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ORIGINAL PAPER

A chemical basis for different oviposition rates of *Helicoverpa assulta* across two *Nicotiana* species

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Abstract

The tobacco *Nicotiana rustica* is widely used as a trap crop in the fields of *Nicotiana tabacum* in China, by attracting oviposition of *Helicoverpa assulta* females, thus preventing damage to *N. tabacum*. The mechanism underlying the differential oviposition rates of *H. assulta* across these two tobacco species, however, is largely unknown. We investigated the mechanism of host plant acceptance of *H. assulta* with respect to these two tobaccos by using a two-choice behavioral bioassay and GC–MS. Our results indicate that both the leaves and inflorescences of *N. rustica* attracted significantly more eggs than the corresponding parts of *N. tabacum*. Extracts of leaves and inflorescences of *N. rustica* with two different solvents elicited similar oviposition patterns to the corresponding parts of the plants. Chemical analysis by GC–MS revealed that the volatiles of *N. rustica* contain larger amounts of nicotine than those of *N. tabacum* at the flowering stage. In addition, γ-terpinolene and β-elemene are found only in extracts of *N. rustica*. A two-choice bioassay on the individual compounds showed that γ-terpinolene, which is specific to the vegetative stage of *N. rustica*, and nicotine attracted oviposition by *H. assulta*. The volatile β-elemene, which is present only in *N. rustica*, was also attractive. We conclude that the larger amount of nicotine, and the species-specific γ-terpinolene and β-elemene may mediate the different oviposition rates of *H. assulta* females across *N. rustica* and *N. tabacum*.

Keywords *Helicoverpa assulta* · *Nicotiana rustica* · *Nicotiana tabacum* · Volatile · Oviposition attraction

Introduction

Helicoverpa assulta (Guenée) is an oligophagous pest that uses several Solanaceae species as its host plants, including tobacco and hot pepper (Wu et al. 2018). The larvae of *H. assulta* cause severe yield reductions and subsequent economic losses in tobacco fields in China (Sun et al. 2012). Currently, *Nicotiana tabacum* L. is the main cultivated tobacco species for the production of flue-cured tobacco, and many varieties are used, including Zhongyan 100, NC89, and K326 (Zhong 2019). Another tobacco species, *Nicotiana rustica*, is planted across a considerably smaller scale than *N. tabacum*. Long-term field surveys indicated that 6–20 times more eggs were deposited on *N. rustica* than on *N. tabacum* plants by *H. assulta* and *H. armigera* (Luo et al. 2006; Xue et al. 2009). In 2000, a total of 15,120 eggs was found per 100 plants of *N. rustica*, while only 42 eggs were found on a variety of *N. tabacum*, RG17, in a tobacco field in Yiyang County, Henan Province, China (N34° 37′, E112° 05′) (Jiang et al. 2003). Therefore, *N. rustica* could be used as a trap crop for attracting oviposition of *H. assulta* females in tobacco fields (Long et al. 2012).

The life cycle of the female insect involves them searching for suitable sites to lay eggs (Jones et al. 2019). Plant volatiles are critical cues by which lepidopteran moths locate suitable hosts for feeding and oviposition (Morris et al. 2005; Reisenman et al. 2013; Renwick and Chew 1994; Zhang et al. 2011; Rajapakse and Walter 2007). Volatile compounds from tobacco plants affect female *H. assulta* behavior in wind tunnels (Sun et al. 2012) and oviposition site selection (Xue et al. 2009). However, the mechanism driving

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the differential oviposition rates by *H. assulta* females across these tobacco species has not been well characterized.

The most consistent pattern in the host associations of all *Helicoverpa* species is a strong attraction to the flowering stage of their hosts (Fitt 1989). This pattern is also true for *H. assulta* (Sun et al. 2012). In Henan Province, China, the peak oviposition of *H. assulta* occurs from late June to early July, in synchrony with the seedling and flowering stages of *N. rustica* plants (Jiang et al. 2003), during which 88.7% and 94.4% of eggs were deposited on reproductive organs in a seed-breeding tobacco field in June and July, respectively (Guo et al. 1995). Thus, we hypothesize that the different oviposition rates of *H. assulta* females across *N. rustica* and *N. tabacum* may be explained by some specific volatiles in flowering *N. rustica* plants.

In this study, we selected two varieties of each tobacco species and investigated the chemical basis for the differential oviposition rates of *H. assulta* females across the two *Nicotiana* species. We used oviposition bioassays and gas chromatography–mass spectrometry (GC–MS) analysis.

Materials and methods

Insects

Helicoverpa assulta were originally collected as mature larvae from a tobacco field on Xuchang campus, Henan Agricultural University. The larvae were reared on an artificial diet (Ahn et al. 2011) under controlled conditions at 27 ± 1 °C and 75 ± 5% relative humidity under a 16 h day: 8 h night cycle. The pupae were sexed and placed separately in cages. Adults were provided with 10% sucrose solution on eclosion. *H. assulta* used in this study had been maintained for at least 10 generations in the laboratory.

Plants

Two representative varieties of *N. rustica,* "Hanxiaoyan" (abbreviated to "Han") and "Xianfengxiaolanhua" (abbreviated to "XF"), donated by the Institute of Tobacco, Chinese Academy of Agricultural Sciences, and two conventional cultivars of *N. tabacum*, "Zhongyan 100" (abbreviated to "ZY") and "K326," donated by the National Tobacco Cultivation Physiological and Biochemical Research Centre, were used in this experiment. All tobacco seedlings were grown in a greenhouse (25 ± 1 °C) regulated using an air conditioner. Potted plants that had 5–6 fully expanded true leaves were transplanted outside on May 5, 2018. These plants were carefully reared with no mechanical or pest damage, and no nutrient or drought stress.

Relative oviposition rates (leaves and inflorescences) across the tobacco treatments

Ovipositional responses of *H. assulta* to different tobacco varieties were examined with two-choice bioassays in cylindrical screened cages (50 cm diameter × 50 cm height). Terminal leaves and inflorescences were examined separately. The terminal leaves of different tobacco varieties were obtained by cutting each one at the base of the petioles (two leaves per variety in each replicate). Each leaf was placed in a conical flask (15 cm height) containing water to prevent leaf wilting (Thöming et al. 2013; Proffitet al. 2015), with the mouth of the flask tightly sealed with parafilm around the petiole. Each flask was positioned at the edge of the cage equidistant from one another (Fig. 1). Then, three females and five males, aged 3 days after eclosion, were released into the cage at 1900 h. A Petri dish (6 cm inner diameter × 1 cm height) containing cotton wicks saturated with 10% sucrose solution was provided in the center of the bottom to feed the moths. For the inflorescence experiment, the method used was similar, by replacing the leaves with inflorescences. The following morning, the number of eggs that had been deposited on each plant part was counted. Between replicates, the cages were cleaned carefully with alcohol and water to avoid the influence of residual odor on the next test. The treatment groups ($n = 10$/treatment) were as follows:

(1) XF leaves *vs*. K326 leaves;
(2) XF leaves *vs*. ZY leaves;
(3) Han leaves *vs*. K326 leaves;
(4) Han leaves *vs*. ZY leaves;
(5) XF inflorescences *vs*. K326 inflorescences;
(6) XF inflorescences *vs*. ZY inflorescences;
(7) Han inflorescences *vs*. K326 inflorescences; and
(8) Han inflorescences *vs*. ZY inflorescences.

