Electroantennogram responses of *Dendrolimus superans* (Lepidoptera: Lasiocampidae) to six volatiles of *Larix gmelinii*

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**Abstract** *Dendrolimus superans* is one of the important pests feeding on the needles of *Larix gmelinii*. Six standard compounds, (1R)-(+)-x-pinene, (1S)-(−)-x-pinene, ocimene, (1S)-(−)-β-pinene, R(−)-x-phellandrene and camphene, the main volatiles of *L. gmelinii* were used to test the electroantennogram (EAG) responses of moths. The results show that the order of the EAG responses of moths to seven concentrations of these six compounds were as follows: mated females > virgin females > unmated males, except for 0.1 µL/µL R(−)-x-phellandrene, where the order was: virgin females > mated females > unmated males. There are statistically significant differences between the EAG responses of virgin females and unmated males (p < 0.05), and also between mated females with virgin females and mated females and unmated males (p < 0.01), which suggests that the mated females are more sensitive to the volatiles of host plant. The results of EAG responses of the mated females, virgin females and unmated males indicate that they are more sensitive to R(−)-x-phellandrene than to the other volatile components. The active time for the EAG responses of both mated females and unmated males occurs during the night, i.e., from 20:00 to 04:00 hours. This is consistent with their eclosion, mating and oviposition periods.

**Keywords** *Dendrolimus superans*, plant volatiles, activity time, EAG response, *Larix gmelinii*

1 Introduction

*Dendrolimus superans* (Lepidoptera: Lasiocampidae) is one of the most important pests in the forests of northeastern China which endangers *Larix* as well as *Pinus koraiensis, P. tabulaeformis* and *P. sylvestris* var. *mongolica*. During outbreaks, the limbs of these tree species become bare and appear burned. The insects attack larch needles and large areas of tree clusters succumb to these severe attacks which has caused serious losses in forest production (Xiao, 1992). In the past, chemicals were used to control this pest. With the more recent developments in chemical insect ecology, secondary plant substances may be used to control pests through regulating pest behavior. The use of biologically active substances, extracted and separated from plants or plant productions to control pests, has become a world wide trend of integrated pest management (Nehlin et al., 1994; Deepa et al., 2004; Wei et al., 2004; John et al., 2004, 2005). A single terpene, a volatile of the tree *Platycladus orientalis*, stirred up strong electroantennogram responses to *Semanotus bifasciatus* and affected the behavior of pests searching for their host plants (Kong et al., 2005). The EAG responses of *Anoplophora glabripennis* to eleven volatile monomers of *Acer negundo* declined with a decrease in the concentration of the volatiles. Furthermore, each monomer, when in liquid form, had the strongest effect on the activity of EAG response (Li et al., 1999). The EAG responses of mated females of *Acantholyda posticalis* to *Pinus tabulaeformis, P. densiflora* and *P. armandii* were sensitive and the females show high optional taxis to these host plants indicating that the olfaction function of antennae plays an important role in host selection (Zhang et al., 2005),

The main volatile components of *L. gmelinii* were terpenes, including x-pinene, β-pinene, ocimene, camphene and R(−)-x-phellandrene (Guo et al., 1996; Yan et al., 1999). There are few reports on whether these terpene substances can arouse olfaction responses of moths. To determine this and discover their active concentration range, the EAG responses of moths were tested to standard samples of terpene substances of *L. gmelinii* at various concentrations. The results would provide a scientific basis for further studies on the effects of volatiles on the behavior of moths and controlling the pest damage.
2 Material and methods

The pupa of *Dendrolimus superans* were collected from Yakeshi and Zhirui towns of the Keshiketengqi forest region and were raised until the moths emerged in the laboratory at a temperature about (25 ± 1)°C. The photoperiod followed a circadian rhythm: the light period was from 05:00–18:00 hours, and the dark period was from 19:00 to 04:00, the L:D is 14:10. The relative humidity was (75 ± 5)%. The collected moths were divided into two groups and all were fed with 10% honey water. Males and females in group one were fed separately, and in another group were fed together with a sex ratio of 1:2 (female: male) and were separated after mating. The EAG responses of moths to terpenes were tested in both light and dark period. All the males and females were tested within one day after they emerged.

