did (Fig. 2). Tobacco leaves treated with artificial damage plus caterpillar regurgitant or water emitted the same levels of volatiles, which were higher than that emitted by undamaged tobacco leaves (Fig. 2). The total amounts of volatiles emitted from mechanically damaged tobacco leaves are similar to that emitted from

Table 1 Amounts (±SE) of single compounds collected from undamaged and differently treated tobacco leaves[a)]

Code	Compound	Retention time/min	Undamaged and herbivore-infested plants/ng·3 leaves^{-1}·12 h^{-1} b)			Mechanically damaged plants/ng·3 leaves^{-1}·12 h^{-1} b)		
			UN	H. ar	H. as	Water	R(H.ar)	R(H.as)
1	*β*-pinene	6.55	0a	87.62±27.04b	0a	0a	0a	0a
2	(*Z*)-3-hexenal	6.82	0a	595.01±125.40b	146.02±35.50a	0a	0a	0a
3	(*E*)-2-hexenal	7.87	0a	1561.33±386.53c	1012.06±224.95b	256.83±57.47a	145.95±59.53a	189.93±17.01a
4	*γ*-terpinene	8.27	0a	454.58±173.03ab	279.64±138.52ab	746.91±406.24b	257.92±150.36ab	312.86±147.75ab
5	hexyl acetate	8.57	0a	0a	0a	151.26±43.44bc	90.09±17.59b	200.35±44.62c
6	(*Z*)-3-hexenyl acetate	9.23	101.15±29.86a	1083.35±271.45a	709.64±152.53a	8804.17±2862.02b	6782.53±1093.77b	8045.72±1978.00b
7	(*E*)-2-hexenyl acetate[c)]	9.46	0a	68.47±40.38ab	46.39±22.83ab	94.61±57.71ab	37.34±37.34a	159.35±28.22b
8	1-hexanol	9.74	0a	133.47±35.33bc	107.51±11.26ab	245.43±31.75c	247.00±50.72c	498.25±78.88c
9	(*Z*)-3-hexen-1-ol	10.24	0a	1633.13±291.38bc	891.68±172.65ab	1842.43±447.46bc	2007.34±834.87bc	2548.49±698.07c
10	*n*-nonanal[c)]	10.45	0a	106.43±12.17b	35.47±9.82a	47.10±29.96a	44.69±12.23a	30.71±8.87a
11	(*E*) -2-hexen-1-ol	10.56	0a	276.77±92.86b	243.83±48.42b	207.79±29.96b	160.46±43.38b	300.77±61.42b
12	1,4-dichlorobenzene[c)]	11.25	207.11±128.31a	195.00±123.33a	512.17±68.30a	333.49±101.15a	259.35±100.28a	98.30±43.96a
13	(*Z*)-3-hexenyl butyrate[c)]	11.43	0a	140.28±33.84ab	44.25±30.23a	344.42±118.86b	334.89±126.12b	337.04±101.18b
14	methyl salicylate	16.19	0a	268.53±78.74abc	229.46±106.77ab	690.74±143.09c	474.79±71.91bc	653.01±253.68c
15	nicotine	17.55	528.21±266.12a	575.42±183.96a	768.18±386.87a	476.48±152.31a	889.22±329.31a	502.94±124.02a
16	unknown compound	18.03	445.32±12.42a	1906.67±296.77c	972.73±285.02ab	2760.32±468.96d	1340.31±243.17bc	1371.02±207.10bc

a) UN, undamaged; H. ar, infested by *H. armigera*; H. as, infested by *H. assulta*; Water, mechanically damaged and treated with water; R(H.ar), mechanically damaged and treated with regurgitant of *H. armigera*; R(H. as), mechanically damaged and treated with regurgitant of *H. assulta*. b) Different letters on the same line mean significant differences between amounts of single compounds emitted from differently treated plants (Ducan's new multiple range test, $n = 5$; $p<0.05$). c) Compounds were tentatively identified when their mass spectra showed > 90% identity with those of the mass spectra library NIST.

