

# Influences of host volatiles on feeding behaviour of the Japanese pine sawyer, *Monochamus alternatus*

J. T. Fan<sup>1,2</sup> and J. H. Sun<sup>1</sup><sup>1</sup>State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China; <sup>2</sup>Graduate School of the Chinese Academy of Sciences, Beijing, China*Ms. received: November 29, 2005; accepted: December 28, 2005*

**Abstract:** Choice and no-choice feeding assays on the twigs of three host species demonstrated the following feeding preference sequence by *Monochamus alternatus*: *Pinus massoniana* > *Cedrus deodara* > *Pinus thunbergii*. There were significant differences in the concentrations of  $\alpha$ -pinene, camphene, D-limonene,  $\beta$ -phellandrene, longifolene and  $\beta$ -caryophyllene in volatiles emitted by twigs among the three species. We tested the effects of six monoterpenes ( $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, myrcene, limonene and  $\beta$ -caryophyllene) added to an artificial diet consisting of bark from *P. thunbergii* on consumption rates by *M. alternatus*. The addition of  $\alpha$ -pinene at all four concentrations 0.4, 1.2, 3.6 and 10.8  $\mu$ l/ml resulted in increases of a twofold greater consumption rate than the control at a concentration of 3.6  $\mu$ l/ml. Limonene inhibited diet consumption at concentrations >0.4  $\mu$ l/ml. The concentration of  $\alpha$ -pinene in volatiles emitted by twigs was significantly higher for *P. massoniana* than for *P. thunbergii*, whereas the reverse was true for limonene. There were no differences for any of the other host components, suggesting that  $\alpha$ -pinene and limonene may play an important role in the adult's selection and acceptance of suitable and unsuitable feed host. Mixed compounds promoted the consumption of artificial diet at a concentration of 0.4  $\mu$ l/ml, whereas consumption was inhibited at a concentration of 10.8  $\mu$ l/ml. There were significant linear correlations ( $\beta$ -pinene:  $r^2 = 0.930$ ,  $P < 0.05$ ; myrcene:  $r^2 = 0.933$ ,  $P < 0.05$ ) between the amount of diets consumed and diet concentrations of  $\beta$ -pinene and myrcene. In conclusion, host volatile terpenes may stimulate or repel *M. alternatus* depending on terpene concentrations they encounter during initial feeding and then possibly inhibit further feeding activity once concentrations increase to threshold levels.

**Key words:** *Monochamus alternatus*, artificial diet, feeding behaviour, GC-MS, host volatiles, Japanese pine sawyer, terpenes

## 1 Introduction

The pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhner) Nickle (Tylenchida, Aphelenchoididae) is native to North America and was introduced into Japan, South Korea, Mexico and Portugal (Sun 2005) where it is the causal agent of the destructive pine wilt disease (Mamiya and Enda 1972). Since it was first detected in Nanjing province of China in 1982 (Sun 1982), the range of the pine wood nematode has expanded to Jiangsu, Hubei, Anhui, Zhejiang, Guangdong and Shandong provinces by 2002. More than 40 million pines over an area of 87 000 hectares have been killed in China alone, with a direct economic loss of 25 billion RMB (US\$3 billion) (Yang et al. 2003; Sun 2005).

The Japanese pine sawyer, *Monochamus alternatus* Hope (Col., Cerambycidae) is the key vector of the pine wood nematode in China with the two species sharing similar hosts (Kobayashi et al. 1984; Yang and Wang 1989; Yang et al. 2003; Chai and Jiang 2004). Newly emerged *M. alternatus* adults feed on the phloem of

healthy hosts with transmission of the pine wood nematode into healthy pine trees occurring through these feeding wounds within 25 days of emergence (Togashi 1985; Jikumaru and Togashi 2001; Yang et al. 2003). *Monochamus alternatus* has been recorded from > 15 species of *Pinus* as well as several species of *Abies*, *Cedrus*, *Picea*, *Larix* and *Malus* (Yang et al. 2003). Among these, the masson pine (*Pinus massoniana* Lamb.) is the main indigenous host species in 19 southern provinces of China, a region accounting for 40–50% of dead pines caused by pine wood nematodes in China (Zhao and Xu 1998; Chai and Jiang 2004).

Feeding preference behaviours of phytophagous insects are often mediated by host-produced chemical attractants and feeding stimulants (Maxwell et al. 1963; Daskotch et al. 1970; Levy et al. 1974; Matsuda and Matsumoto 1975; Jermy 1976; Lin et al. 1998). Compounds extracted from the bark of pine twigs, such as  $\beta$ -sitosterol, campesterol, sucrose, glucose, arabinoside, fructose, pinitol, mannitol, xylitol, galactose and inositol, stimulate feeding by adult

*M. alternatus* (Kobayashi et al. 1984; Yang et al. 2003).

