Introduction

The gypsy moth Lymantria dispar (L.) (Lepidoptera: Lymantriidae) is a major defoliator of deciduous trees throughout the northern hemisphere (Elkinton and Liebhold, 1990) showing widespread outbreaks in temperate holarctic regions (Keena et al., 2008). L. dispar includes three subspecies: L. dispar dispar, L. dispar asiatica, and L. dispar japonica with different geographical ranges, habitats, and flight capabilities (Pogue and Schaefer, 2007).

Several outbreaks of the gypsy moth have been reported in Europe (McManus and Csóka, 2007). In North America, the gypsy moth is an invasive species, which was accidentally introduced around 1869. It has been gradually expanding its range (Liebhold et al., 1992) and has recently been defoliating millions of hectares of forest each year with economic losses amounting to millions of dollars (Leuschner et al., 1996). In China, the gypsy moth is native and distributed in most regions (Yang, 1996). More than 500 tree species have been reported as its suitable hosts (Schaefer et al., 1984) including the genera Quercus, Populus, Prunus, Cerarias, Malus, Crataegus, Armeniaca, Salix, Ulmus, Betula, Acer, Tilia, Picea, and Larix (Xiao, 1992). In recent years, the gypsy moth has been epidemic in the forests of Da Hingan Ling mountains in Inner Mongolia, especially on the Dahurian larch, Larix gmelinii. The sex pheromone of L. dispar was identified in the early 1970’s (Bierl et al., 1970). The synthetic sex pheromone, 2-methyl-7R,8S-epoxy-octadecane (disparlure), has been commonly used as a monitoring tool and applied in various pest management practices such as mass-trapping and mating disruption against this serious pest insect in many countries (Beroza and Knippling, 1972; Carde, 1976; Leonhardt et al., 1996). The same synthetic sex pheromone also strongly attracted L. dispar males in China (Miao et al., 1982; Wallner et al., 1984), and it was considered as an effective approach for both monitoring and mass-trapping of the moth (Miao et al., 1988). In contrast to the thoroughly studied sex-pheromone, on both basic and applied aspects, little is known about the potential roles of volatile organic compounds (VOCs, host plant volatiles) of the host trees in the host selection of the gypsy moth.
Our objectives in the present study were to 1) study whether the gypsy moth adults are attracted by the major volatile components of *Larix gmelinii* needles, 2) compare the attractiveness of the plant volatiles with that of the synthetic sex pheromone, 3) determine if there is any synergistic effect between the plant volatiles and sex pheromone, and 4) test electroantennogram (EAG) responses of major host plant volatiles on *Lymantria dispar*.

**Material and Methods**

**Study area**

The field study was carried out in the Aershan forestry region, located in the Inner Mongolia Autonomous Region in northern China (47° 07′ – 47° 55′ N, 119° 51′ – 120° 57′ E). Man-made forests cover 20% of the area, and the main tree species is Dahurian larch. Semi-natural forests have Asian white birch, *Betula platyphylla* Sukatschev, as an admixed deciduous tree species. The area has a cold, temperate, continental, monsoon climate with an elevation from 820 to 1745 m. Mean annual precipitation and temperature are 445.3 mm and –3.1 °C, respectively. Mean monthly minimum and maximum temperatures range between –25.6 and 16.6 °C.

**Experimental design in the field**

Field experiments were carried out in purely man-made *Larix* forests with three different age categories: young mean age [17 years; altitude, (934 ± 6) m above sea level (Sd); tree height, 8 m; diameter at breast height, 13 cm], middle-aged [29 years; (938 ± 8) m above sea level; tree height, 14 m; diameter at breast height, 20 cm], and mature stands [34 years; (912 ± 10) m above sea level; tree height, 16 m; diameter at breast height, 23 cm]. There were three replicates for each age category.

Cross-window traps were used to collect *L. dispar* adults. Each trap consisted of two perpendicular intercepting transparent polymethyl methacrylate (PMMA) panes (30 cm x 19 cm) and a basin below the panes, half-filled with water, salt, and a small amount of detergent for collecting insects. Traps baited with different lures or blank control were set up in lines in the larch stands as blocks, with 50 m between the traps within each block and more than 2 km between the trap blocks. They were hung on the larch trunks 1.3 m above ground. All traps were checked weekly from late July to early September, and the lures were refreshed at the same time.