Relative oviposition rates (leaf and inflorescence extracts) across the tobacco treatments

The extraction of terminal leaves and inflorescences of the two tobacco species was performed with two solvents of different polarities, i.e., *n*-hexane or dichloromethane. One plant part was dipped into *n*-hexane and another, equivalent one, into dichloromethane, by dipping samples for 72 h at room temperature (27 ± 1 °C) with 1 g equivalent of whole leaves or inflorescences for 1 mL of solvent. Each pair of treatments was replicated ten times. When testing, four filter paper disks (1.5 cm diameter) dipped in each extract (20 µL per disk) (two disks per species in each replicate) were used as the odor sources. The bioassay to

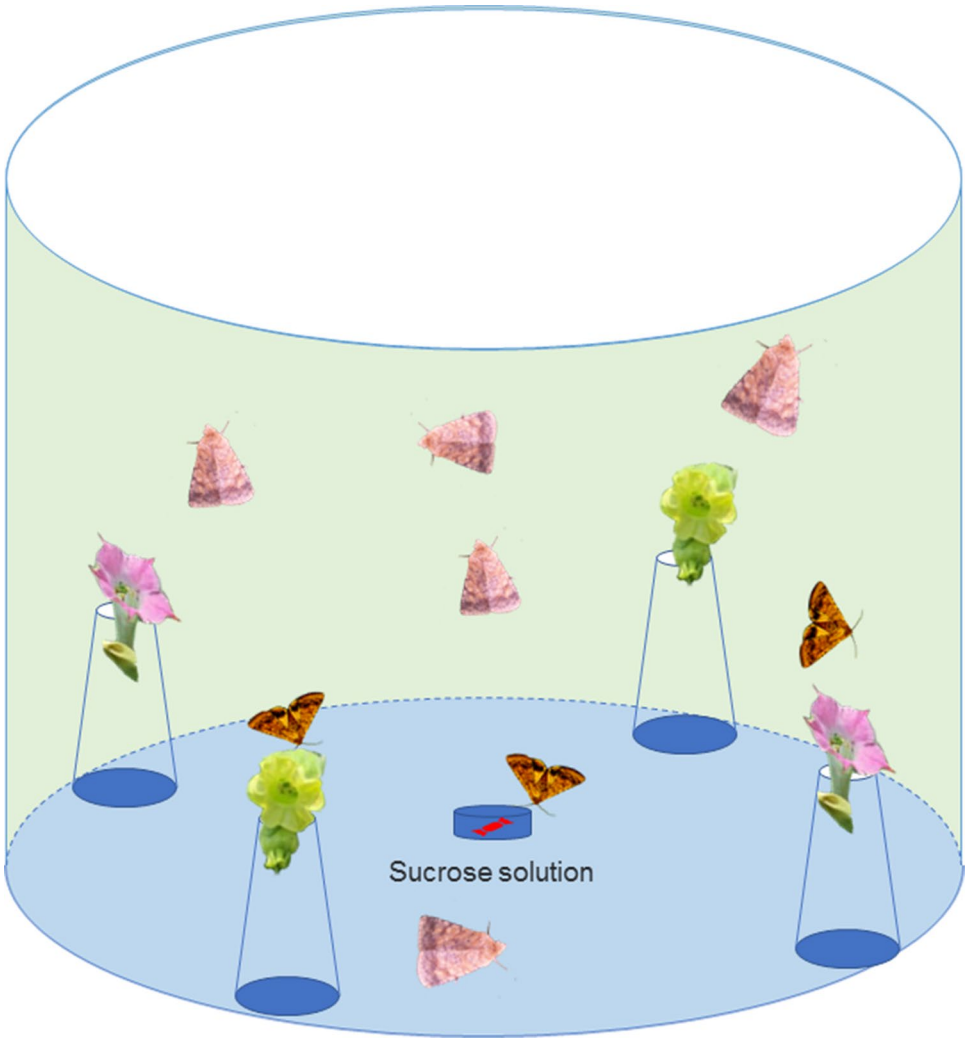

Fig. 1 Schematic drawing of the oviposition assay. Yellow flowers represent the leaves or inflorescences of *N. rustica*, red flowers represent those of *N. tabacum*. A 10% sucrose solution (w:v) was provided as a diet for the moths

test the extracts was similar to that mentioned above, by replacing the leaves with the odor sources (filter paper disks) and the conical flasks covered with cotton gauze to serve as an oviposition substrate, and the odor source placed on the cotton gauze.

Collection and GC–MS analyses of tobacco headspace volatiles

Tobacco headspace volatiles were collected by a dynamic air entrainment system (PYE volatile collection kit, Kings Walden, Herts, UK) integrated with an air cleaning unit (charcoal filters). For each tobacco variety, the above-ground part of an intact plant in the vegetative or flowering stage was enclosed in a PET (polyethylene terephthalate) oven bag (48 cm × 60 cm, EasyOven kitchensources Ltd.) with parafilm around the stem base. Air flow was maintained at 600 mL/min for 12 h. Volatiles were trapped in an adsorbent tube (6 mm × 150 mm) filled with 100 mg Tenax TA adsorbent resin (Zhengzhou Puxi Technology Ltd). Before each collection, the adsorbent was cleaned using HH-10 type adsorbent reactivator (Zhengzhou Puxi Technology Ltd). After collection, the adsorbent tube was eluted with 2 mL *n*-hexane (chromatographic purity, 98%, J. T. Baker, USA), and the extractant was condensed to 100 µL by blowing pure nitrogen over it. A total of eight samples were obtained from the headspace of each tobacco variety at each growth stage.

Chemical analyses were conducted on an Agilent 7890 B GC + 5977 B MS system equipped with an HP5 ms column (30 m × 250 µm × 0.25 µm). Helium was used as a carrier gas. The oven temperature was programmed as follows: 40 °C for 1 min; 5 °C/min to 150 °C and then held for 1 min; 10 °C/min to 250 °C and then held for 5 min. The temperatures of the injector and the transfer line were set to 250 °C. A 1 µL sample was injected at a 10:1 split ratio. The ionization source was EI, operating at 250 °C and scanning from 1.2 to 1100 amu. Compounds were identified by comparing mass spectra with NIST library spectra (Agilent Technologies, USA) and further confirmed by comparing other reports about tobacco volatiles (Raguso et al. 2003; Sun et al. 2012) and standard compounds.

Relative oviposition rates across individual tobacco volatiles or blends of volatiles

Based on the results of the GC–MS analysis as well as the principal component analysis, individual volatile compounds were selected for examination at two concentrations (0.001 mol/L, and 0.01 mol/L, dissolved in paraffin oil), using the bioassay method described above.

In the bioassay for each volatile compound, four filter paper disks (1.5 cm diameter) were used, two of them were impregnated with the compound (20 µL per disk), and the other two were impregnated with paraffin oil (20 µL per disk). Each was placed centrally on the cotton gauze that covered each of the four conical flasks, and the conical flasks were placed across from one another, as in Fig. 1. Each chemical compound at each concentration was replicated 7–14 times.