It is possible that initial selection of host trees by *M. alternatus* may be mediated by olfactory cues with acceptance of hosts mediated by gustatory cues. The importance of volatile terpenes present in coniferous trees as attractants, and feeding and oviposition stimulants has been discussed in studies of feeding and oviposition behaviour for several other species of Coleoptera (Alfaro et al. 1980; Faccoli et al. 2005). Therefore, the aim of this study was to isolate and identify volatile substances emitted by twigs of preferred host species of pines in China and then to evaluate the role of these compounds in the feeding behaviour of *M. alternatus*.

## 2 Materials and Methods

### 2.1 Insects and plant materials

The study was conducted at Jingtingshan Forestry Centre, Xuancheng, Anhui, China, in 2003–2005. In order to attract large numbers of wild *M. alternatus*, we stressed masson pines by cutting three wounds on the stem in different directions with an axe. These trees were then injected with 5 ml of 5% aqueous solution of purified paraquat dichloride to create baited trees in June to July 2003. A week later, a large number of male and female *M. alternatus* were attracted to baited trees which proceeded to mate and oviposit on the stems of the baited trees. In April 2004, baited trees were felled and piled in large cages (4 m × 5 m × 3 m) made of an iron framework and a plastic window screen. Every day, fresh twigs of masson pine were placed in the cages as food for newly emerged adults. Adults in the cages were collected and kept at room temperature for 4 h without food prior to use in the feeding bioassays. All twigs used for feeding assays and volatiles collection were collected fresh from three pine species at the Jingtingshan Forestry Centre in 2004: 16–20-year-old masson pine; 20–22-year-old black pine (*Pinus thunbergii* Parl.); and 18–20-year-old deodar cedar (*Cedrus deodara* Loud.).

### 2.2 Chemicals

(*R*)-(+)- $\alpha$ -Pinene (chemical purity, 98%) was purchased from Acros Organics (Geel, Belgium). (–)- $\beta$ -Pinene (chemical purity, 80%), (*R*)-(+)-limonene (chemical purity, 96%) and *trans*-caryophyllene (chemical purity, 98%) were obtained from Fluka Chemie AG (Buchs, Switzerland). Myrcene (chemical purity, 98%) and (+)-3-carene (chemical purity, 90%) were obtained from Aldrich Chemical Co. (Milwaukee, WI).

### 2.3 Feeding on twigs

No-choice tests were conducted separately with 300 g of 1–3-year-old twigs of deodar masson pine, black pine and deodar cedar, placed separately into different cages (10 cm × 10 cm × 10 cm) that are made of wire netting and poplar batten. In the choice tests, 100 g of 1–3-year-old twigs of the three species were placed together in the same cage. Five replications, each with different groups of 10 adult *M. alternatus* (five females, five males) per cage, were run for both choice and no-choice tests. The feeding traces on the twigs were recorded with a drawing paper, and the drawing

paper was laid on 1 mm graph paper to measure the feeding area (mm<sup>2</sup>) for each test 48 h later. Each beetle was used for only one test and then discarded.

### 2.4 Volatiles collection and analysis

Volatile compounds emitted by the three species of host were collected from 200 g of twigs (1–3 years old) freshly cut from host plants using an absorbent trap collection method. Twigs were placed in polyvinyl plastic bags (Reynolds, Richmond, VA) with the cut wound kept outside the bag. Air was purified by passing over glass tubing [7 cm × 1 cm internal diameter (ID)] containing 5 cm of activated charcoal and allowed to flow into the plastic bags containing the test twigs. Each bag was attached to glass tubing (7 cm × 0.5 cm ID) sealed at the end with glass wool (Supelco, Bellefonte, PA) and containing 0.5 cm of XAD-2 (Amberlite, Philadelphia, PA). The tubing was then connected to the air inlet of QC-1 gas sampler (Beijing Municipal Institute of Labour Protection, Beijing, China), and another tube containing charcoal was connected to its air outlet. The flow meter ensured a constant airflow of 1.5 l/min. All components of the sampling set-ups were connected with silica gel tubing. Plant volatiles were sampled for 5 h (10:00–15:00 hours) and collected from each treatment in five replicates. Volatiles of each sample were separately eluted from the Supelco pack with 500  $\mu$ l hexane containing dodecane (150 ppb) as an internal standard. Dodecane serves as a qualitative internal standard, as it is not present in pine volatiles and is easily separated from the naturally present terpenes. Collected samples were stored at –5°C until analysis.