H. armigera infested tobacco leaves, but larger than that emitted from *H. assulta* infested tobacco leaves. The emission amounts of single compounds were also different between differently treated plants (Table 1). The differences were large between herbivore-induced and mechanical damage-induced compounds, and small between *H. armigera*- and *H. assulta*-induced compounds, and among compounds emitted from mechanically damaged plants treated with water or caterpillar regurgitant (Table 1).

3 Discussion

Tobacco plant is considered as one of the model systems for the study of plant-herbivore interactions[27]. Tobacco plants are able to defense herbivores directly and indirectly, by producing nicotine and releasing herbivore-induced volatiles respectively[28,29]. In the current study, *C. chlorideae* was found to be strongly attracted by *H. armigera*- and *H. assulta*-induced tobacco volatiles (Fig. 1). This means tobacco plants could indirectly defense the two insects by releasing herbivore-induced volatiles as attractants for *C. chlorideae*.

A considerable degree of specificity in herbivore-induced plant volatiles has been observed by comparing different plants and herbivore species, as well as different plant ages and the developing stages of the herbivore[11−13]. Exploitation of this specificity has also been documented in several parasitoids[11,12,30]. For example, the specialist parasitoid *Cardiochiles nigriceps* exploited herbivore-specific plant volatiles to distinguish tobacco plants infested by its host *Heliothis virescens* from that infested by non-host *Helicoverpa zea*[12]. However, the degree at which natural enemies make use of specific differences in volatile blends depend on their dietary specialization and/or their host/prey species[31]. Specialists more frequently use specific cues, whereas generalists more frequently use general cues for host location[32]. The use of general chemical cues present in all hosts or their respective food plants is considered to be an adaptive strategy for generalist parasitoids[31,33]. In tobacco, the same volatile compounds could be induced by *H. virescens*, *H. zea* and *Manduca sexta*[34]. Tobacco plants fed on by herbivores with different feeding habits could also result in similar volatile emissions and similar attraction to *Geocoris pallens*[29,35]. Several single compounds, (*Z*)-3-hexen-1-ol, linalool and *cis*-*a*-bergamotene, were sufficient to attract *G. pallens* when they were used individually[29]. In the present study, a majority of the same compounds could be induced by *H. armigera* and *H. assulta* (Fig. 2 and Table 1), and *C. chlorideae* could be attracted by both blends of herbivore-induced volatiles. This indicates that the generalist parasitoid may use compounds induced commonly by the two insects as host foraging cues. Interestingly, mechanically damaged plants, whether treated with caterpillar regurgitant or water, attracted more parasitoids than undamaged plants did (Fig. 1). This strongly suggests that *C. chlorideae* uses wound-induced general compounds, such as some green leaf volatiles or related compounds, as attractive cues.

Damaged plants typically release a blend of green leaf volatile compounds immediately from ruptured plant cells, which are products (six-carbon aldehydes, alcohols, and acetates) of the breakdown of lipids through the fatty acid/lipoxygenase pathway[36,37]. Green leaf volatiles are probably the most common volatiles released by plants damaged by herbivores[38]. Many parasitoids have been found to be attracted by green leaf volatiles. For example, the parasitoid *Cotesia marginiventris* was strongly attracted by odors released by freshly damaged cotton and cowpea plants (mainly green leaf volatiles)[38,39]. In a flight tunnel test the parasitoids *Microplitis croceipes* and *Netelia heroica* were found to be attracted by green leaf volatiles, especially by different hexenols and hexenals[40]. In a Y-tube olfactometer, the parasitoid *Aphidius rhopalosiphi* strongly responded to (*Z*)-3-hexenyl acetate and (*E*)-2-hexenal[41]. In our current study, more than half of herbivore-induced compounds were green leaf volatiles (Fig. 2 and Table 1). Particularly, compounds (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, (*Z*)-2-hexenyl acetate, 1-hexanol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol and (*Z*)-3-hexenyl butyrate were shared among blends of herbivore-induced and mechanical damage-induced tobacco volatiles. These compounds were not or only released in trace amounts by undamaged plants., thereby implying they function to attract *C. chlorideae*.

