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SHORT COMMUNICATION

Effects of volatiles of non-host plants and other chemicals on oviposition of *Monochamus alternatus* (Coleoptera: Cerambycidae)

Shui-Qing Li · Yu-Ling Fang · Zhong-Ning Zhang

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Abstract The response of adult females of *Monochamus alternatus* to volatiles of non-host plants was evaluated using a Y-tube olfactometer. Eight non-host plant species were tested, namely *Broussonetia papyrifera*, *Cedrus deodara*, *Firmiana simplex*, *Metasequoia glyptostroboides*, *Platycladus orientalis*, *Populus xiaohei*, *Salix babylonica* and *Sophora japonica*. The volatiles of all test species, except those of *C. deodara*, repelled the females in the olfactometer bioassays. Oviposition by adult females on the host plant, *Pinus massoniana*, was deterred by the volatiles of non-host plants and by some other chemicals. Volatiles of *P. orientalis* had the strongest oviposition-deterring activity and those of *S. japonica* the least. Volatiles of *C. deodara* had no influence on oviposition of the females. Selected chemicals, namely benzaldehyde, citronellal, essential oil of *Mentha spicata*, eucalyptol, nerolidol, nicotine and salicylaldehyde, strongly deterred the females from oviposition, and nicotine caused maximum decrease in egg-laying and scar-excavation.

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S.-Q. Li · Y.-L. Fang · Z.-N. Zhang (✉)
State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 25 Beisihuan Xilu, Haidian 100080, Beijing, China
e-mail: zhangzn@ioz.ac.cn

S.-Q. Li
College of Chemical and Environmental Engineering, Yangtze University, Jingzhou 434023, Hubei, China

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Introduction

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle, is the causal agent of the destructive pine wilt disease, which causes considerable mortality of *Pinus densiflora* Sieb. et Zucc. and *Pinus thunbergii* Parl. in Japan (Mamiya and Enda 1972). In China, the pinewood nematode was first discovered in Nanjing in 1982 and then spread rapidly into the Anhui Province, Guangdong Province, Zhejiang Province and Shandong Province (Sun 1982). The pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), is a primary vector of the pinewood nematode. The preferred host of *M. alternatus* in China is *Pinus massoniana* Lamb. (Hu et al. 1997). To date, *M. alternatus* and *B. xylophilus* have caused the death of about 6.7 million trees of *P. massoniana*. In recent years, there have been many studies on integrated pest management of *M. alternatus*, including biological control (Shimazu 1994; Shimazu and Sato 2003), insecticide application (Togashi 1990) and control of attractants (Ikeda et al. 1980; Sakai and Yamasaki 1990, 1991).

Oviposition by an insect is an important step in its reproduction and in determining the size of its population in a locality. So deterrence of oviposition by a pest insect can reduce its population and help in its management. The oviposition behaviour of some phytophagous insects can be modified by volatiles of the host and non-host species. Examples include *Acanthoscelides obtectus* (Say) (Papachristos and Stamopoulos 2002), *Callosobruchus maculatus* (F.) (Boeke et al. 2003), *Heliothis virescens* (F.) (Tingle and Mitchell 1984), *Callosobruchus maculatus* (F.) (Elhag 2000), *Diorhabda elongata deserticola* (DeLoach et al. 2003) and *Pieris rapae* (L.) (Renwick and Radke 1985; Renwick et al. 1989).

However, at present, we have no information on the influence of volatiles from non-host plants on the oviposition of *M. alternatus*. According to our investigation, *Broussonetia papyrifera* (Linn.) Vent (Moraceae), *Cedrus deodara* (Roxb.) Loud (Pinaceae), *Firmiana simplex* (Linn.) F.W. Wight (Sterculiaceae), *Metasequoia glyptostroboides* Hu et Cheng (Taxodiaceae), *Platycladus orientalis* (Linn.) Franco (Cupressaceae), *Populus xiaohei* T.S. Hwang et Liang (Salicaceae), *Salix babylonica* Linn. (Salicaceae) and *Sophora japonica* Linn. (Leguminosae) are primary non-host plants of *M. alternatus* on Jingting Mountain, Xuancheng, Anhui Province. So, these eight non-host plant species were chosen to test the influence on the females of *M. alternatus* in olfactory and oviposition tests.

Four chemicals, which are secondary metabolites in some plants, namely nicotine, eucalyptol, nerolidol and essential oil of *Mentha spicata* L., and three, which produced very strong electroantennography (EAG) responses on the antennae of female beetles in preliminary tests, namely citronellal, benzaldehyde and salicylaldehyde, were also tested for their effects on the oviposition of *M. alternatus*.

Materials and methods

Insect source

An experimental colony of *M. alternatus* was established from insects collected on Jingting Mountain, Xuancheng, Anhui Province, China. Newly emerged adults were kept, males separate from females, in iron-screened cages (50 cm long × 40 cm wide × 30 cm high) on 1–2-year old *P. massoniana* twigs at 25 °C and under a 12:12 h (light/dark) photoperiod. Two weeks later, the females and males were paired. Female adults were used in olfactory and oviposition tests 18–25 days after emergence. The females were allowed to oviposit before being used in oviposition tests.