Collected samples were diluted by a factor of 20 with analysis-pure hexane and subsequently analysed by gas chromatograph-mass spectrometry (Agilent 6890N-5973N GC/MSD, Agilent Technologies, Palo Alto, CA, USA). The carrier gas was helium (99.999%) at a flow rate of 1 ml/min. A 60 m (length) × 0.25 mm (ID) 0.25  $\mu$ m (film) capillary column (DB-5MS; J&W Scientific, Folsom, CA, USA) was employed with 2- $\mu$ l sample injections in a 50:1 split ratio. The temperature was set at 50°C for 2 min and rose to 200°C at a rate of 5°C/min, and then maintained at 220°C for 5 min. The flame ionization detector temperature was 300°C, the injector temperature was 250°C. The mass spectrometer was operated in the 70-eV EI ionization mode. Spectra were continuously scanned in a mass range from 30 to 300 amu.

### 2.5 Feeding in the artificial diet

The bark-based artificial diet consists of ground black pine bark, agar, potassium sorbate, formaldehyde and water (table 1). Black pine was chosen as the surrogate diet species because it is the least favoured species among the three used

**Table 1.** The components of the artificial diet of *M. alternatus*

Component	Source of supply	Content
1 Pine powder*	<i>Pinus thunbergii</i>	55 g
2 Agar	Putian Heji Food Co. Ltd.	10 g
3 Potassium sorbate	Zhejiang Bossen Ingredients Co. Ltd.	0.7 g
4 Formaldehyde	Beijing Chemical Plant	0.7 ml
5 Water	–	300 ml

\*The phloem was removed from of 1–3-year-old twigs of *P. thunbergii* located in the Jingtingshan Forestry Centre, Xuancheng, Anhui.

in this study. The phloem of black pine twigs (1–3 years old) was finely chopped with a knife and ground into a powder with a blender (Geenwah, Jiangmen, Guangdong, China), and then filtered using a 25-mesh filter. Agar was put into the cooker after the water boiled. While the agar was completely dissolved, the pine powder and two common antiseptics, potassium sorbate and formaldehyde, were added to the mixture and completely mixed. After a few minutes of heating, the diet was poured into several 50-ml beakers.

Six chemicals, (+)- $\alpha$ -pinene, (-)- $\beta$ -pinene, myrcene, 3-carene, (+)-limonene and *trans*-caryophyllene, were selected to assess the effects of plant volatile terpenes on the feeding behaviour of *M. alternatus* adults. These terpenes of 20, 60, 180 or 540  $\mu$ l solution were separately incorporated into the bark-based artificial diet of 50 ml, after the diet was allowed to cool down a few minutes to avoid excessive volatilization of terpenes.

To further assess the role of plant volatile terpenes in regulating the feeding behaviour of *M. alternatus* adults, another artificial diet based on ground maize flour, instead of pine powder, was prepared as above. A blend of six terpenes of 20, 60, 180 and 540  $\mu$ l in  $\alpha$ -pinene :  $\beta$ -pinene :  $\beta$ -myrcene : 3-carene : D-limonene :  $\beta$ -caryophyllene = 92.76 : 12.23 : 7.76 : 0.74 : 5.75 : 4.18, as present naturally in the twig volatile profile for masson pine, was added to the maize-based artificial diet of 50 ml.

After the diet dried, it was chipped into equal four square nubs with a knife. In choice tests, the inside areas of glass Petri plates (120 mm diameter) were separated into two equal parts with a strip of filter paper attached to the bottom. The diet nub treated with terpenes was placed in one side of a Petri plate, and that not treated with terpenes was placed on the other side as a control. Thirty replications, each with one adult per Petri plate (15 females and 15 males), were performed for each treatment at a temperature regime of 18–28°C and a photoperiod of 14 : 10 h (light : dark). For each compound and each concentration, 10 Petri dishes containing two nubs with the same diet composition were used to estimate the natural loss of the water in the artificial diet. The nubs were weighed with an electronic precision balance (Kerndy, Fuzhou, Fujian, China) before and after a feeding period of 48 h. The ingested diets were recorded and calculated for each test. Meanwhile, the death rate of beetles were recorded. Each beetle was used for only one test.

## 2.6 Statistical analysis

Quantitative compositions of volatiles were calculated from peak areas using dodecane as the internal standard. The identification of the chemical constituents of each sample was based on a comparison of their retention times (Rt) and mass spectra with those obtained from the standard compounds and from the library spectra. Data analyses were carried out using statistical software SPSS 11.0 for Windows (SPSS Inc 2001). Differences between feeding areas and absolute contents of volatile compounds were compared by ANOVA at  $P \leq 0.05$ . Values were compared with Bonferroni multiple-

comparison test. Differences of consumed amount between artificial diet treated with terpenes and artificial diet with no terpenes (control) were analysed by paired-samples *t*-test. The correlations of the consumed amounts of artificial diet and concentrations of volatile chemicals were tested by linear regression.