Five different VOCs from *L. gmelinii* foliage damaged by the moth larvae (Guo et al., 1996; Yan, 1999) were used to set up the lures. In Lure I, five different VOCs (α-pinene, 1S-β-pinene, camphene, 3-carene, and 1-hexanol, see Table I) were placed in five different polyethylene bottles (Pherobio Tech, Beijing, China). In Lure II, the same five VOCs were placed in a single bottle with a total volume of 15 ml (Table I). Five polyethylene bottles were used as blank control (CK). Lure III was the synthetic gypsy moth sex pheromone in a polyethylene bottle (Trece, Inc., Salinas, CA, USA). Lure IV was a combination of Lure II and Lure III to test whether the VOCs and the sex pheromone had any synergistic effects. The sex pheromone can remain effective for three years. To test which VOC used in Lure II was the most attractive to *L. dispar*, five new traps baited with one VOC each at a time were erected.

The attractiveness of the different volatiles and the sex pheromone were tested in three successive years, 2008–2010. In 2008, experiments were carried out during July 28 to September 1, to test Lure I, Lure II, and a blank control in nine blocks. In 2009, experiments were carried out during the same period to test Lure I, Lure II, Lure III, Lure IV, and a blank control in nine blocks. In 2010, experiments were carried out during the same period to test Lure II, Lure III, Lure IV, the five separate volatiles, and a blank control in nine blocks.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source</th>
<th>Purity (%)</th>
<th>Proportion (%)</th>
<th>Lure I [µl]</th>
<th>Lure II [µl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>Fluka, Valencia, Spain</td>
<td>98</td>
<td>39.50</td>
<td>15000</td>
<td>4637.1</td>
</tr>
<tr>
<td>Camphene</td>
<td>Aldrich, Seelze, Germany</td>
<td>95</td>
<td>21.42</td>
<td>5678</td>
<td>2514</td>
</tr>
<tr>
<td>1S-β-Pinene</td>
<td>Alfa Aesar, Lancashire, UK</td>
<td>99</td>
<td>14.95</td>
<td>4772.6</td>
<td>1755.3</td>
</tr>
<tr>
<td>3-Carene</td>
<td>Aldrich, Seelze, Germany</td>
<td>90</td>
<td>12.57</td>
<td>8132.2</td>
<td>1475.4</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>Alfa Aesar, Lancashire, UK</td>
<td>99</td>
<td>11.56</td>
<td>4391.2</td>
<td>1357.5</td>
</tr>
</tbody>
</table>
Captured *L. dispar* were identified and counted. Voucher specimens were stored at the Aershan Forestry Bureau, Aershan, China and Beijing Forestry University, Beijing, China.

**Electroantennogram (EAG) responses**

In 2010 the egg masses of gypsy moths from Aershan were cultivated in an incubator and then divided into virgin males and females after their eclosion. The antenna was excised from the head of each animal so that all segments and the basal nerve were still attached. An indifferent glass capillary electrode, filled with Kaissling saline and grounded via a silver wire, was inserted into the severed moth’s head with antenna, while the recording electrode was placed in contact with the distal end of the antennal club. Signals were stored on a PC equipped with an intelligent data acquisition controller card and analysed with the program EAG 2000. The stimulation was amplified by a stimulus controller CS-55. All equipment was from Synthech, Hilversum, the Netherlands.

Reactions produced by Lure II and each of the five different VOCs were tested separately. Stimuli were prepared by applying VOCs (0.001 to 1000 µg/µl) in 10 µl of *n*-hexane on a piece of filter paper (25 mm x 5 mm) in a Pasteur pipette. The stimuli were tested at seven concentrations from the lowest to the highest. The antenna was mounted between two Kaissling saline-filled Ag/AgCl electrodes. A solvent blank (10 µl of *n*-hexane) and an active standard control (10 µg cis-3-hexen-1-ol per filter paper) stimulus were interspersed between two tested samples. Each stimulus lasted 0.1 s and was followed by a minimum of 60 s of filtered, humid air passing over the antenna to ensure recovery of antennal receptors. Three repetitive tests were done for each concentration of each stimulus, and six antennae were tested with each stimulus. The EAG responses were normalized to the relative response (%) of the active standard control.

**Data analysis**

EAG dose-response values of female and male gypsy moths to five volatiles and their mixture at seven dosages were calculated as the ratio to active standard control and presented as mean value ± standard deviation. Differences between values were analysed using analysis of variance (ANOVA) and Fisher LSD (least significant difference) test, with a *p* value of 0.05 set as the limit for statistical significance. The Fisher LSD test is a method for comparing treatment group means after the ANOVA null hypothesis of equal means has been rejected. The different responses of males and females to a chemical at the same dosage were compared by paired samples *T* test. Statistical analyses were performed using SPSS for Windows 16.0™.