According to the peak area ratio of each compound in GC–MS analysis, blends of these compounds were prepared for oviposition bioassay. The blends, with a total of 1 mL, were embedded in 10 mL of 2% agar pectin. With respect to the blends obtained from the vegetative stage or flowering stage plants, oviposition rates were examined across the *N. rustica* varieties and *N. tabacum* varieties, as detailed for the oviposition attraction tests described above. Each pair of blends was replicated 10–12 times.

Data analysis

The significance of the mean number of eggs in the two-choice bioassays in each trial was analyzed by a paired *t*-test. In those cases in which the normality assumption could not be met, a non-parametric Mann–Whitney *U*-test was used. For all statistical tests, the results were considered significant at $P \leq 0.05$ and $P \leq 0.01$. Principal component analysis (PCA) was performed on a correlation matrix for the visual comparison of the headspace volatile profiles across the two tobacco species during each stage. The proportion of each compound in the blends of volatiles was quantified based on the percentage of the peak area of the GC–MS analysis. All statistical analyses were conducted using SPSS 19.0 for Windows.

Results

Relative oviposition rates of *H. assulta* on leaves and inflorescences across tobacco treatments

In all experiments, significantly more eggs were deposited by *H. assulta* on *N. rustica* leaves and inflorescences compared with the corresponding paired *N. tabacum* plants (leaves: $t_{\text{XF vs. K326}} = 11.55$, $t_{\text{XF vs. ZY}} = 6.16$, $t_{\text{Han vs. K326}} = 6.03$, $t_{\text{Han vs. ZY 100}} = 6.71$, $P < 0.01$. Inflorescences: $t_{\text{XF vs. K326}} = 12.33$, $t_{\text{XF vs. ZY}} = 5.09$, $t_{\text{Han vs. K326}} = 5.05$, $t_{\text{Han vs. ZY}} = 7.64$, $P < 0.01$) (Fig. 2, Table S1).

Relative oviposition rates of *H. assulta* on the extract treatments across the two tobacco species

Significantly more eggs were deposited on the dichloromethane leaf extracts from *N. rustica* varieties compared with those from *N. tabacum* varieties ($t_{\text{XF vs. K326}} = 6.16$, $t_{\text{Han vs. K326}} = 3.67$, $U_{\text{Han vs. ZY}} = 2.81$, $P < 0.01$), except for the comparison of XF with ZY ($t = 0.28$, $P > 0.05$) (Fig. 3a, Table S2).

The number of eggs laid on the dichloromethane inflorescence extract of one *N. rustica* variety (XF) was not significantly different from that of the two *N. tabacum* varieties ($t_{\text{XF vs. K326}} = 0.89$, $t_{\text{XF vs. ZY}} = 1.39$, $P > 0.05$). In contrast, significantly more eggs were laid on the inflorescence extracts of the other *N. rustica* variety (Han) compared with the two *N. tabacum* varieties ($t_{\text{Han vs. K326}} = 2.63$, $P < 0.05$; $t_{\text{Han vs. ZY}} = 3.35$, $P < 0.01$) (Fig. 3b, Table S2).

The statistical results of the leaves extracted by *n*-hexane were similar to those of the leaf bioassay in Fig. 2. In other words, significantly more eggs were laid on extracts from *N. rustica* varieties than on those from *N. tabacum* varieties ($t_{\text{XF vs. K326}} = 2.61$, $P < 0.05$; $t_{\text{XF vs. ZY}} = 4.10$, $t_{\text{Han vs. K326}} = 3.37$, $t_{\text{Han vs. ZY}} = 6.06$, $P < 0.01$) (Fig. 3c, Table S2).

Significantly more eggs were laid on the *n*-hexane inflorescence extracts from *N. rustica* varieties than on those from *N. tabacum* varieties ($t_{\text{XF vs. K326}} = 3.03$, $P < 0.05$; $t_{\text{XF vs. ZY}} = 8.30$, $P < 0.01$; $t_{\text{Han vs. ZY}} = 2.72$, $P < 0.05$), except for the comparison across Han and K326 ($t = 0.10$, $P > 0.05$) (Fig. 3d, Table S2).

Identification of the volatiles from the two tobacco species

The identity retention time and relative peak area of volatiles identified from tobacco by GC–MS are shown in Table 1 (see also Figs. S1–S9, Table S5). Compounds commonly associated with the earth's atmosphere, as well as compounds associated with the analytical system (e.g., toluene, benzene, siloxanes, and phthalates) were excluded from the list (Warneke et al. 2001; Jansen et al. 2009; Megido et al. 2014). D-limonene was always the most abundant volatile emitted from both *N. tabacum* varieties, vegetative or flowering stage (86.1% and 80.0% in the K326 vegetative and flowering stages, and 83.7% and 81.2% in the ZY vegetative and flowering stages, respectively). At the flowering stage, the most striking difference in volatiles across the *N. rustica* and *N. tabacum* species was that the *N. rustica* varieties emitted much more nicotine than *N. tabacum* varieties.

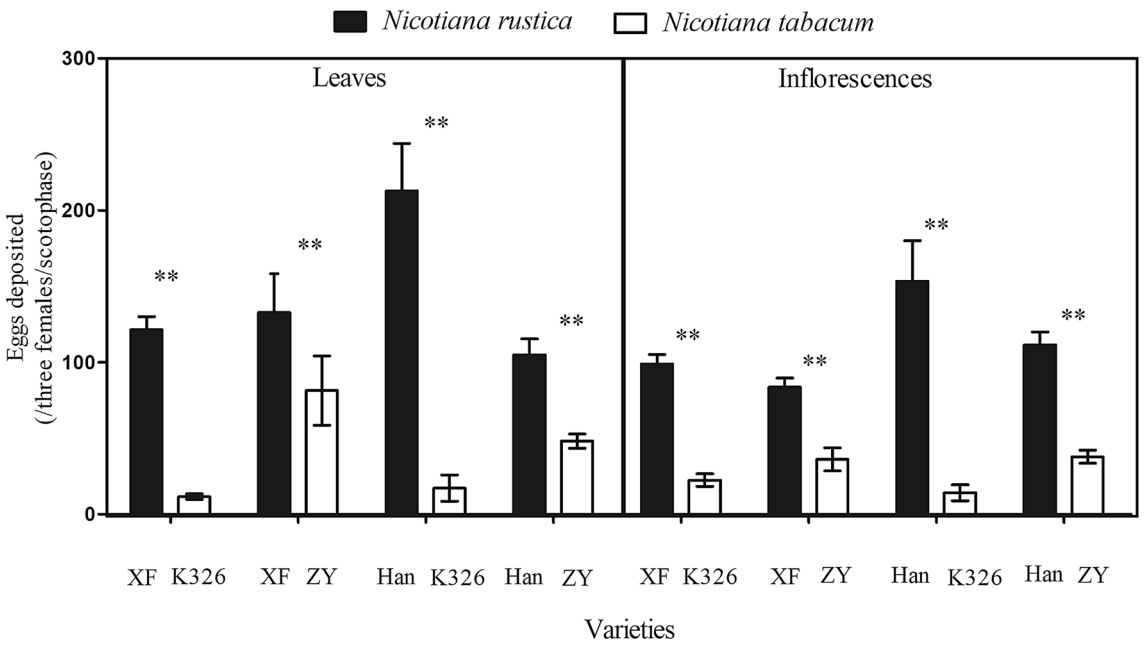

Fig. 2 Eggs deposited by *H. assulta* under dual-choice conditions of leaves (left) or inflorescences (right) across *N. rustica* varieties and *N. tabacum* varieties, as specified on the *x*-axis. **Above bars indicate a significant difference in number of eggs deposited across the two test substrates at the $P = 0.01$ level based on a paired *t*-test ($n = 10$)

Volatiles emitted from the *N. rustica* vegetative stage exhibited a relatively large variation across the two varieties.