## 3 Results

### 3.1 Feeding in twigs

In the no-choice tests, beetles fed more on twigs of masson pine and deodar cedar than on twigs of black pine with no significant difference between masson pine and deodar cedar (table 2). In the choice tests, beetle consumption was highest on twigs of masson pine and lowest on twigs of black pine with consumption of deodar cedar slightly less than that of masson pine. The preference sequence for adult *M. alternatus* on twigs of the three host species is as follows: masson pine > deodar cedar > black pine.

### 3.2 Volatiles analysis

Nine terpenes were identified from the volatiles emitted by twigs of host trees (fig. 1).  $\alpha$ -Pinene, camphene,  $\beta$ -phellandrene, longifolene and  $\beta$ -caryophyllene were more abundant in the volatiles emitted by twigs of masson pine than in volatiles emitted by twigs of black pine and deodar cedar. Limonene was more abundant in volatiles emitted by black pine than in volatiles emitted by masson pine and deodar cedar. The concentration of  $\beta$ -pinene,  $\beta$ -myrcene and 3-carene in volatiles did not differ among the three species.

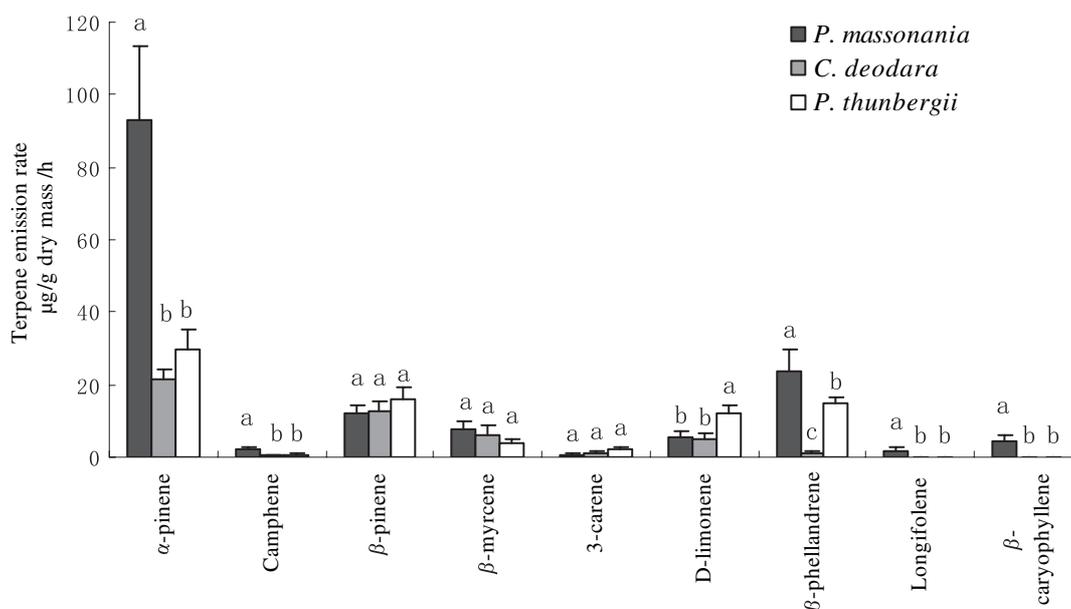
### 3.3 Feeding in the artificial diet

Consumption of the bark-based artificial diet was increased by the addition of  $\alpha$ -pinene at all four concentration levels of 0.4, 1.2, 3.6 and 10.8  $\mu$ l/ml (fig. 2). At a concentration of 3.6  $\mu$ l/ml, the consumption of diet enhanced with  $\alpha$ -pinene was twice as much as that of control diet. The consumed amount of artificial diet enhanced with limonene was no different than the control at 0.4  $\mu$ l/ml but significantly lower than the control at 1.2, 3.6 and 10.8  $\mu$ l/ml (fig. 2).

$\beta$ -Pinene,  $\beta$ -myrcene and 3-carene had a similar effect on consumption rates of enhanced bark-based artificial diet. At 0.4  $\mu$ l/ml, consumption of the enhanced diet was higher, whereas at 10.8  $\mu$ l/ml, it was lower. There were no differences in diet consumption between control and enhanced diets at 1.2 and 3.6  $\mu$ l/ml (fig. 2). Consumption of diet enhanced with

	No-choice (mm <sup>2</sup> /adult; mean $\pm$ SEM)	Choice (mm <sup>2</sup> /adult; mean $\pm$ SEM)
Feeding area of masson pine	230.56 $\pm$ 12.61 a	92.64 $\pm$ 8.87 a
Feeding area of deodar cedar	218.56 $\pm$ 15.68 a	58.34 $\pm$ 6.87 b
Feeding area of black pine	116.76 $\pm$ 10.54 b	36.38 $\pm$ 4.25 c
Mean values in the same column followed by different letters differ significantly at $P \leq 0.05$ using the Bonferroni approach ( $n = 50$ ).		