Nicotine is one of the most broadly effective plant defense metabolites, in that it poisons acetocholine receptor and thereby is toxic to all heterotrophs with neuromuscular junctions. Nicotine plays a defensive role to some opportunistic and generalist herbivores. However, insects feeding on tobacco usually have adaptive mechanisms to tolerate or detoxify this compound. For example, tobacco-feeding coleopterans and orthopterans can metabolize nicotine to cotinine or other alkaloids[42]. The aphid, *Myzus persicae* (Sulz.) avoids nicotine by selectively feeding in the phloem[43], and *M. sexta* rapidly excretes most of the nicotine it ingests[44]. The defensive use of nicotine against nicotine-adapted herbivores will not be profoundly effective. On the contrary, this toxin may be sequestered by specialist herbivores for their own defense against their parasitoids and predators. For example, *M. sexta* larvae are thought to use dietary nicotine against larval parasitoid, *Cotesia congregata*[45]. Therefore, the ecological complexity of chemical defense may make certain combinations of direct and indirect defenses incompatible. It seems that tobacco plants can tailor their defense responses to different herbivores, and optimize the defenses against nicotine-adapted herbivores by integrating the deployment of direct and indirect defenses. For example, when it was fed on by *M. sexta*, tobacco plants

suppressed the accumulation of nicotine, but not the emission of volatiles[46]. A burst of *M. sexta*-induced ethylene, which suppresses the wound-induced accumulation of nicotine biosynthetic genes *NaPMT1* and *NaPMT2*, explained the attenuated nicotine response[46,47].

Both *H. armigera* and *H. assulta* are nicotine-adapted insects. No effects could be found on the growth of *H. armigera* and *H. assulta* when they were fed with artificial diets containing 0.5% nicotine[48]. At present, we know nothing about the effects of nicotine sequestered by host insects on *C. chlorideae*, but the ingestion of another toxic secondary substance gossypol by host *H. armigera* was clearly detrimental to the parasitoid[49]. In previous experiments, we have demonstrated that when tobacco plants were fed on by *H. armigera* and *H. assulta*, or when the two insects' oral secretions were applied to leaf punctures, a suppression of the accumulation of nicotine occurred[50]. Based on the previous and current results, we speculate that the emission of herbivore-induced plant volatiles and the suppression of the accumulation of nicotine might be an adaptive strategy for tobacco defending against nicotine-adapted *H. armigera* and *H. assulta*. Based on field experiments, Kester et al. have demonstrated that the pressure exerted by natural enemies is more important than the impact of nicotine in determining feeding site location of nicotine-tolerant *M. sexta* and *Manduca quinquemaculata*[51].

In China, the parasitoid *C. chlorideae* mainly distributes in the Yellow River Valley and the Yangtze River Valley, giving rise to 8—10 generations per year[23,52]. The parasitoid preferentially lays eggs in the second and the third instar larvae of *H. armigera*[52,53]. Parasitism by *C. chlorideae* greatly deters the development and reduces the consumption of the host larvae within 2—3 d[53], and totally disables their feeding ability after one week. So most parasitized *H. armigera* larvae die before they molt into voracious stages, and their consumption is only 20% of that of unparasitized larvae[23], which could be translated into a fitness benefit to the plant. Therefore, it is possible to exploit *C. chlorideae* against some noctuid species in biological control programs. Techniques for mass rearing of the parasitoid are being developed[54]. The current study gives evidence in support of the potential use of induced volatiles in improving the foraging efficacy of the parasitoid.

In summary, the direct function of herbivore-induced PIs and PPO in defending against *H. armigera* and *H. assulta* may occure, but the attraction of herbivore-induced plant volatiles to *C. chlorideae* should be also an adaptive strategy for tobacco plants to defend the two nicotine-adapted insects. Moreover, it was reported that the emission of herbivore-induced tobacco volatiles could cause a reduction in oviposition rates of moths[29,34], which implies that a direct defensive function of volatiles also occurs. That plant releases volatiles in response to herbivory seems to be an adaptive strategy, suggesting that the artificial regulation of the emissions of plant volatiles may have agricultural uses[35]. The problem as to whether or not the emission of *H. armigera*- and *H. assulta*-induced tobacco volatiles repel the adults of the two species is under investigation.

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