Front. For. China 2008, 3(2): 237–242
DOI 10.1007/s11461-008-0028-5

RESEARCH ARTICLE

Shanchun YAN, Yingsheng LIU, Qi WANG, Hong CHENG, Jian ZHANG, Defu CHI

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

Abstract *Dendrolimus superans* is one of the important pests feeding on the needles of *Larix gmelinii*. Six standard compounds, (1R)-(+)-α-pinene, (1S)-(−)-α-pinene, ocimene, (1S)-(−)-*β*-pinene, R(−)-α-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(−)-α-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(−)-α-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 α-pinene, *β*-pinene, ocimene, camphene and R(−)-α-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.

Translated from *Scientia Silvae Sinicae*, 2007, 43(7): 55–60 [译自: 林业科学]

Shanchun YAN (✉), Yingsheng LIU, Qi WANG, Hong CHENG, Jian ZHANG, Defu CHI
College of Forestry, Northeast Forestry University, Harbin 150040, China
E-mail: yanchanchun@126.com

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 feeded with 10% honey water. Males and females in group one were feeded separately, and in another group were feeded 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 Tianjun 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). Camphene was bathed in 80°C water for 5 min before preparation. All solutions were stored at 4°C. Liquid paraffin was used as CK.

Table 1 Name, purity and sources of six standard volatiles

name of compound	purity/%	source of supply
(1R)-(+)-α-pinene	⩾99	Sigma
(1S)-(−)-α-pinene	>99.5	Sigma
Ocimene	70 (cis-ocimen) and 25 (limonene)	Fluka
(1S)-(−)-β-pinene	99	Sigma
R-(−)-α-phellandrene	50	Fluka
Camphene	95	Aldrich

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 mated females to phellandrene improved with an increase in concentration. There were no significant differences among the EAG responses of mated females to phellandrene of adjacent concentrations that ranged from 0.00001 to 0.001 μL/μL. Significant differences ($p <$ 0.01) were found between 0.001 and 0.1 μL/μL concentration of phellandrene. When the concentrations were greater than 0.0001 μL/μL phellandrene, the differences in EAG responses of moths were not significant. These results indicate that the sensitivity of the EAG response of *Dendrolimus superans* to phellandrene concentrations ranges from 0.001 to 0.1 μL/μL.

EAG responses of adults to 0.00001 μL/μL (1R)-(+)-α-pinene, (1S)-(−)-α-pinene, ocimene, (1S)(−)-*β*-pinene, R (−)-α-phellandrene and camphene were lower than the control. These differences were statistically not significant (Fig. 1). The results of Xiao et al. (2000) show that when the EAG responses of female *Helicoverpa armigera* to cineole were significantly lower than control, these volatiles would shield the smelling reflection of female moths. It also indicated that the methods affecting the behavior of insects to volatiles are of vital importance for the implementation of modern technology in pest management. The results of our study need more evidence from practical experience.

The maximum of EAG responses of the three physiological stages of moths to 0.1 μL/μL (1R)-(+)-α-pinene and camphene was observed. For virgin females and unmated males, the maximum of EAG responses to four volatiles occurred at the same concentration, i.e., 0.1 μL/μL for ocimene, phellandrene and at 0.2 μL/μL of (1S)-(−)-α-pinene. For mated females, the maximum EAG responses at (1S)-(−)-*β*-pinene were lower than those of virgin females and unmated males. EAG responses of mated females at concentrations of 0.2 and 0.5 μL/μL for ocimene, phellandrene, (1S)-(−)-α-pinene