**Table 2.** Feeding results of *M. alternatus* on three species of pine twigs



**Fig. 1.** The quantitative composition analysis of twig volatiles in different hosts. a, b, c: different letters mean terpene emission rates (mean  $\pm$  SEM,  $n = 5$ ) are significantly different among different host species for each composition at  $P \leq 0.05$  using the Bonferroni approach

$\beta$ -caryophyllene was higher than control at 3.6  $\mu\text{l/ml}$  and then lower than control at 10.8  $\mu\text{l/ml}$ , whereas there were no differences at 0.4 and 1.2  $\mu\text{l/ml}$ .

The consumed amount of the maize-based artificial diet enhanced with a mixture of the seven compounds was significantly higher than that of the control at 0.4  $\mu\text{l/ml}$ , whereas consumption of enhanced diet was lower than that of the control at 10.8  $\mu\text{l/ml}$ . There were no significant difference in consumption rates of enhanced and control diets at 1.2 and 3.6  $\mu\text{l/ml}$  (fig. 2). Besides, the death rate of beetles reared on the maize-based diet was 10.35% after 48 h, whereas the death rate on bark-based diet was  $< 1\%$  after 48 h.

There were significant linear regressions between the consumed amounts and increasing concentrations of  $\beta$ -pinene and myrcene ( $\beta$ -pinene:  $r^2 = 0.930$ ,  $P < 0.05$ ; myrcene:  $r^2 = 0.933$ ,  $P < 0.05$ ). Moreover, there were no significant linear correlation between the consumed amounts and increasing concentrations of  $\alpha$ -pinene, 3-carene, limonene,  $\beta$ -caryophyllene and mixed compounds ( $\alpha$ -pinene:  $r^2 = 0.055$ ,  $P = 0.766$ ; 3-carene:  $r^2 = 0.805$ ,  $P = 0.103$ ; limonene:  $r^2 = 0.484$ ,  $P = 0.304$ ;  $\beta$ -caryophyllene:  $r^2 = 0.475$ ,  $P = 0.311$ ; mixed compounds:  $r^2 = 0.750$ ,  $P = 0.134$ ).