**Results**

For Lure I only data of the two years, 2008 and 2009, are available. In 2008, the traps baited with Lure I caught 1208 male gypsy moths (Table II). The highest number of moths was captured on 11th August 2008 and also in 2009, on 4th August and 18th August (Fig. 1). The control traps caught only 15 moths. The number of individuals decreased in 2009: only 435 moths were captured by Lure I, but the control traps got 704 individuals.

For Lure II a three-year data set from 2008 to 2010 was obtained (Fig. 1). For Lure II, 4th August and 11th August were the two peak dates of caught moths in 2008 and 2009. There was a decrease in the number of captured moths from 2008 to 2009, however, In 2010, a rebound was found in the catch of moths: 4713 moths were captured in 2010. The number of moths caught with the control traps was 2376, which be due to the relatively high population density. These results indicate that the main flying time of the gypsy moth is in early to mid August in Inner Mongolia, but some variation between the years may exist because of weather conditions or forest management.

The field experiments examined the attractiveness of several types of lures in three successive years. The experiment in 2008 showed that the number of captured moths was significantly higher in the traps baited with Lure II than in those baited with Lure I. Both lures attracted a significantly higher number of moths than the control traps (Table II). Lure III and Lure IV were more effective than Lure I and Lure II (*p* < 0.05) in the field experiments in 2010, while Lure IV was more effective than Lure III (*p* < 0.05). Also the control traps captured many moths, and the number of moths was even higher in the control traps than in the traps baited with Lure I (Table II). In the field experiment in 2010, the five separate VOCs used as lures were found much less effective than Lure II, Lure III, and Lure IV indicating
that the subtraction of any of the five VOCs will decrease the effect of Lure II. The effect of Lure II was found similar to that of Lure III, while the attractiveness of Lure IV was the highest \((p < 0.05)\) (Table II).

EAG responses to compounds and dosages were tested in male and female gypsy moths (Table III). There was a significant effect of the dosage on the response. This response increased as the dosage increased from 0.001 to 1000 \(\mu\)g/\(\mu\)l for both male and female moths and for all chemicals. EAG responses to the mixture of the chemicals significantly peaked except the response of female moths to 3-carene with the dosages of 0.001 and 0.01 \(\mu\)g/\(\mu\)l. The results indicate that the largest amplitude of EAGs \((> 0.8 \mu\)g/\(\mu\)l) mostly in male moths was observed in responses to the mixture of 3-carene and camphene (Table I). Low EAGs \((< 0.15 \mu\)g/\(\mu\)l) were recorded in responses of females to \(\alpha\)-pinene and 1S-\(\beta\)-pinene at their lowest dosage (Table I). The EAG responses to 3-carene had the highest level in average compared to the other compounds, followed by camphene or 1-hexanol depending on the dosage and the sex of the moth. Compared to the other compounds, relatively low responses of the moths to 1S-\(\beta\)-pinene and \(\alpha\)-pinene were obtained. Comparison of EAG responses between female and male gypsy moths by paired samples \(T\) test indicated that the responses of male moths were significantly stronger than those of females at most dosages, especially to 1-hexanol.

**Discussion**

The gypsy moth \((Lymantria dispar)\) has become a major pest of deciduous trees, multiple countermeasures have been taken to control this pest. Entomopathogenic fungi such as *Entomophaga maimaiga* Humber, Shimazu & Soper (Hajek et al., 1998; Nielsen et al., 2005) and *Fusarium pallidoroseum* Sacc. (Dukes et al., 2009; Munshi et al., 2008), as well as entomopathogenic bacteria such as *Bacillus thuringiensis* (Martin et al., 2009) and nuclear polyhedrosis virus (NPV) (Grove and Hoover, 2007; Martemyanov et al., 2009) have been proven to be effective in control. In addition, applying pheromones for controlling the moth has long been under research (Carde, 1976). The application of the sex pheromone by mass-trapping or permeation of the air with pheromone disruption has been tried to prevent their propagation (Beroza and Knipling, 1972), as well as various management practices (Witzgall et al., 2010) and control treatments to slow down its spread (Leonhardt et al., 1996).

The relationship between phytophagous insects and their host plants is partially mediated by VOCs (Pophof et al., 2005). Some VOCs have been applied to manage the insect pests (Imai et al., 1998;
The electrophysiological and behavioural responses of various insects have proven that the emitted or synthetic volatiles can function as an attractant of e.g. several wood-dwelling insect taxa (Schlyter et al., 1987; Vrkocova et al., 2000; Zhang et al., 2001; Sullivan, 2005; Kendrick and Raffa, 2006; de Groot et al., 2008). Previous research has revealed that the emission of VOCs from holm oak (Quercus ilex L.) leaves can be affected by gypsy moth feeding (Staudt and Lhoutellier, 2007). Our present study firstly indicated that VOCs of damaged Dahurian larch were effective in attracting gypsy moth males especially in the peak flight period. Besides, the synergistic effect between host plant volatiles and sex pheromone was also obvious.