2.1 Standard compounds

Standard volatiles used by us are listed in Table 1. The six standard volatile compounds were dissolved in liquid paraffin (chemically pure, from the Tianjin Taixing Chemical Regent Factory) to concentrations of 0.00001, 0.0001, 0.001, 0.01, 0.1, 0.2 and 0.5 μL/μL (v/v).

Table 1 Name, purity and sources of six standard volatiles

<table>
<thead>
<tr>
<th>name of compound</th>
<th>purity/%</th>
<th>source of supply</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1R)-(+)–α-pinene</td>
<td>≥99</td>
<td>Sigma</td>
</tr>
<tr>
<td>(1S)-(−)–α-pinene</td>
<td>&gt;99.5</td>
<td>Sigma</td>
</tr>
<tr>
<td>Ocimene</td>
<td>70 (cis-ocimen) and 25 (limonene)</td>
<td>Fluka</td>
</tr>
<tr>
<td>(1S)-(−)–β-pinene</td>
<td>99</td>
<td>Sigma</td>
</tr>
<tr>
<td>R(−)–α-phellandrene</td>
<td>50</td>
<td>Fluka</td>
</tr>
<tr>
<td>Camphene</td>
<td>95</td>
<td>Aldrich</td>
</tr>
</tbody>
</table>

2.2 Electroantennogram responses

Standard samples were swirled for 30 s and then 10 μL of it were dripped on the 10 mm × 20 mm × 20 mm triangle filter paper inside the Pasteur tube. One end of the Pasteur tube was connected to gas purging equipment and another was inserted into an odor mixing cuvette of a vibration apparatus. The antennae whose tops were cut off were put on the poles with conductive glue. The distance from the odor mixing cuvette to the antennae was about 1 cm. The velocity of the stimulated airflow was 60 mL/s. The period of stimulation was 0.5 s and the time interval 30 s, to ensure that the antennae activity would completely return. All the tests of EAG responses of virgin females, unmated males and mated female to the six volatiles were performed from low to high concentrations. Ten antennae were tested at each concentration of one standard sample and the response of each antenna was repeated three times, and 10 antennae were tested to one standard compound. The EAG equipment consisted of four parts: data obtained from an IDAC-4 controller, a Syntech CS-55, a Syntech MN-151 and a software disposal system made by the Syntech Company from the Netherlands.

2.3 Statistical analysis

All data were analyzed by one-way analysis of variance and Duncan tests were performed for making multiple comparisons among means. The SPSS 10.0 software package was used (SPSS 2001). Figures were plotted by the Excel Software.

3 Results

3.1 EAG responses to six standard volatiles under light condition

The differences in the EAG responses of moths to (1R)-(+)-α-pinene, (1S)-(−)-α-pinene, ocimene, (1S)-(−)-β-pinene, R(−)-α-phellandrene and camphene increased when the concentrations of the volatiles were increased, then declined after they reached a peak. The results show that the order of the EAG responses of moths to the seven concentrations of the six compounds were mated females > virgin females > unmated males, except for 0.1 μL/μL R(−)-α-phellandrene, where the rated order was: virgin females > mated females > unmated males. There are statistically significant differences between the EAG responses of virgin females and unmated males (*p* < 0.05), as well as mated females with virgin females and unmated males (*p* < 0.01), which indicates that these volatiles play an important role in oviposition locating by moths.

The EAG responses of the moths of the three physiological stages were greater to (1S)-(−)-α-pinene, R(−)-α-phellandrene and camphene than that to control at concentration 0.0001 μL/μL, however, the EAG responses to (1R)-(+)–α-pinene, (1S)-(−)-β-pinene and ocimene were lower than the control (*p* < 0.05). This shows that the limit of olfactory detection by moths was more sensitive to the first three volatiles than to the others. It may be caused by the long-distance orientation to the volatile components. When concentrations were greater than or equal to 0.0001 μL/μL, the EAG responses of moths to the six volatile components were greater than that of the control (*p* < 0.01). The EAG responses, to phellandrene in various concentrations, of virgin females and unmated males were significant (*p* < 0.05). So, we concluded that phellandrene played a key role for moths in searching for host plants. When the concentrations were less than or equal to 0.1 μL/μL, the responses of...