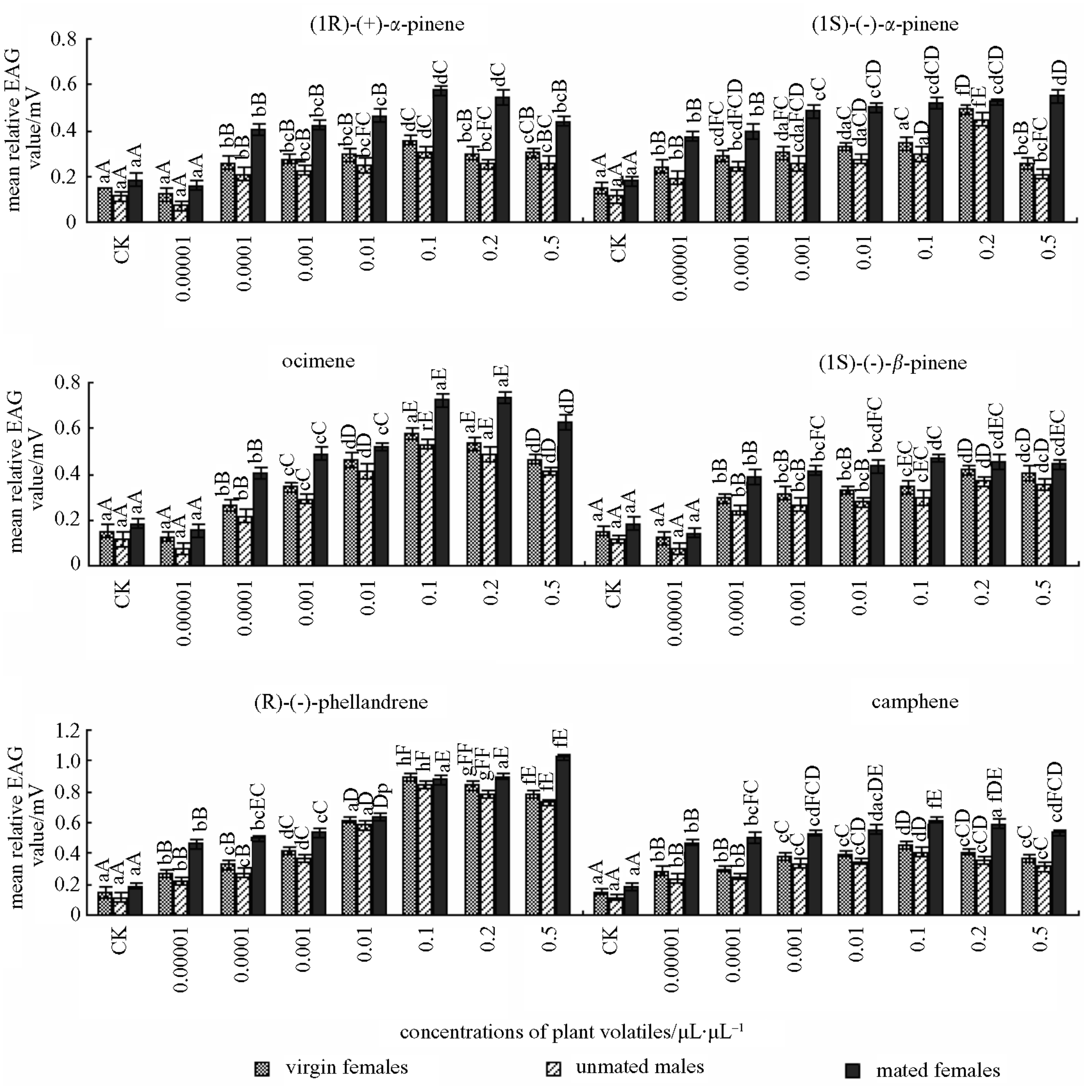

Fig. 1 EAG responses of *Dendrolimus superans* to seven concentrations of six standard volatiles
Note: different letters indicate significantly differences (small letters, $p < 0.05$; capital letters, $p < 0.01$)

were greater than those of virgin females and unmated males. These results demonstrate that mated females possess a wider range of perception to phellandrene and (1S)-(−)-α-pinene than the responses of moths. Phellandrene and (1S)-(−)-α-pinene are the long-distance directional infochemicals of moths searching for oviposition sites and host-plants. When the concentrations of (1R)-(+)-α-pinene, ocimene, (1S)-(−)-β-pinene and camphene exceed the range over which moths can be stimulated, EAG values present smooth trends or clearly decreased than that to threshold values of the concentrations of these four volatile components.

3.2 EAG responses to six standard volatiles under dark condition

Mated females were more sensitive to volatile components and more active than unmated males in the dark at the same time. The EAG responses were as follows: unmated males were responsive to 0.01 μL/μL phellandrene at 02:00, to 0.001, 0.01 and o.1 μL/μL phellandrene at 04:00, to 0.01 μL/μL ocimene at 20:00, and to 0.1 μL/μL ocimene at 02:00 and 04:00, which was greater than that of mated females. The EAG responses of mated females and unmated males to the six volatiles increased with the increases in concentrations (Figs. 2 and 3). Active time for the EAG responses of mated females and unmated males were from 20:00 to 04:00, the same with their eclosion, mating and oviposition periods (Li et al., 2002).