Comparison of EAG responses between female and male gypsy moths indicated that the response of male moths were significantly stronger than those of females at most dosages, except for 1-hexanol. The EAG response is considered as the expression of generator potentials of many simultaneously stimulated receptor cells (Schneider, 1969; Light et al., 1988). The EAG results indicated significant differences in the size of acceptor cell populations for the various examined stimuli (Payne, 1975; Light et al., 1988). A previous study (Topazzini et al., 1990) on the EAG responses of Lepidoptera species to 26 odorants showed that among α-pinene, β-pinene, and 1-hexanol, 1-hexanol had the strongest response of gypsy moths, significantly higher than that of the other two volatiles at all dosages for female moths and at most dosages for male moths. The response of 1-hexanol was followed by that of β-pinene and α-pinene while these two had no significant differences at all dosages for female moths. We also found an obvious increase in sex pheromone catches when baited with the plant volatiles. It was concluded that the volatiles could be applied as attractants of gypsy moths or as an supplementary of sex pheromone in controlling gypsy moths.

The attraction of male gypsy moths by VOCs of larch could be explained by the physiological responses (EAG tests) of gypsy moths, especially for the male moths. The fact that only the male moths were trapped may be explained by the life history of gypsy moth. The most important function of female gypsy moths is mating and laying eggs in relatively stable sites during the adult period, which poses limitations to their flying capabilities. Actually, suppression of male gypsy moths can already significantly limit the development of the moth population through mating disruption. However, the possibility of volatile compounds to attract female gypsy moths should be investigated in future studies.

The present study provides a new tool for monitoring the population dynamics of gypsy moths. However, only the males were caught in
Table III. EAG responses of female and male gypsy moths to five volatiles and their mixture at seven dosages. The comparisons were made among
the responses of individuals with same sex to each chemical at each dosage. Different letters indicate significant differences at the 0.05 level.