Principal component analysis based on the GC–MS data was used to identify the specific volatiles in *N. rustica* that may be responsible for attracting relatively more oviposition by mated *H. assulta* females. In the vegetative stage, β-phellandrene, β-elemene, and γ-terpinolene were specific to the *N. rustica* varieties. Salicylaldehyde, acetophenone, benzyl acetate, naphthalene, methyl salicylate, β-pinene, β-myrcene, *p*-cymene, eucalyptol, D-limonene, linalool, caryophyllene oxide, nonanal, decanal, and nicotine were specific to the *N. tabacum* varieties. The shared components in the vegetative stage included 3-hexanol, 3-carene, and β-caryophyllene (Fig. 4, left, Table 1). In the flowering stage, salicylaldehyde, 3-carene, and isopentyl butyrate were specific to the *N. tabacum* varieties. The specific components in flowering *N. rustica* varieties included 3-hexanol, (*Z*)-3-hexen-1-ol, benzaldehyde, benzyl alcohol, benzeneactaldehyde, ethyl benzoate, α-pinene, geraniol, (*E*)-*β*-ocimene, ketoisophorone, β-elemene, (*E*)-*β*-caryophyllene, nonanal, ethyl caprylate, decanal, (*Z*)-3-hexenyl acetate, nicotine, dodecanal, dhelwangin, and pentanoic acid. The shared components in the flowering stage included linalool, naphthalene, D-limonene, benzyl acetate, and *p*-cymene (Fig. 4, right, Table 1). Intraspecific volatile variations in the vegetative stage of *N. tabacum* and the flowering stage of *N. rustica* were larger than those in the vegetative stage of *N. rustica* and flowering stage of *N. tabacum*, respectively, as shown in the distribution of scatter points in Fig. 4 and Table 1.

Relative oviposition rate of *H. assulta* on the blends of volatile compounds across the two tobacco species

To test further whether any of these volatiles play a role as chemical signals for attracting oviposition by *H. assulta* females among these tobacco varieties, we selected 26 individual compounds (Table S6) and prepared the blends of these compounds according to the GC–MS and PCA analyses of different tobacco varieties (Fig. 4).

The oviposition rates of *H. assulta* in relation to the blends of these compounds from the vegetative stage were similar to those of the *n*-hexane leaf extracts in Fig. 3c as well as those of the leaf bioassay in Fig. 2. In summary, significant more eggs were laid on the blends from *N. rustica* varieties than on those from *N. tabacum* varieties ($t_{\text{XF } vs.\ \text{K326}} = 4.55$, $t_{\text{XF } vs.\ \text{ZY}} = 5.17$, $t_{\text{Han } vs.\ \text{K326}} = 7.08$, $t_{\text{Han } vs.\ \text{ZY}} = 4.29$, $P < 0.01$) (Fig. 5, left panel, Table S3). Unexpectedly, more eggs were deposited on the blends of both varieties of *N. tabacum* than those of *N. rustica* at the flowering stage, that is, in three of four cases, the blends of flowering stage volatiles from *N. tabacum* varieties exhibited stronger ovipositional attraction to *H. assulta* females

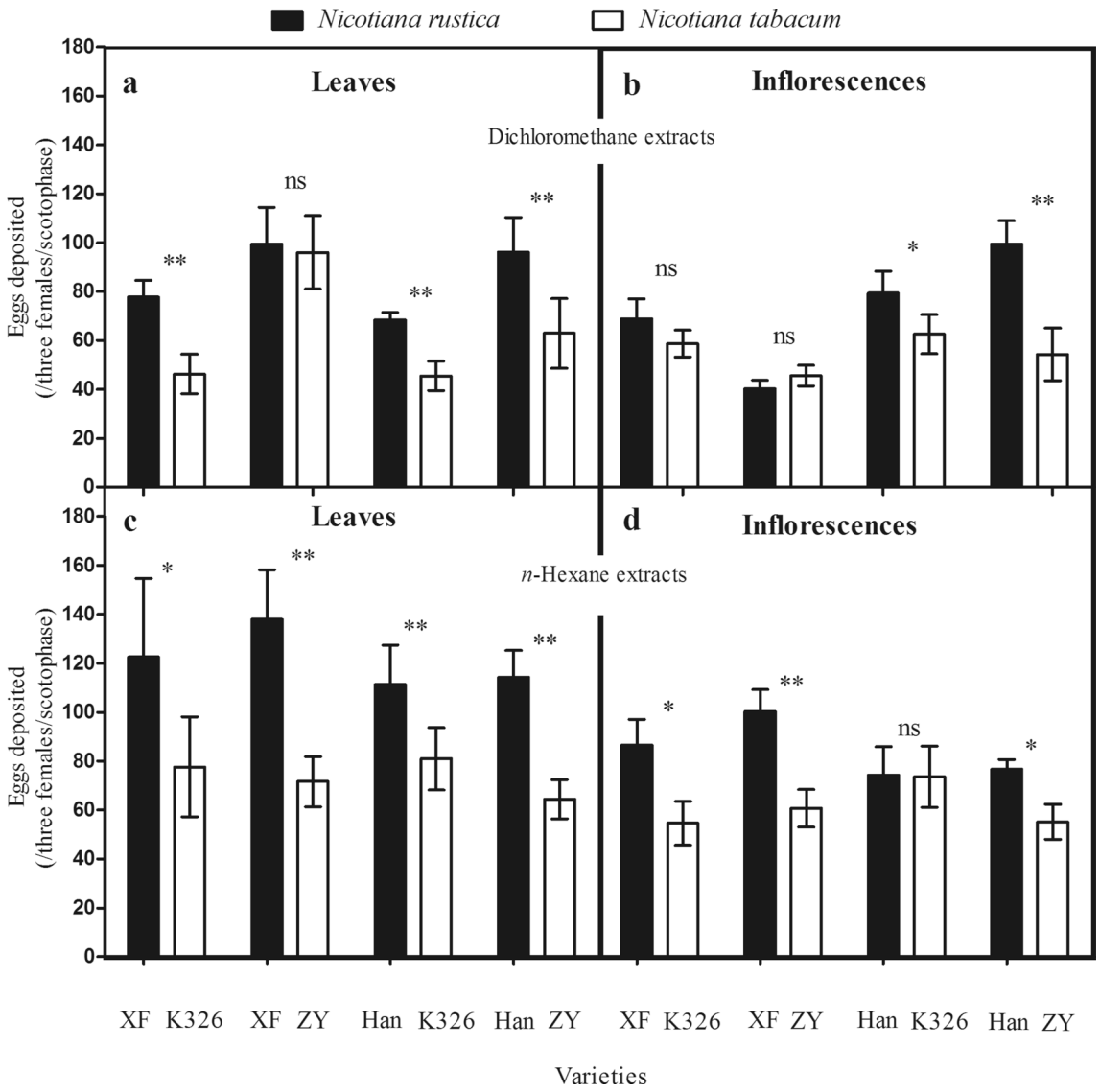

Fig. 3 Eggs deposited by *H. assulta* across two extracts, presented simultaneously, from different varieties of *N. rustica* and *N. tabacum:* **a** dichloromethane extracts of leaves, **b** dichloromethane extracts of inflorescences, **c** *n*-hexane extracts of leaves, and **d** *n*-hexane extracts of inflorescences. In the comparison of dichloromethane extracts of leaves across Han and ZY, the data did not meet the assumptions of the paired *t*-test, so the Mann–Whitney *U*-test was used, and the difference was significant at the $P=0.05$ level. In all other cases, ** and *above the bars indicate significant differences at the $P=0.01$ and $P=0.05$ levels, respectively, and "ns" indicates no significant difference based on a paired *t*-test ($n=10$)