Non-host plants

For testing the influence of volatiles of non-host plants on the females, 10 g freshly excised leaves of each of the eight non-host plants were used in olfactory and oviposition tests.

Chemicals

The sources and purity of the chemicals were: nicotine (95%), eucalyptol (99%), New Jersey, USA; citronellal (97%), salicylaldehyde (≥96%), Xudong Chemical Plant of Beijing, China; benzaldehyde (≥98%), Wuhan Shengshi Fine Chemical Co. Ltd., China; nerolidol (95%), essential oil of *M. spicata* (95%), Department of Chemistry, Capital Normal University, China.

Olfactometer bioassay

A Y-tube olfactometer made of glass (9 cm diameter; main tube, 32 cm long; arm length, 15 cm; angle between arms, 70°) was used to determine the influence of volatiles of non-host plants on the behaviour of the female beetles. In each test, a brown bottle containing 10 g freshly excised leaves of one of the non-host plants was connected to one arm of the Y-tube with a silicon tube, and an empty brown bottle was connected to the other arm of the Y-tube as a control. A vacuum pump drew air into the system through activated charcoal. The airflow was set at 2.5 l/min. A female beetle was then released downwind of the main tube and allowed to walk along the tube and to choose either the arm containing a sample odour or clean air. Each beetle was allowed 15 min to make its choice, and the choice was recorded when the beetle reached the end of one of the arms. Connections of the odour sources to the arms were reversed after testing five insects to avoid asymmetrical bias. The olfactometer was rinsed with alcohol and dried between trials. Each test was repeated using 43–50 females.

Effect of volatiles of non-host plants on oviposition

Healthy *P. massoniana* trees about 8–10-year old were cut into 15-cm long bolts without nodes on 26 May 2005. The cut ends of the pine bolts were sealed with liquid paraffin (melting point: 56–58 °C) and stored in sealed black plastic bags at room temperature until used in oviposition tests. The bolts were 3.6–4.5 cm in diameter (mean ± SE = 4.1 ± 0.1 cm) and had a bark thickness of 1.1–2.2 mm (mean ± SE = 1.7 ± 0.1 mm).

To test the effects of volatiles of eight non-host plants on oviposition, a petri dish (8.5 cm diameter × 3.2 cm high) containing 10 g freshly excised leaves of a non-host plant was placed in a transparent plastic container (20 cm long × 20 cm wide × 20 cm high). One pine bolt was placed vertically in the centre of the container and two 1–2-year old *P. massoniana* twigs were placed in the cage as food. One gravid female of *M. alternatus* was then released on the *P. massoniana* twigs at the onset of dusk. The female was removed 48 h later and the number of scars and eggs counted. Each test was replicated 25 times using 25 females aged 18–25 days.

Effect of selected chemicals on oviposition

For testing the influence of selected chemicals on the ovipositional behaviour of *M. alternatus*, each chemical was diluted 100-fold in analytical pure hexane and was then applied to the bark surface of pine bolts using the tip of a calligraphy brush, 1 ml per bolt.

Each test was accompanied by controls in which 25 female beetles were given untreated bolts for oviposition.

Data analysis

Data obtained from tests were subjected to statistical analysis using the SPSS 11.0 for Windows software. The χ^2-test was used to analyse differences obtained in olfactometer bioassays. The effects of volatiles of non-hosts and chemicals on oviposition were analysed with independent-samples *t*-test. If the *t*-test result was significant, then Scheffe's test of the analysis of variance (ANOVA) was used to compare the differences between the means of test volatiles of non-host plants and chemicals.

Results

Olfactory response

Female beetles showed no difference in choice between volatiles of *C. deodara* and clean air. However, significant differences were found when the beetles preferred clean air to air containing volatiles of seven other non-host plants, namely *B. papyrifera*, *F. simplex*, *M. glyptostroboides*, *P. orientalis*, *P. xiaohei*, *S. babylonica* and *S. japonica*.

Effect of volatiles of non-host plants on oviposition

In our study, all tests with volatiles of non-host plants and with selected chemicals were accompanied by controls in which female beetles were given the pine bolts for oviposition. Thus, the mean numbers of eggs and scars from the controls was more than 15. In order to check if the mean numbers of eggs and scars in different controls were significantly different, they were analysed by ANOVA. The results showed that the mean numbers of eggs and scars in these 15 batches were not significantly different at the 5% probability level. According to this result, one egg data of batch 9 (mean number of eggs 5.28 ± 0.19) and the scar data of batch 4 (mean number of scars 5.52 ± 0.31) were closest to the average of the means of all 15 batches and were therefore used for comparisons of egg and scar data in tests on the effects of volatiles of non-host plants and selected chemicals.

In the presence of volatiles of each of the test non-host plants except *C. deodara*, the number of eggs laid and scars excavated by female beetles were significantly fewer than in the controls (Table 1). Maximum reduction in egg-laying and scar-excavation occurred in the presence of volatiles from leaves of *P. orientalis*, the mean number of eggs laid and scars excavated by the beetles being 1.72 ± 0.16 and 1.92 ± 0.15, respectively, and were about one-third of those of the controls ($P < 0.001$). The volatiles of *B. papyrifera*, *F. simplex*, *M. glyptostroboides*, *P. xiaohei*, *S. babylonica* and *S. japonica* also deterred the female beetles from oviposition to different degrees, and those of *S. japonica* caused the least reduction in the number of both eggs and scars.