Active time for the EAG responses of mated females to 0.1 μL/μL (1R)-(+)-α-pinene presented a double peak, while to the other concentrations and volatiles a single peak was observed. The EAG responses of mated females to (1R)-(+)-α-pinene, (1S)-(−)-α-pinene, ocimene, (1S)-(−)-β-pinene were steadily increasing from the start of the dark period. The peak occurred at 23:00 and then decreased. The EAG responses of mated females to ocimene, phellandrene and camphene had little fluctuation before 21:00 and increased over time since then. The peaks of EAG values in response to phellandrene and camphene occurred at 01:00. To the other volatiles at different concentrations, the peaks appeared at 23:00. The EAG response of mated females to the volatiles decreased to a minimum at 05:00, but all of the EAG responses to 0.1 μL/μL (1R)-(+)-α-pinene, (1S)-(−)-α-pinene, ocimene, (1S)-(−)-β-pinene, R(−)-α-phellandrene and 0.01 μL/μL ocimene show a rising trend. The values of the EAG responses to the other concentrations of these five volatiles and various concentrations of camphene remained the same (Fig. 2).

The EAG response of unmated males to (1R)-(+)-α-pinene was a single peak, an exception, to the other five volatiles presented double peaks at 22:00 and 02:00. At midnight the EAG values dropped to the bottom and then reached a second maximum. The EAG responses increased to 0.001 μL/μL (1R)-(+)-α-pinene and (1S)-(−)-α-pinene and decreased considerably or appeared invariant to the other concentrations and volatiles after 04:00 (Fig. 3).

4 Conclusions

1) There were statistically significant differences in EAG responses between mated females, virgin females

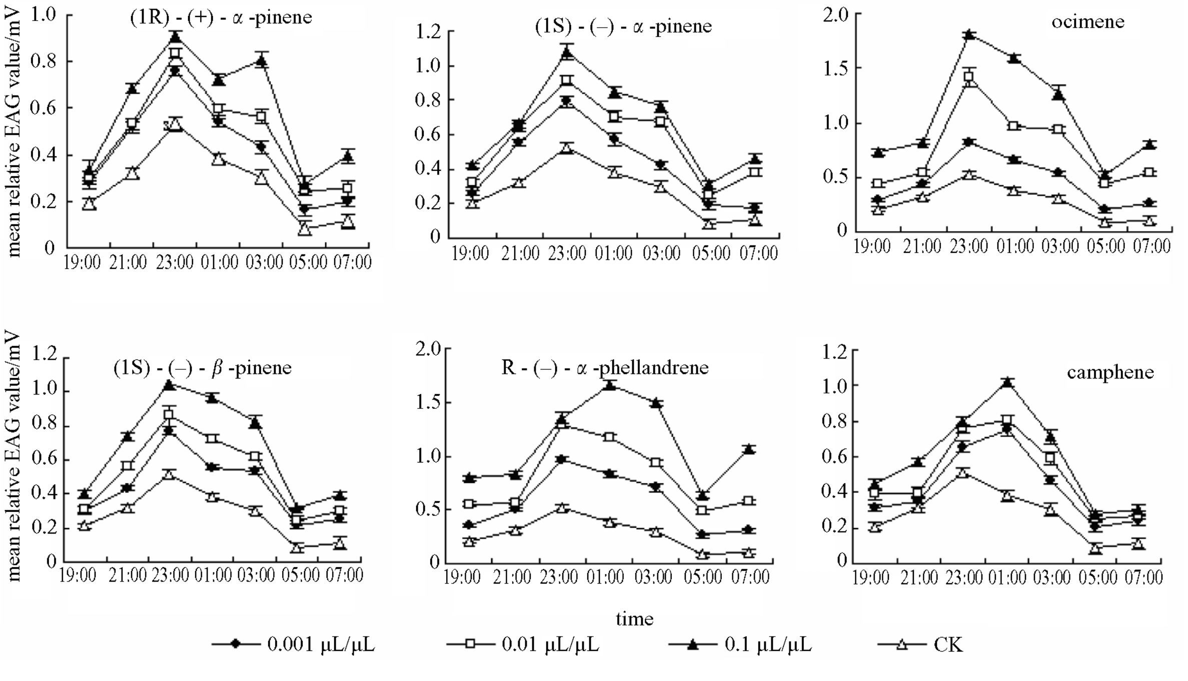

Fig. 2 EAG responses of females mated to three concentrations of six standard volatiles in the dark