($t_{\text{XF }vs.\text{ ZY}}=2.42$, $P<0.05$; $t_{\text{Han }vs.\text{ K326}}=8.79$, $t_{\text{Han }vs.\text{ ZY}}=5.16$, $P<0.01$) (Fig. 5, right panel, Table S3).

Ovipositional response of *H. assulta* to individual tobacco volatiles

To verify which volatile compounds determined oviposition attraction, we calculated the oviposition preference index (OPI) of the females with respect to different odor sources at the concentrations of 0.01 mol/L and 0.001 mol/L. In our two-factor analysis of variance of odors, we found both the odor and the concentrations exhibited significant effects on the OPIs of the females. Therefore, we analyzed the differences across the OPIs at different concentrations (at concentration of 0.01 mol/L, $F_{25,194}=4.936$, $P<0.01$; at 0.001 mol/L, $F_{25,200}=4.840$, $P<0.01$). Our results indicated that most of the individual tobacco volatiles detered oviposition by *H. assulta* at the two test concentrations (including decanal, caryophyllene oxide, β-caryophyllene, linalool, ketoisophorone, D-limonene, 3-carene, benzyl acetate, benzyl alcohol, phenylacetaldehyde, benzyl dehyde, and (*Z*)-3-hexen-1-ol).

Table 1 Volatile compounds identified from different varieties of *N. rustica* and *N. tabacum* at different growth stages

Compounds	Rt (min)	Relative peak area (%)*							
		N. rustica Han		*N. rustica* XF		*N. tabacum* K326		*N. tabacum* ZY	
		Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage
Green leaves									
3-Hexanol	4.40	15.38	0.66		2.44	0.56		0.51	
(*Z*)-3-hexen-1-ol	5.75		0.57		1.60				
(*Z*)-3-hexenyl acetate	15.93				1.60				
Aromatic compounds									
Benzaldehyde	8.53		11.30		9.50				
Benzyl alcohol	8.93		1.66		2.35				
Benzeneactaldehyde	9.23		1.60		3.02				
Salicylaldehyde	10.97					1.46	1.54	1.20	1.89
Acetophenone	11.66							0.38	
Ethyl benzoate	12.84		0.81						
Benzyl acetate	14.56				0.92	1.57	2.78	1.57	2.98
Naphthalene	15.08		0.42		1.43	0.64	2.25	0.51	2.23
Methyl salicylate	15.43					1.27		0.72	
Terpenoids									
α-Pinene	7.80				2.18				
Geraniol	8.70		0.42						
β-Pinene	9.03					0.37			
β-Ocimene	9.36		0.36						
β-Myrcene	9.47					1.87		1.81	
3-Carene	10.01			29.41		0.52	5.73	0.51	4.56
p-Cymene	10.44				1.26		5.35	3.87	5.64
Eucalyptol	10.65					0.37			
D-limonene	10.59		1.59		36.05	86.13	80.00	83.74	81.24
γ-Terpinolene	11.05	50.00							
Ketoisophorone	12.06		0.33						
β-Phellandrene	12.13	34.62		26.47					
Linalool	12.68				1.26	1.20	1.36	1.68	1.46
β-Elemene	18.76		4.49	20.59	1.76				
β-Caryophyllene	21.57		10.43	23.53	5.71			0.48	
Caryophyllene oxide	25.65							0.41	
Others									
Isopentyl butyrate	11.39						0.98		
Nonanal	12.81		0.30		1.68	0.79		0.62	
Ethyl caprylate	13.57		0.54						
Decanal	15.74		0.30		1.43	0.54		0.41	
Nicotine	19.59		59.46		22.60	2.80		1.57	
Dodecanal	21.20		0.81		1.60				
Dhelwangin	21.27		3.59						
Pentanoic acid	6.47				1.60				

*Relative peak area indicates the ratio of each peak area in the sum of the peak areas of identified compounds

Only nonanal and γ-terpinolene showed positive oviposition attractiveness at both test concentrations. Meanwhile, nicotine and naphthalene exhibited repellent effects on the oviposition of *H. assulta* at a concentration of 0.01 mol/L and attractive effects at a lower concentration (0.001 mol/L). By comparison, the OPI of nicotine was the largest, followed by nonanal, γ-terpinolene, naphthalene, and ethyl benzoate, but the differences among them were not significant (Fig. 6, Table S4).

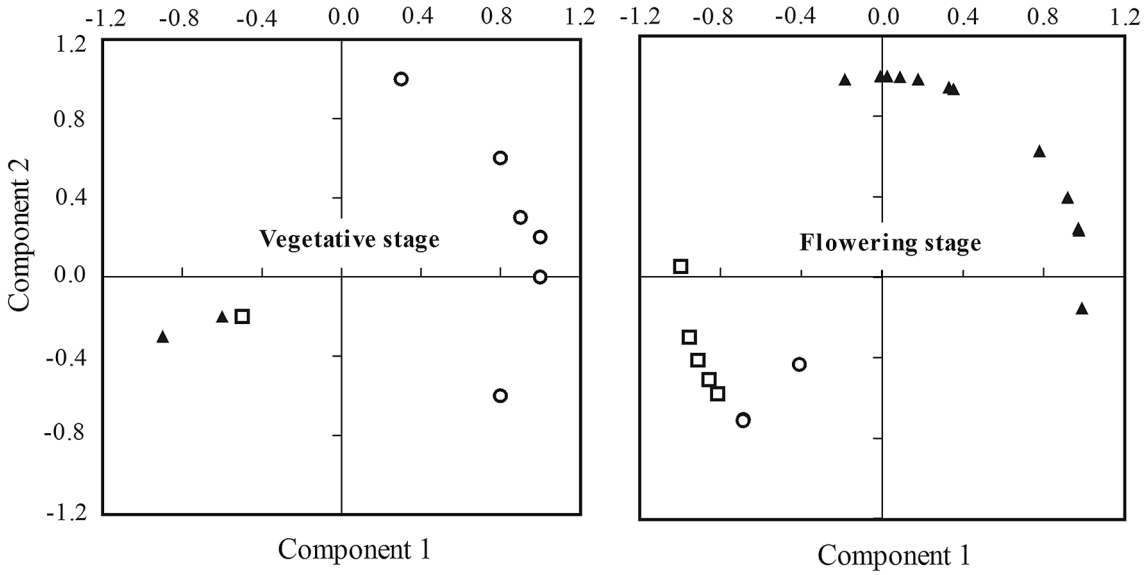

Fig. 4 PCA plots of headspace odor profiles of two tobacco species at the vegetative stage (left) and flowering stage (right). Triangles and circles indicate the volatiles from *N. rustica* varieties and *N. tabacum* varieties, respectively, and squares represent shared eigenvalues of these two species. All compounds identified in Table 1 were included in the analysis. It should be noticed that the coordinate values of some scatters are same or similar and difficult to distinguish from each other by the naked eye. For example, in the left panel, the "square" included 3-hexanol ($x=-0.495$, $y=-0.175$), 3-carene ($x=-0.548$, $y=-0.211$), and β-caryophyllene ($x=-0.559$, $y=-0.197$)