Effect of selected chemicals on oviposition

The results in Table 2 show that each of the seven chemicals deterred female beetles from oviposition. In the presence of benzaldehyde, citronellal, essential oil of *M. spicata*, eucalyptol, nerolidol, nicotine and salicylaldehyde, the number of eggs laid and scars excavated by the beetles were significantly fewer than in the controls ($P < 0.001$). Nicotine caused maximum

Table 1 Oviposition response of *M. alternatus* to pine bolts in the presence of volatiles of some non-host plants

Non-host plant[a]	Number of eggs (mean ± SE)[b]	Number of scars (mean ± SE)
C. deodara	4.88 ± 0.19 (NS)	5.04 ± 0.18 (NS)
S. japonica	4.68 ± 0.19 a	4.76 ± 0.18 a
F. simplex	3.88 ± 0.19 b	4.08 ± 0.19 b
B. papyrifera	3.68 ± 0.17 bc	3.96 ± 0.18 b
M. glyptostroboides	3.56 ± 0.15 bc	4.12 ± 0.17 b
S. babylonica	3.16 ± 0.18 cd	3.56 ± 0.21 bc
P. xiaohei	2.84 ± 0.21 d	3.20 ± 0.26 c
P. orientalis	1.72 ± 0.16 e	1.92 ± 0.15 d
Control[c]	5.28 ± 0.19	5.52 ± 0.31

[a] The source of volatiles was 10 g of freshly excised leaves of each non-host plant

[b] Mean values within *columns* followed by the *same letters* are not significantly different ($P > 0.05$) by Scheffe's test of the analysis of variance (*ANOVA*). *NS* no significant difference from controls by independent-samples *t*-test

[c] Batches 9 and 4, with mean numbers of eggs and scars closest to the average of the means of all 15 batches, were used as the controls

Table 2 Oviposition response of *M. alternatus* to pine bolts in the presence of selected chemicals

Chemical[a]	Number of eggs (mean ± SE)[b]	Number of scars (mean ± SE)
Citronellal	3.44 ± 0.21 a	3.76 ± 0.23 a
Essential oil of *M. spicata*	2.68 ± 0.23 b	2.92 ± 0.24 b
Benzaldehyde	2.60 ± 0.17 b	2.88 ± 0.20 b
Nerolidol	2.32 ± 0.11 bc	2.52 ± 0.14 bc
Eucalyptol	2.12 ± 0.11 c	2.20 ± 0.13 c
Salicylaldehyde	2.08 ± 0.13 c	2.28 ± 0.16 c
Nicotine	1.12 ± 0.09 d	1.45 ± 0.15 d
Control[c]	5.28 ± 0.19	5.52 ± 0.31

[a] Each chemical was diluted 100-fold with analytical pure hexane

[b] See footnote b of Table 1

[c] See footnote c of Table 1

decrease in egg-laying and scar-excavation, and citronellal the least.

Discussion

Oviposition deterrents can be very useful in integrated pest management. Deterrence to oviposition by insects can serve as an important measure to reduce the populations of the pests. A number of authors have reported their investigation results about oviposition deterrence pheromones (Anderson et al. 1993; Fettköther et al. 2000; Li et al. 2001; Li and Ishikawa 2004). However, most oviposition deterrents serve as contact antiovipositants.

Possibly, volatile oviposition deterrents would be more effective than contact oviposition deterrents because the former would diffuse around the host plants. Successful examples dealing with the advantageous effect of volatile deterrents in the field included *Heliothis virescens* (F.) (Tingle and Mitchell 1984) and *Dendroctonus valens* (L.) (Zhang et al. 2006). Thus, volatile oviposition deterrents would seem to have great potential in the management of pest insects.

In our study, the volatiles of all test non-host plants, except those of *C. deodara*, deterred oviposition of the females. These investigation results showed the presence of putative oviposition deterrents in volatiles of non-host plants. Jactel et al. (2001) reported the influence of non-host volatiles on the behaviour of *Ips sexdentatus*. Of the seven selected chemicals, nicotine was the most effective in deterring oviposition. The influence of selected chemicals, such as benzaldehyde (Kelley and Schilling 1998), citronellal (Corbet et al. 2000; Sen et al. 2005), eucalyptol (Zhu et al. 2001) and nerolidol (Wheeler et al. 2003), on the behaviour of some pest insects has been reported.

Further investigation is needed to identify the constituents of the volatiles of the non-host plants and to identify the bioactive compounds so that their potential value in controlling *M. alternatus* can be realised. Our observations indicate that volatiles of *P. orientalis* are promising candidates for forest trials to deter oviposition by *M. alternatus*. In a subsequent study, nicotine, which caused the largest decrease in egg-laying and scar-excavation in the present study, will also be tested in forest trials.

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