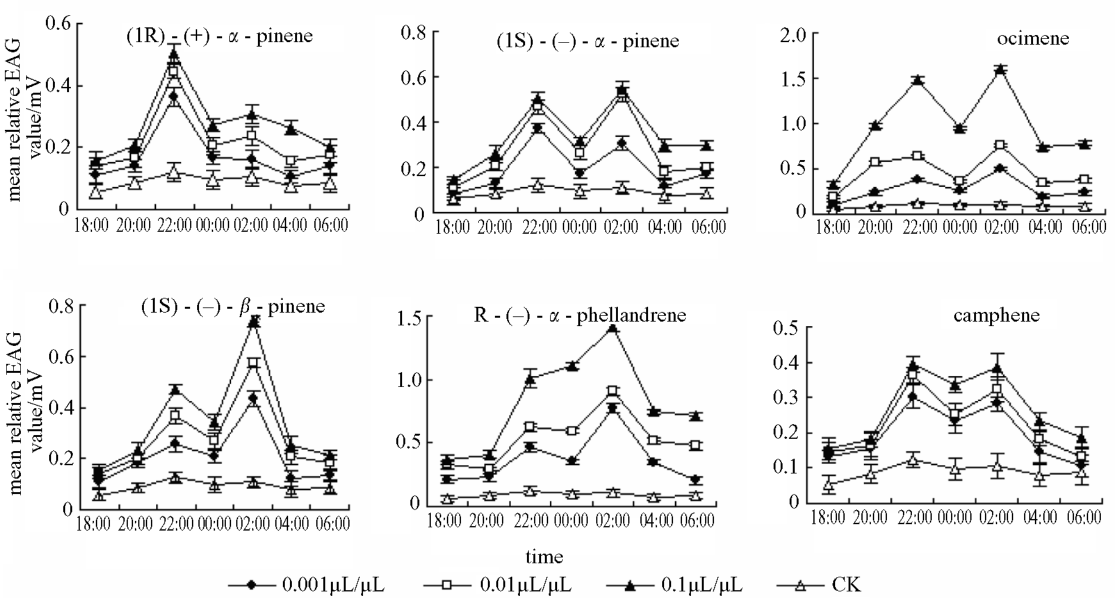

Fig. 3 EAG responses of unmated males to three concentrations of six standard volatiles in the dark

and unmated males to the standard compounds (1R)-(+)-α-pinene, (1S)-(−)-α-pinene, ocimene, (1S)-(−)-β-pinene, R(−)-α-phellandrene and camphene, the main components of the volatiles from *L. gmelinii*, which agrees with what had been reported in the studies of *D. punctatus* by Zhao et al. (1995). There were no obvious EAG responses being monitored to the six volatiles at low concentrations, but EAG responses increased with increases in volatile concentrations, reached a maximum and towards the end gradually decreased. Our research indicated that the EAG responses had an optimum range of volatile concentration. It provides a reliable theoretical basis for further research on the biological significance of volatiles.

2) The ordered EAG responses of moths to seven concentrations of the six compounds were mated females > virgin females > unmated males, except for the 0.1 μL/μL concentration of R(−)-α-phellandrene, which show an order of virgin females > mated females > unmated males. There were statistically significant differences between the EAG responses of virgin females and unmated males ($p < 0.05$) and also significant differences between mated females with virgin females and unmated males ($p < 0.01$), which show that the mated females were more sensitive to the volatiles of their host plants.

3) In the period of darkness, the active time for the EAG responses of both mated females and unmated males was from 20:00 to 04:00, the same with their eclosion, mating and oviposition periods.

4) Mated females gave significant olfactory responses to six standard volatiles of *L. gmelinii* during the periods of darkness and light, which showed that mated females were more sensitive to host-plant volatiles that related to their oviposition orientation. Tha unmated males showed the lowest EAG responses to the six standard volatiles at various concentrations during the period of light, but they may be more sensitive to female sex pheromones.

The bark of non-host plants releases *n*-hexanol inhibiting the behavioral response of *Ips typographus* to pheromone (Zhang et al., 2000). But the EAG responses of unmated males were greater than mated females to specific concentrations of ocimene and phellandrene in the dark. We presumed a synergistic effect with the combination of ocimene and phellandrene of host-plant volatiles and sex pheromone on male moth sensillums which induced significant EAG responses. We propose that sexual dimorphism exists in the sensillums of female and male moths.

5) Monoterpene deficiency is the reason for the moth outbreaks (Baranchikov et al., 1987). The terpenoid emission of needles increased four times after insects feed leaves of *L. gmelinii* and the possibility of damage by moths will be decreased (Guo et al., 1996). As our result showed, the moths showed significant EAG responses to terpenoid compounds of *L. gmelinii*. It is possible to disturb insect oviposition and feeding behavior with the using of exogenous terpenoid compounds, but the components and concentrations of terpenoid must be determined by biological assays before implementation.