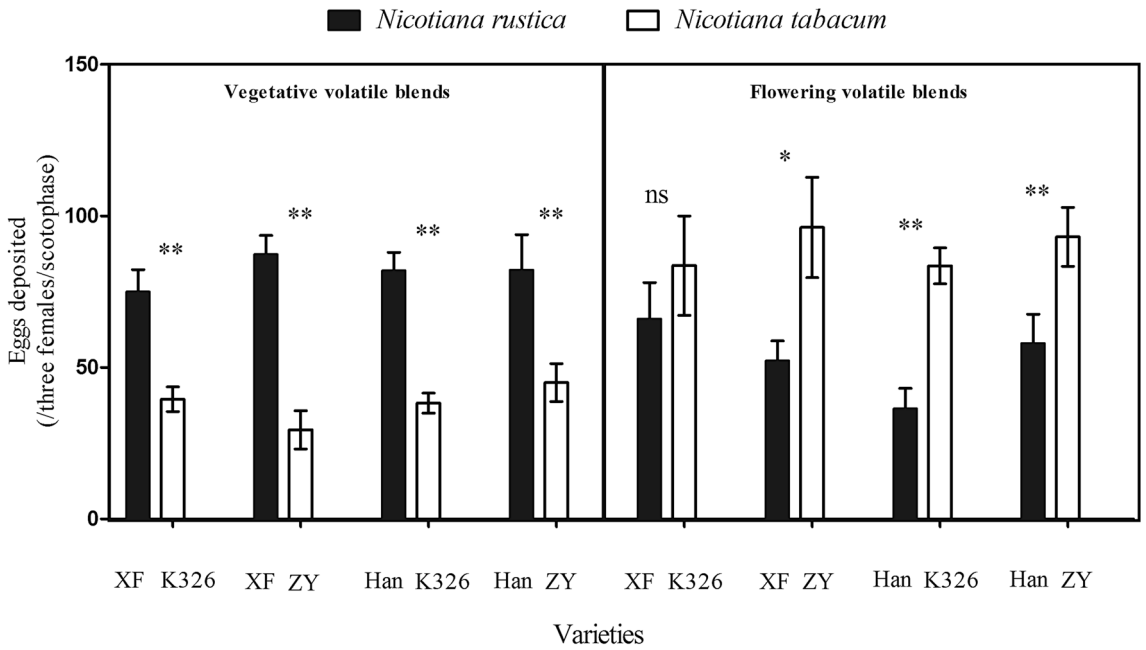

Fig. 5 Eggs deposited by *H. assulta* across two blends of volatile compounds, presented simultaneously, from different varieties of *N. rustica* and *N. tabacum* at the vegetative (left) and flowering (right) stages. ** And *above the bars indicate a significant difference at the $P=0.01$ and $P=0.05$ levels, respectively, and "ns" means no significant difference based on a paired *t*- test, $n=10$–12

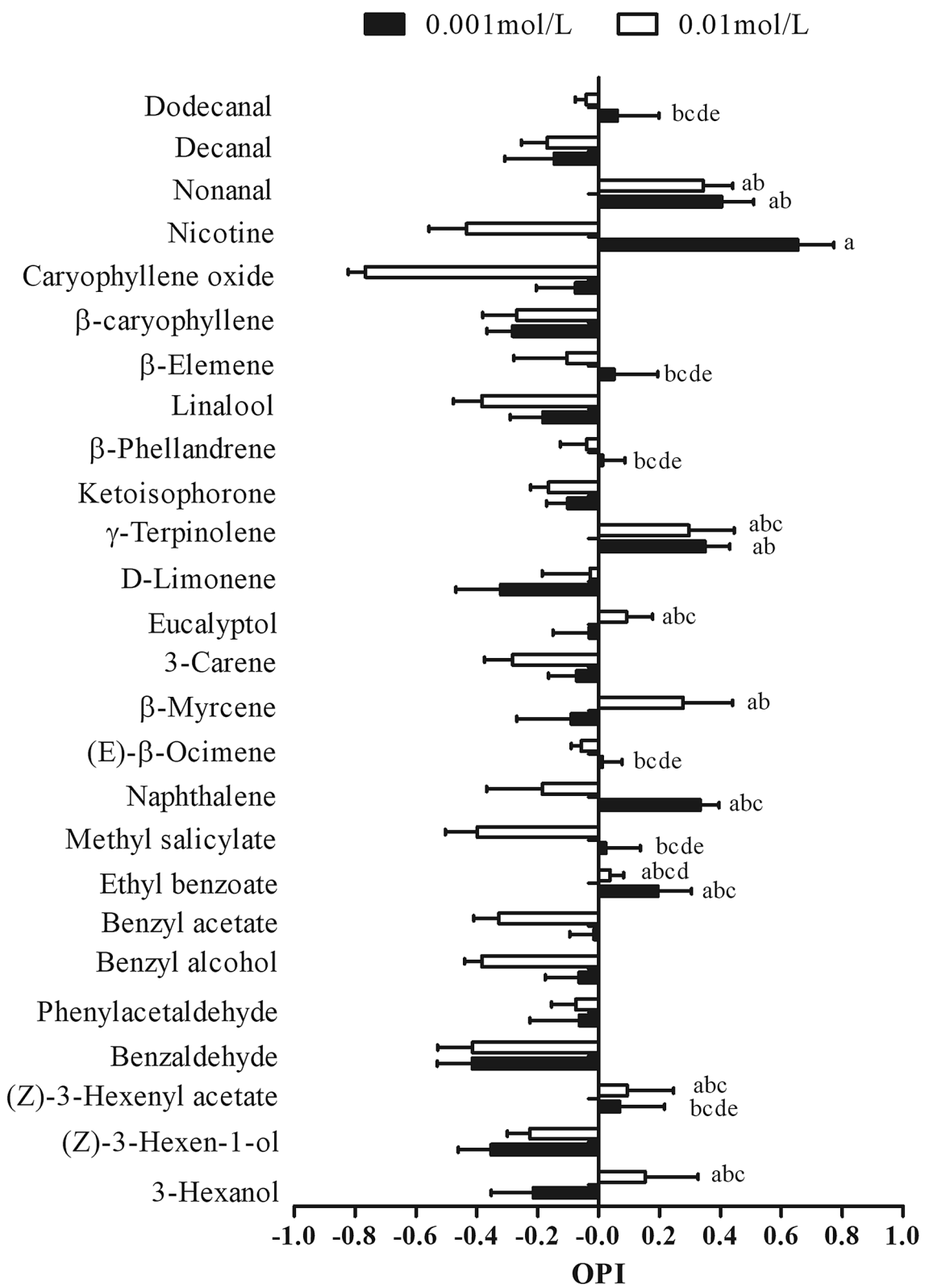

Fig. 6 Oviposition preference index of *H. assulta* in response to different compounds from tobacco headspace volatiles. Different small letters attached to the bars indicate a significant difference at the $P = 0.05$ levels at the same concentrations pooled (one-way ANOVA followed by a Student–Newman–Keuls test, $P < 0.05$) ($n = 7$–14). Significant difference symbols have been omitted for OPIs less than zero