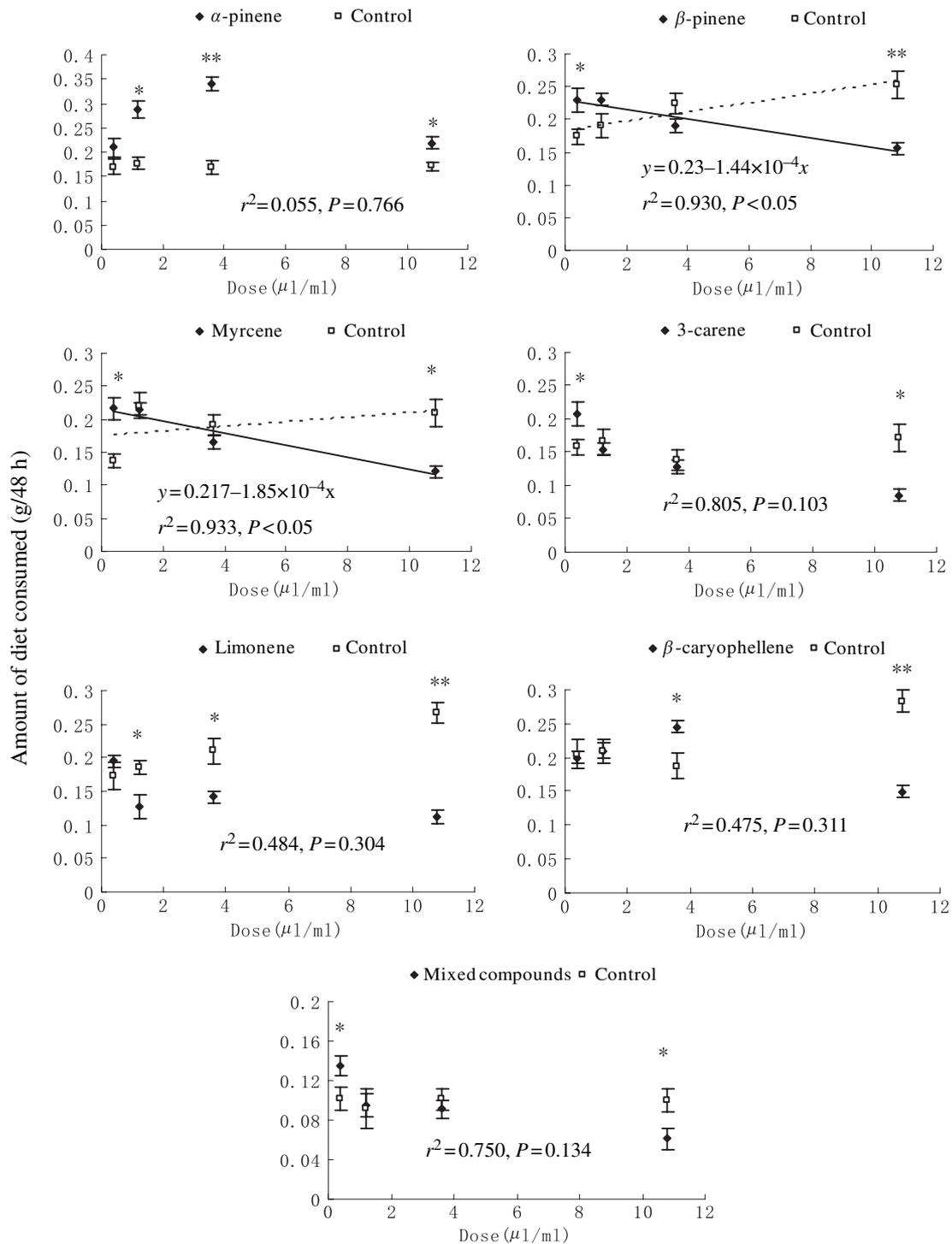
#### 4 Discussion

In the no-choice tests, consumption of deodar cedar was similar to that of masson pine (table 2), suggesting that deodar cedar may be susceptible to feeding by *M. alternatus* in the wild. Such damage would have significant consequences because deodar cedar is an important ornamental pine species and distributed widely in northern China. Currently, damage by *M. alternatus* is mainly limited to southern and central parts of China, areas dominated by masson pine. If the

northern distribution limit of *M. alternatus* in China is limited only by the  $-10^\circ\text{C}$  isotherm of January mean air temperature (Ma et al. 2005) then both suitability of deodar cedar as a host and climatic conditions could lay a foundation for the potential dispersal of *M. alternatus* into northern China. A critical issue yet to be determined is the suitability of deodar cedar for the whole life history of *M. alternatus*.

Black pine was the least favoured host of *M. alternatus* among the three species tested in this study. Differences in volatile blends emitted by twigs may aid beetles in differentiating between the three hosts. The concentrations of  $\alpha$ -pinene and  $\beta$ -phellandrene were significantly higher for masson pine twigs than for black pine twigs (fig. 1).  $\alpha$ -Pinene significantly promoted the consumption of bark-based artificial black pine diet at 0.4, 1.2, 3.6 and 10.8  $\mu\text{l/ml}$ ; consumption was doubled at 3.6  $\mu\text{l/ml}$ . This diet enhancement mirrored the natural condition in masson pine, the most preferred host, suggesting that part of the basis for preference of masson pine by *M. alternatus* may simply relate to the concentration of  $\alpha$ -pinene in the phloem tissue. Field studies show that  $\alpha$ -pinene is one of the most important components in the attractant of *M. alternatus* and is a key olfactory cue in finding the suitable ovipositional place (Ikeda et al. 1980, 1986).

Enhancement with limonene inhibited diet consumption at concentrations  $> 0.4 \mu\text{l/ml}$ . These results are consistent with our finding that the concentration of limonene in masson pine is significantly lower than that in black pine. Tiberi (1999) demonstrated that (*R*)-(+)-limonene deters oviposition by the pine processionary caterpillar, *Thaumetopoea pityocampa*. Limonene inhibits attraction of the pine weevils *Hylobius abietis* and *Hylobius pinastri* to  $\alpha$ -pinene in field tests (Nordlander 1990, 1991; Lindgren et al. 1996). Studies of *Pinus ponderosa* and *Dendroctonus*



**Fig. 2.** Influence of host volatile chemicals on the amount of artificial diet consumed by *M. alternatus*. Differences of consumed amount between artificial diet treated with terpenes and artificial diet with no terpenes (control) were analysed by paired-samples *t*-test. Significant differences at \* $P \leq 0.05$  and \*\* $P \leq 0.01$ . The correlation of the amount of artificial diet consumed and increasing quantities of volatile chemicals was tested by linear regression

*brevicomis* LeConte show that tree resistance is linked to higher limonene contents (Sturgeon 1979).  $\beta$ -Pinene, myrcene, 3-carene and  $\beta$ -caryophyllene also inhibited consumption of bark-based diet although  $\beta$ -pinene and myrcene at low concentrations seemed to enhance consumption (fig. 2).