6) Studies of plant volatiles and pheromones were interdependent and represent two routes for pest control. Although the synthesis of moths pheromones had been completed (Kong et al., 2006), satisfactory results had not been obtained by using pheromone trapping or disturbance alone. Repellent volatiles of plant origin from plant and sex pheromones used for pest control simultaneously would be a much better option. Both routes are equally important for integrated pest management.

References

Baranchikov Y N, Ryzhkova T S (1987). Transformation of essential oils and monoterpenes in the caterpillar of sibirian *Dendrolimus superans*. Ekologiya, 1: 41–46

Byers J A, Zhang Q H, Birgersson G (2004). Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors. Naturwissenschaften, 91(5): 215–219

Deepa S P, Regine G, John H B (2004). Antennal responses of four species of tree-killing bark beetles (Coleoptera: Scolytidae) to volatiles collected from beetles, and their host and nonhost conifers. Chemoecology, 14:59–66

Guo T Q, Yue S K, Liu N, Guo X F, Guo X H, Teng W X, Ping G Y (1996). Relations between odour of larch needles and the infection of *Dendrolimus superans*. J Northeast For Univ, 24(4): 80–86 (in Chinese)

John A B, Zhang Q H, Göran Birgersson (2004). Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors. Naturwissenschaften, 91: 215–219

John F T, Amy L C, Lawrence M H (2005). Plant volatiles are behavioral cues for adult females of the gall wasp *Antistrophus rufus*.Chemoecology, 15: 85–88

Kong X B, Zhang Z, Wang H B, Wang Y J, Kong Q H (2006). Investigation on sex pheromones in *Lasiocampidae*: advance and prospect. Sci Silv Sin, 42(6): 115–123 (in Chinese)

Kong X B, Zhang Z, Wang H B, Yang J, Hu Y T (2005). Analysis of *Platycladus* orientalis volatiles and their electroantennogram responses with *Semanotus bifasciatus*. For Res, 18(3): 260–266 (in Chinese)

Li J G, Luo Y Q, Jin Y J (1999). Electroantennogram activity of ashleaf maple (*Acer negundo*) volatiles to *Anoplophora glabripennis* (Motsch.). J Beijing For Univ, 21(4): 1–5 (in Chinese)

Li L, Meng H W, Sun X (2002). Bionomics of *Dendrolimus superans* Butler. J Inner Mongolia Inst Agric Anim Husban, 23(1): 101–103 (in Chinese)

Nehlin G, Valterová I, Barg-karlson A K (1994). Use of conifer volatiles to reduce injury caused by carrot psyllid, *Trioza apicalis* Forster (Homoptera, Psylloidea). J Chem Ecol, 20(3): 771–783

Pureswaran D S, Gries R, Borden J H (2004). Antennal responses of four species of tree-killing bark beetles (Coleoptera: Scolytidae) to volatiles collected from beetles, and their host and nonhost conifers. Chemoecology, 14(2): 59–66

Tooker J F, Crumrin A L, Hanks L M (2005). Plant volatiles are behavioral cues for adult females of the gall wasp *Antistrophus rufus*. Chemoecology, 15(2): 85–88

Wei H, Hou Y M, Yang G, You M S (2004). Repellent and antifeedant effect of secondary metabolites of non-host plants on *Plutella xylostella*. Chin J Appl Ecol, 15(3): 473–476 (in Chinese)

Xiao C, Du J W, Zhang Z N (2000). Electroantennogram responses of cotton bollworm (*Helicoverpa armigera*) to several plant volatiles. Acta Agric Jiangxi, 12(3): 27–31 (in Chinese)

Xiao G R (1992). Chinese Forest Insect. 2nd ed. Beijing: China Forestry Publishing House (in Chinese)

Yan S C, Hu Y Y, Sun J H, Alain R, Sun F (1999). Larch cone volatile profile and its damage by cone fly (*Strobilomyia* spp.) in northeastern China. Sci Silv Sin, 35(3): 58–62 (in Chinese)

Zhang Q H, Schlyter F, Birgersson G (2000). Bark volatiles from nonhost angiosperm trees of spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae): Chemical and electrophysiological analysis. Chemoecology, 10(2): 69–80

Zhang T X, Cui W Z, Sun X G, Zhang W G, Liang Z G (2005). Electroantennogram responses of *Acantholyda posticalis* Matsumura to volatiles of different trees. Acta Entomol Sin, 48(4): 514–517 (in Chinese)

Zhao C H, Wu D M, Yan Y H, Li Q (1995). Analysis of Masson pine needle volatiles and their electroantennogram activity with Masson pine caterpillar. Sci Silv Sin, 31(2): 125–131 (in Chinese)