<table>
<thead>
<tr>
<th>Volatile</th>
<th>Sex</th>
<th>Dosage EAG response (%)</th>
<th>0.001 µg/ml</th>
<th>0.01 µg/ml</th>
<th>0.1 µg/ml</th>
<th>1 µg/ml</th>
<th>10 µg/ml</th>
<th>100 µg/ml</th>
<th>1000 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.12 ± 0.01 b</td>
<td>0.20 ± 0.06 b</td>
<td>0.24 ± 0.00 c</td>
<td>0.27 ± 0.01 d</td>
<td>0.39 ± 0.07 c</td>
<td>0.41 ± 0.06 c</td>
<td>0.54 ± 0.04 d</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>Female</td>
<td>0.001</td>
<td>0.12 ± 0.01 b</td>
<td>0.20 ± 0.06 b</td>
<td>0.24 ± 0.00 c</td>
<td>0.27 ± 0.01 d</td>
<td>0.39 ± 0.07 c</td>
<td>0.41 ± 0.06 c</td>
<td>0.54 ± 0.04 d</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0.19 ± 0.01 b</td>
<td>0.22 ± 0.02 c</td>
<td>0.26 ± 0.03 c</td>
<td>0.37 ± 0.03 d</td>
<td>0.48 ± 0.02 d</td>
<td>0.55 ± 0.03 c</td>
<td>0.64 ± 0.03 d</td>
<td>0.71 ± 0.03 d</td>
</tr>
<tr>
<td></td>
<td>T test</td>
<td>0.03</td>
<td>0.65</td>
<td>0.58</td>
<td>0.62</td>
<td>0.67</td>
<td>0.72</td>
<td>0.76</td>
<td>0.83</td>
</tr>
<tr>
<td>1S-β-Pinene</td>
<td>Female</td>
<td>0.11 ± 0.01 b</td>
<td>0.20 ± 0.03 b</td>
<td>0.24 ± 0.03 c</td>
<td>0.27 ± 0.03 d</td>
<td>0.31 ± 0.02 c</td>
<td>0.34 ± 0.03 b</td>
<td>0.37 ± 0.03 c</td>
<td>0.40 ± 0.03 d</td>
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<tr>
<td></td>
<td>Male</td>
<td>0.21 ± 0.03 b</td>
<td>0.24 ± 0.02 c</td>
<td>0.27 ± 0.02 c</td>
<td>0.37 ± 0.01 d</td>
<td>0.49 ± 0.03 d</td>
<td>0.52 ± 0.04 c</td>
<td>0.55 ± 0.04 d</td>
<td>0.64 ± 0.04 d</td>
</tr>
<tr>
<td></td>
<td>T test</td>
<td>0.03</td>
<td>0.33</td>
<td>0.57</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>3-Carene</td>
<td>Female</td>
<td>0.18 ± 0.02 a</td>
<td>0.32 ± 0.03 a</td>
<td>0.38 ± 0.06 b</td>
<td>0.43 ± 0.03 b</td>
<td>0.56 ± 0.01 b</td>
<td>0.65 ± 0.03 b</td>
<td>0.73 ± 0.01 b</td>
<td>0.82 ± 0.07 ab</td>
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<tr>
<td></td>
<td>Male</td>
<td>0.23 ± 0.04 ab</td>
<td>0.32 ± 0.02 b</td>
<td>0.34 ± 0.04 b</td>
<td>0.59 ± 0.03 b</td>
<td>0.71 ± 0.04 b</td>
<td>0.82 ± 0.02 b</td>
<td>0.88 ± 0.07 ab</td>
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<tr>
<td></td>
<td>T test</td>
<td>0.10</td>
<td>0.96</td>
<td>0.46</td>
<td>0.06</td>
<td>0.03</td>
<td>0.01</td>
<td>0.07</td>
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<tr>
<td>Camphene</td>
<td>Female</td>
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<td>0.28 ± 0.02 a</td>
<td>0.31 ± 0.02 bc</td>
<td>0.34 ± 0.03 c</td>
<td>0.54 ± 0.04 b</td>
<td>0.60 ± 0.02 b</td>
<td>0.68 ± 0.03 c</td>
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<td>Male</td>
<td>0.20 ± 0.01 ab</td>
<td>0.25 ± 0.01 b</td>
<td>0.28 ± 0.01 c</td>
<td>0.46 ± 0.04 c</td>
<td>0.62 ± 0.03 c</td>
<td>0.77 ± 0.02 c</td>
<td>0.83 ± 0.01 b</td>
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<tr>
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<td>0.03</td>
<td>0.13</td>
<td>0.06</td>
<td>0.07</td>
<td>0.02</td>
<td>0.00</td>
<td>0.01</td>
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</tr>
<tr>
<td>1-Hexanol</td>
<td>Female</td>
<td>0.16 ± 0.002 a</td>
<td>0.32 ± 0.003 a</td>
<td>0.36 ± 0.01 b</td>
<td>0.38 ± 0.02 c</td>
<td>0.53 ± 0.03 b</td>
<td>0.63 ± 0.02 b</td>
<td>0.71 ± 0.03 bc</td>
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<tr>
<td></td>
<td>Male</td>
<td>0.22 ± 0.01 ab</td>
<td>0.24 ± 0.03 bc</td>
<td>0.28 ± 0.02 c</td>
<td>0.40 ± 0.04 cd</td>
<td>0.61 ± 0.05 c</td>
<td>0.73 ± 0.02 c</td>
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<tr>
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<td>0.04</td>
<td>0.05</td>
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<td>0.01</td>
<td>0.01</td>
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<tr>
<td>Mixture</td>
<td>Female</td>
<td>0.16 ± 0.03 a</td>
<td>0.32 ± 0.05 a</td>
<td>0.45 ± 0.04 a</td>
<td>0.53 ± 0.04 a</td>
<td>0.66 ± 0.03 a</td>
<td>0.75 ± 0.03 a</td>
<td>0.84 ± 0.04 a</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0.24 ± 0.02 a</td>
<td>0.35 ± 0.01 a</td>
<td>0.43 ± 0.05 a</td>
<td>0.66 ± 0.05 a</td>
<td>0.79 ± 0.03 a</td>
<td>0.92 ± 0.04 a</td>
<td>0.95 ± 0.04 a</td>
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<tr>
<td></td>
<td>T test</td>
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</table>
the plant volatiles-baited traps. This is also true for the commonly used expensive sex pheromone lures. The physiological interpretation of this finding deserves more attention.

The following questions guide future studies: 1) How would two or more additional tree species releasing the same or similar volatiles like the existing host tree species affect the moth behaviour? 2) How would tree species releasing non-specific volatiles affect the moth behaviour? 3) How will the two kinds of volatiles affect together the moth behaviour?


Acknowledgement

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