The three species of hosts vary in terms of host volatile composition, particularly with respect to  $\alpha$ -pinene

and limonene. Our data demonstrate that olfactory terpenes can either stimulate or repel *M. alternatus* depending on the concentration; they can first increase and then inhibit feeding activity once they have reached a certain threshold.

The consumed amounts of the maize-based artificial diet with or without mixed compounds were significantly lower than those of the bark-based artificial diet

of black pine. Moreover, the death rate of beetles reared on the maize-based diet was largely higher than that on bar-based diet. These data suggest either that black pine has some additional components or that there are some lethal compounds in the maize-based diet.  $\beta$ -Phellandrene may well play a role as it is found in the volatiles. However, we were unable to obtain  $\beta$ -phellandrene for our studies.

Moreover, the importance of visual cues in host selection behaviour has been reported in many phytophagous insects (e.g. Prokopy and Owens 1983; Aluja and Prokopy 1993; Harris et al. 1993; Szentesi et al. 1996; Butkewich and Prokopy 1997). However, the interaction of olfactory cues with visual cues is still poorly understood in the host-finding behaviour of cerambycid species. Further studies on the role of visual cues of *M. alternatus* and potential interaction between visual and chemical cues should be carried out.

### Acknowledgements

We greatly appreciate a thorough review of an earlier draft of the manuscript by Dr Daniel R. Miller, USDA Forest Service. We sincerely thank Dr Li Shuiqing (Institute of Zoology, Chinese Academy of Sciences) and Mr Ma Sheng'an (Director of Xuancheng Forest protection Station, Anhui) for his assistance with the experiment work. Thanks are also extended to Dr Zhang Longwa (Institute of Zoology, Chinese Academy of Sciences) for his helpful comments on the manuscript. This work was funded by the CAS Knowledge Innovation Program (KSCX1-SW-13).