Discussion

The oviposition attraction of tobacco to *H. assulta* females is temporally and spatially associated with flowering tobacco and tobacco species (Guo et al. 1995; Jiang et al. 2003). Therefore, volatiles specific to the flowering stage of tobacco might be responsible for the discrepancy in oviposition rates of *H. assulta* across the two tobacco species, *N. rustica* and *N. tabacum*. The results obtained from our preliminary experiment support this hypothesis (i.e., significantly more eggs were deposited on leaves and inflorescences of *N. rustica* than on the corresponding parts of *N. tabacum*). Furthermore, our results obtained from bioassays on solvent extracts partially support this hypothesis. Nonetheless, the disparities in the number of eggs deposited in these latter tests were not as large as those in our first experiment. We speculate that either the solvent or the extraction method we used were inadequate to obtain complete volatile profiles of these tobacco plants. Alternatively, stimuli from the tobacco leaf surface (e.g., leaf trichomes, flower color, flower pattern or contact chemicals) are also needed to elicit *H. assulta* oviposition.

To obtain volatile profiles under natural conditions, we collected the headspace volatiles emitted from living plants of different tobacco varieties at different stages. Relative oviposition rates of *H. assulta* females across the blends of volatile compounds based on the GC–MS analyses were examined. Regardless of the variety, the volatile blends of *N. rustica* plants at the vegetative stage were consistently more attractive than those from *N. tabacum* plants at the same stage. In contrast, at the flowering stage, more eggs were laid on the volatile blends of *N. tabacum* than on those of *N. rustica* varieties.

Incomplete complements of cues in the blend bioassays may explain the differences across the results obtained from of the bioassay of tobacco organs and the bioassay of tobacco headspace volatile blends. During oviposition, females need additional cues, including contact chemicals and visual signals, to evaluate the characteristics of a potential host plant (Awmack and Leather 2002; Olsson et al. 2006). These additional cues, however, were lacking in our blend bioassays. Some contact chemicals, or color, present in *N. rustica* flowers, may be more important cues for *H. assulta* oviposition than in *N. tabacum* flowers.

In this study, phenylacetaldehyde and benzyl acetate had no oviposition attraction to *H. assulta*, regardless of concentration, but previous long-term field trapping studies showed that these two floral volatiles may be key components of generic floral attractants to noctuid moths (Li et al. 2014). This may be true for nectar-foraging behavior, but our results indicate that is not the case for ovipositional attraction in *H. assulta*. Likewise, the most abundant component in both growth stages of *N. tabacum*, D-limonene, repelled oviposition of *H. assulta* when examined on its own, regardless of its concentration, the same cases were found for β-caryophyllene, linalool, and benzaldehyde. When tested individually, (*E*)-*β*-ocimene and (*Z*)-3-hexenyl acetate had only a slight effect on the oviposition response of *H. assulta,* nonanal showed significant oviposition attraction at both tested concentrations in our study, but the mixture of (*E*)-*β*-ocimene, (*Z*)-3-hexenyl acetate, nonanal and (*E*)- β-caryophyllene was identified as the major olfactory cue for attracting ovipositing *H. assulta* females to another *N. tabacum* variety, NC89 (Sun et al. 2012). Regardless, further study is necessary to reconcile these contradictory findings and determine whether differences in results, i.e., those presented in the current paper and those reported by Sun et al. (2012), are due to different bioassays (ovipositional context or wind tunnel attraction) or sampling bias (volatiles examined individually or combined). Because the volatiles emitted by tobacco plants vary with varieties, growth stages, collection method, and adsorbent (Raguso et al. 2003; Li et al. 2015), we suggest that there may be other volatile compounds mediating the ovipositional behavior of *H. assulta*, which need to be further studied.

Many volatile compounds of plants have been identified as ovipositional stimulants or attractants for moths (Mozuraitis et al. 2002; Morris et al. 2005, 2009; Lee et al. 2006; Gregg et al. 2010; Feng et al. 2017; Cui et al. 2018). In our study, several compounds from tobacco plants were identified to be ovipositional attractants for *H. assulta.* γ-Terpinolene, specific to vegetative *N. rustica*, showed ovipositional attractiveness at concentrations of 0.01 mol/L and 0.001 mol/L. A similar results were obtained from nicotine at 0.001 mol/L, nonanal, (*Z*)-3-hexenyl acetate, and ethyl benzoate at both concentration. In contrast, β-elemene was present only in *N. rustica* volatiles and showed moderate ovipositional attractiveness at 0.001 mol/L and repellence at a higher concentration (0.01 mol/L).

In summary, headspace volatile profiles were dependent on both the species of the tobacco plants tested and the stages of their growth, while the volatile profile across conspecific varieties showed somewhat convergent characteristics. Volatiles partially explained the difference in the ovipositional rates of *H. assulta* across the two tobacco species, *N. rustica* and *N. tabacum*. The differences in nicotine, γ-terpinolene, and β-elemene emissions may explain this ovipositional attraction.

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References

Ahn SJ, Badenes-Pérez FR, Heckel DG (2011) A host-plant specialist, *Helicoverpa assulta*, is more tolerant to capsaicin from *Capsicum annuum* than other noctuid species. J Insect Physiol 57(9):1212–1219. https://doi.org/10.1016/j.jinsphys

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Ann Rev Entomol 47(1):817–844. https://doi.org/10.1146/annurev.ento.47.091201.145300

Cui WC, Wang B, Guo MB, Liu Y, Jacquin-Joly E, Yan SC, Wang GR (2018) A receptor-neuron correlate for the detection of attractive plant volatiles in *Helicoverpa assulta* (Lepidoptera: Noctuidae). Insect Biochem Mol Biol 97:31–39. https://doi.org/10.1016/j.ibmb.2018.04.006

Feng B, Qian K, Du YJ (2017) Floral volatiles from *Vigna unguiculata* are olfactory and gustatory stimulants for oviposition by the bean pod borer moth *Maruca vitrata*. Insects 8:60. https://doi.org/10.3390/insects8020060

Fitt GP (1989) The ecology of *Heliothis* species in relation to agroecosystems. Ann Rev Entomol 34(1):17–52. https://doi.org/10.1146/annurev.en.34.010189.000313

Gregg PC, Del Socorro AP, Henderson GS (2010) Development of a synthetic plant volatile-based attracticide for female noctuid moths. II. Bioassays of synthetic plant volatiles as attraction for the adults of cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Austral Entomol 49(1):21–30. https://doi.org/10.1111/j.1440-6055.2009.00734.x