### References

- Alfaro RI, Pierce HD Jr, Borden JH, Oehlschlager AC, 1980. Role of volatile and nonvolatile components of Sitka spruce bark as feeding stimulants for *Pissodes strobi* Peck (Coleoptera: Curculionidae). *Can. J. Zool.* **58**, 626–632.
- Aluja M, Prokopy RJ, 1993. Host odor and visual stimulus interaction during intratree host finding behavior of *Rhagoletis pomonella* flies. *J. Chem. Ecol.* **19**, 2671–2696.
- Butkewich SL, Prokopy RJ, 1997. Attraction of adult *Plum curculios* (Coleoptera: Curculionidae) to host-tree odor and visual stimuli in the field. *J. Entomol. Sci.* **32**, 1–6.
- Chai XM, Jiang P, 2004. Occurrence and Control of Pine Wilt Disease. Chinese Agricultural Press, Beijing (in Chinese).
- Doskotch RW, Chatterji SK, Peacock JW, 1970. Elm bark derived feeding stimulants for the smaller European elm bark beetle. *Science* **167**, 380–382.
- Faccoli M, Blazenc M, Schlyter F, 2005. Feeding response of host and nonhost compounds by males and females of the spruce bark beetle *Ips typographus* in a tunnelling microassay. *J. Chem. Ecol.* **31**, 745–759.
- Harris MO, Rose S, Malsch P, 1993. The role of vision in the host plant-finding behaviour of the Hessian fly. *Physiol. Entomol.* **18**, 31–42.
- Ikeda T, Enda N, Yamane A, Oda K, Toyoda T, 1980. Attractants for the Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae). *Appl. Entomol. Zool.* **15**, 358–361.
- Ikeda T, Yamane A, Enda N, Oda K, Makihara H, Ito K, Okochi I, 1986. Attractiveness of volatile components of felled pine trees for *Monochamus alternatus* (Coleoptera: Cerambycidae). *J. Jpn. For. Soc.* **68**, 15–19 (in Japanese with English summary).
- Jermy T, 1976. The host-plant in relation to insects behaviour and reproduction. *Symp. Biol. Hung.* **16**, 1–322.
- Jikumaru S, Togashi K, 2001. Transmission of *Bursaphelenchus mucronatus* (Nematoda: Aphelenchoididae) through feeding wounds by *Monochamus saltuarius* (Coleoptera: Cerambycidae). *Nematology* **3**, 325–333.
- Kobayashi F, Yamane A, Ikeda T, 1984. The Japanese pine sawyer beetles as the vector of pine wilt disease. *Ann. Rev. Entomol.* **29**, 115–135.
- Levy EC, Ishaaya I, Gurevitz E, Cooper R, Lavie D, 1974. Isolation and identification of host compounds eliciting attraction and bite stimuli in the fruit tree bark beetle, *Scolytus mediterraneus*. *J. Agric. Food. Chem.* **22**, 374–379.
- Lin S, Binder BF, Hart ER, 1998. Insect feeding stimulants from the leaf surface of *Populus*. *J. Chem. Ecol.* **24**, 1781–1790.
- Lindgren BS, Nordlander G, Birgersson G, 1996. Feeding deterrence of verbenone to the pine weevil, *Hylobius abietis* (L) (Col, Curculionidae). *J. Appl. Entomol.* **120**, 397–403.
- Ma RY, Hao SG, Kong WN, Sun JH, Kang L, 2005. Cold hardiness as factors accessing the distribution and dispersal possibility of the Japanese pine sawyer *Monochamus alternatus* (Coleoptera: Cerambycidae) in China. *Ann. For. Sci.* (in press).
- Mamiya Y, Enda N, 1972. Transmission of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae) by *Monochamus alternatus* (Coleoptera: Cerambycidae). *Nematologica* **18**, 159–162.
- Matsuda K, Matsumoto Y, 1975. Feeding stimulation of the organic acids from the polygonaceous plants [strawberry, *Rumex obtusifolius*] to four species of Chrysomelidae. *Jpn. J. Appl. Entomol. Zool.* **19**, 281–284.
- Maxwell FG, Jenkins JN, Keller JC, Parrott WL, 1963. An arrestant and feeding stimulant for the boll weevil in water extracts of cotton plant parts. *J. Econ. Entomol.* **56**, 449–454.
- Nordlander G, 1990. Limonene inhibits attraction to  $\alpha$ -pinene in the pine weevil, *Hylobius abietis* and *H. pinastri*. *J. Chem. Ecol.* **16**, 1307–1320.
- Nordlander G, 1991. Host finding in the pine weevil, *Hylobius abietis*: effects of conifer volatiles and added limonene. *Entomol. Exp. Appl.* **59**, 229–237.
- Prokopy RJ, Owens ED, 1983. Visual detection of plants by herbivorous insects. *Ann. Rev. Entomol.* **28**, 337–364.
- SPSS Inc, 2001. SPSS for Windows, Rel. 11.0.1. SPSS Inc, Chicago, IL.
- Sturgeon KB, 1979. Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. *Evolution.* **33**, 803–814.
- Sun YC, 1982. *Bursaphelenchus xylophilus* was discovered in Sun Yet-sen's mausoleum in Nanjing. *J. Jiangsu For. Sci. Tech.* **4**, 47 (in Chinese).
- Sun JH, 2005. Forest invasive species: country report – P.R. China. In: *The Unwelcome Guests, Proceedings of the Asia-Pacific Forest Invasive Species Conference*. Ed. by Philip M, Chris B, Sun JH, Wu J, FAO Regional Office for Asia and Pacific, Bangkok, 80–86.
- Szentesi A, Hopkins TL, Collins RD, 1996. Orientation responses of the grasshopper, *Melanoplus sanguinipes*, to visual, olfactory and wind stimuli and their combinations. *Entomol. Exp. Appl.* **80**, 539–549.
- Tiberi R, Niccoli A, Curini M, Epifano F, Marcotullio MC, Rosati O, 1999. The Role of the monoterpene composi-

- tion in *Pinus spp.* needles, in host selection by the pine processionary caterpillar, *Thaumetopea pityocampa*. *Phytoparasitica* **27**, 263–272.
- Togashi K, 1985. Transmission curves of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) from its vector, *Monochamus alternatus* (Coleoptera: Cerambycidae), to pine trees with reference to population performance. *Appl. Ent. Zool.* **20**, 246–251.
- Yang B, Wang Q, 1989. Distribution of the pine wood nematode in China and susceptibility of some Chinese and exotic pines to the nematode. *Can. J. For. Res.* **19**, 1527–1530.
- Yang BJ, Pan HY, Tang J, Wang YY, Wang LF, 2003. *Bursaphelenchus xylophilus*. Chinese Forestry Press, Beijing (in Chinese).
- Zhao ZD, Xu FY, 1998. Recent progress of research on relations between pine chemistry and pine wilt disease caused by PWN. *Chem. Indust. For. Prod.* **18**, 83–88 (in Chinese).
- Author's address:** Jiang Hua Sun (corresponding author), State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China. E-mail: sunjh@ioz.ac.cn