Guo XR, Luo MH, Peng GF, Sun TS, Dang RS, Wang XF, Liu JA, Li A (1995) Studies on the spatial distribution of eggs of *Helicoverpa assulta*/*H. armigera* in tobacco field II. The spatial distribution and density estimation of eggs in the tobacco field after wheat harvest and the field of tobacco seed-plot. Chin Tob Sci 2(4):1–7

Jansen RMC, Hofstee JW, Wildt J, Verstappen FWA, Bouwmeester HJ, Posthumus MA, van Henten EJ (2009) Health monitoring of plants by their emitted volatiles: trichome damage and cell membrane damage are detectable at greenhouse scale. Ann Appl Biol 154(3):441–452. https://doi.org/10.1111/j.1744-7348.2008.00311.x

Jiang JW, Guo XR, Luo MH, Zheng XJ, Zhang SH (2003) The seasonal characters of arthropod community in different types of tobacco. Acta Tab Sin 9(1):35–38

Jones LC, Rafter MA, Walter GH (2019) Insects allocate eggs adaptively across their native host plants. Arthropod-Plant Interact 13:181–191. https://doi.org/10.1007/s11829-019-09688-x

Lee SH, Hieu TT, Ahn YJ (2006) Oviposition-stimulating activity of (*E*)-capsaicin identified in *Capsicum annuum* fruit and related compounds towards *Helicoverpa assulta* (Lepidoptera: Noctuidae). Chemoecology 16(3):153–157. https://doi.org/10.1007/s00049-006-0341-0

Li WZ, Li HL, Wang J, Guo XR, You XF, Yuan GH (2014) Optimization of floral attractant formula with broad-trapping spectrum of moths using uniform design. Chin Agri Sci Bull 30(4):304–311

Li X, Yang Z, Hu BW, Dai X, Dong WX (2015) The release of leaf exudates and plant volatiles of tobacco. Plant Prot 41(1):13–22

Long X, Zeng AP, Zhou ZC, Chen YN, Hu RS, Long JZ, Li XY, Wu CE (2012) Occurrence regularity of *Heliothis assulta* Guenée in Hunan and forecast thereof. Tob Sci Technol 45(2):75–79

Luo MH, Xue WW, Liu XG, Zhao GQ (2006) Studies on the attraction effect of different tobacco varieties to oviposition of *Helicoverpa assulta* Guenée and *H. armigera* Hübner. J Henan Agric Univ 40(2):198–200. https://doi.org/10.16445/j.cnki.1000-2340.2006.02.021

Megido RC, De Backer L, Ettaïb R, Brostaux Y, Fauconnier ML, Delaplace P, Lognay G, Belkadhi MS, Haubruge E, Francis F, Verheggen FJ (2014) Role of larval host plant experience and solanaceous plant volatile emissions in *Tuta absoluta* (Lepidoptera: Gelechiidae) host finding behavior. Arthropod-Plant Interact 8(4):293–304. https://doi.org/10.1007/s11829-014-9315-2

Morris BD, Foster SP, Grugel S, Charlet LD (2005) Isolation of the diterpenoids, ent-kauran-16α-ol and ent-atisan-16α-ol, from sunflowers, as oviposition stimulants for the banded sunflower moth, *Cochylis hospes*. J Chem Ecol 31(1):89–102. https://doi.org/10.1007/s10886-005-0976-2

Morris BD, Charlet LD, Foster SP (2009) Isolation of three diterpenoid acids from sunflowers, as oviposition stimulants for the banded sunflower moth, *Cochylis hospes*. J Chem Ecol 35(1):50–57. https://doi.org/10.1007/s10886-008-9567-3

Mozuraitis R, Stranden M, Ramirez MI, Borg-Karlson AK, Mustaparta H (2002) (-)-Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. Chem Senses 27(6):505–509. https://doi.org/10.1093/chemse/27.6.505

Olsson CPO, Anderbrant O, Löfstedt C (2006) Experience influences oviposition behaviour in two pyralid moths, *Ephestia cautella* and *Plodia interpunctella*. Anim Behav 72(3):545–551. https://doi.org/10.1016/j.anbehav.2005.10.023

Proffit M, Khallaf MA, Carrasco D, Larsson MC, Anderson P (2015) 'Do you remember the first time?' Host plant preference in a moth is modulated by experiences during larval feeding and adult mating. Ecol Lett 18:365–374. https://doi.org/10.1111/ele.12419

Raguso RA, Levin RA, Foose SE, Holmberg MW, McDade LA (2003) Fragrance chemistry, nocturnal rhythms and pollination"syndromes" in *Nicotiana*. Phytochemistry 63(3):265–284. https://doi.org/10.1016/S0031-9422(03)00113-4

Rajapakse CNK, Walter GH (2007) Polyphagy and primary host plants: oviposition preference versus larval performance in the lepidopteran pest *Helicoverpa armigera*. Arthropod-Plant Interact 1:17–26. https://doi.org/10.1007/s11829-007-9003-6

Reisenman CE, Riffell JA, Duffy K, Pesque A, Mikles D, Goodwin B (2013) Species-specific effects of herbivory on the oviposition behavior of the moth *Manduca sexta*. J Chem Ecol 39(1):76–89. https://doi.org/10.1007/s10886-012-0228-1

Renwick JAA, Chew FS (1994) Oviposition behavior in Lepidoptera. Annu Rev Entomol 39(1):377–400. https://doi.org/10.1146/annurev.en.39.010194.002113

Sun JG, Huang LQ, Wang CZ (2012) Electrophysiological and behavioral responses of *Helicoverpa assulta* (Lepidoptera: Noctuidae) to tobacco volatiles. Arthropod-Plant Interact 6(3):375–384. https://doi.org/10.1007/s11829-012-9190-7

Thöming G, Larsson MC, Hansson BS, Anderson P (2013) Comparison of plant preference hierarchies of male and female moths

and the impact of larval rearing hosts. Ecology 94(8):1744–1752. https://doi.org/10.1093/chemse/bjs089

Warneke C, van der Veen C, Luxembourg S, de Gouw JA, Kok A (2001) Measurements of benzene and toluene in ambient air using proton-transfer-reaction mass spectrometry: calibration, humidity dependence and field intercomparison. Int J Mass Spectrom 207(3):167–182. https://doi.org/10.1016/S1387-3806(01)00366-9

Wu H, Li RT, Dong JF, Jiang NJ, Huang LQ, Wang CZ (2018) An odorant receptor and glomerulus responding to farnesene in *Helicoverp aassulta* (Lepidoptera: Noctuidae). Insect Biochem Mol Biol 115:103106. https://doi.org/10.1016/j.ibmb.2018.11.006

Xue WW, Fu XW, Luo MH, Guo XR, Yuan GH (2009) Effects of tobacco volatiles on ovipositing behaviors of two sibling *Helicoverpa* species and volatile chemical analysis. Acta Ecol Sin 29(11):5783–5790

Zhang YF, Huang LQ, Ge F, Wang CZ (2011) Tarsal taste neurons of *Helicoverpa assulta* (Guenée) respond to sugars and amino acids, suggesting a role in feeding and oviposition. J Insect Physiol 57(10):1332–1340. https://doi.org/10.1016/j.jinsphys.2011.06.009

Zhong J (2019) Study of K^+ uptake kinetics of flue-cured tobacco in K^+-enriched and conventional tobacco genotypes. J Plant Nutr 42(7):805–811. https://doi.org/10.1080/01904167.2018.1450418