Ann. For. Sci. 64 (2007) 67–71
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DOI: 10.1051/forest:2006089

Original article

Attraction of the Japanese pine sawyer, *Monochamus alternatus*, to volatiles from stressed host in China

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(Received 15 July 2005; accepted 26 April 2006)

Abstract – Ovipositing female Japanese sawyer beetles, *Monochamus alternatus*, prefer stressed *Pinus massoniana* over healthy trees. Host discrimination by *M. alternatus* suggests that changes in the chemical composition of pines may mediate the host preference of beetles. Volatile compounds from stressed and healthy pine stems were collected using absorbent trap collection method. Significant differences in absolute terpene quantities between stressed and healthy pines occurred for 7 of 10 terpenes. Field trials demonstrated that four terpenes identified from host pines were attractive to *M. alternatus* with (+)-α-pinene as the most attractive compound to *M. alternatus.* Ethanol appeared to be an important synergistic compound causing significant increase in attraction.

***Monochamus alternatus* / pine volatile / trapping / terpene / attractant**

Résumé – Des composés émis par les arbres-hôtes stressés sont attractifs pour le cérambycidé *Monochamus alternatus*. Les femelles du cérambycidé *Monochamus alternatus* préfèrent pondre sur les arbres stressés que sur les arbres sains de *Pinus massoniana*. Cette discrimination dans le choix de l'hôte par l'insecte pourrait être reliée à des modifications intervenues dans la composition chimique des pins. Les composés volatils émis par des pins stressés et sains ont été collectés en utilisant une méthode de piégeage sur résine adsorbante. La quantité absolue de 7 terpènes sur les 10 analysés diffère significativement entre pins stressés et sains. Des essais sur le terrain ont montrés que 4 de ces terpènes sont attractifs pour *M. Alternatus*. l'(+)-α-pinène étant le compose le plus attractif. L'éthanol semble être un composé synergique induisant une augmentation significative de l'attraction.

***Monochamus alternatus* / composés / volatil des pins / piégeage / terpènes / attractif**

1. INTRODUCTION

The Japanese pine sawyer beetle, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), is the most important vector for the transmission of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (Nematoda: Aphelenchoididae), in Japan and China [7, 13, 29, 30]. The direct economic losses caused by the pine wood nematode in China are estimated at approximately 2.5 billion RMB with indirect economic losses exceeding 25 billion RMB [29]. *M. alternatus* has been recorded on more than 15 species of *Pinus*, plus several species of *Abies, Cedrus, Picea,* and *Larix* [29]. Losses of Masson pine, *Pinus massoniana*, an indigenous species found in 19 southern provinces of China, has counted for 40–50% of tree mortality in southern China [7, 32].

Location of suitable host plants by herbivorous insects upon which to feed or oviposit may be mediated by plant volatile semiochemicals [3, 9, 20]. Some insects can identify unsuitable hosts by detecting ratios of common plant components not normally encountered in suitable hosts [5, 25, 26]. The importance of terpenes present in coniferous trees as oviposition stimulants or feeding deterrents to bark and wood boring insects has been well established [1, 12, 14, 21]. Terpenes emitted from or contained in stressed or injured pines may influence host selection during the oviposition period [27]. Ikeda et al. [11] collected the volatiles from the felled *P. densiflora* by means of the cold trap method and identified 11 monoterpenes. In field trials, they demonstrated that the addition of ethanol significantly increased catches of *M. alternatus* to terpene baited traps.

Observations indicated that ovipositing female *M. alternatus* prefer stressed to healthy *P. massoniana*, so host discrimination by *M. alternatus* may be related to changes in the chemical composition of pines following injury or stress conditions. The objective of this study was to investigate the mechanism underlying host discrimination by *M. alternatus* on Masson pine in China. Specifically, we attempted to isolate and compare terpenes emitted by injured, stressed trees versus healthy ones. Once the key compounds were identified,

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trapping experiments were conducted to assess the attractiveness individually and in various ratios.

2. MATERIALS AND METHODS

2.1. Pine material

The study was conducted at Jingtingshan Forestry Centre, Xuancheng, Anhui, in 2004 using 14- to 16-year old Masson pines. Stressed trees were created by cutting three wounds on the stem in different directions with an axe, then injecting wounds with 5 mL of 5% aqueous solution of purified paraquat dichloride.

2.2. Volatiles collection and analysis

Volatiles were collected from the stems of the test trees using an absorbent trap collection method one week post-treatment. Polyvinyl plastic bags (Reynolds, Richmond, VA, USA) were tightly bound on the stems. Each bag was attached to glass tubing (7 cm × 0.5 cm-ID) containing 0.5 cm of XAD-2 (Amberlite, Philadelphia, PA, USA) and sealed at the end with glass wool (Supelco, Bellefonte, PA, USA). The tubing was then connected to the air inlet of a QC-1 gas sampler (Beijing Municipal Institute of Labour Protection, Beijing, China). A second tubing (7 cm × 1 cm-ID) attached to the bags contained 5 cm of activated charcoal was connected to the air outlet of the QC-1 gas sampler allowing purified air flow into the bags. A constant airflow of 1.5 L/min was maintained by a flowmeter. All devices were connected with silica gel tubing. Plant volatiles were sampled for 5 h (10:00–15:00) with five replicates per treatment. Volatiles of each sample were separately eluted from the Supelco pack with 500 μL hexane containing 50 ng/μL dodecane. The dodecane served 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 needed.

Collected samples were diluted by a factor of 20 with analysis-grade pure hexane and subsequently analyzed 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 μm (film) capillary column (DB-5MS, J&W Scientific, Folsom, CA, USA) was employed with 2 μL sample injections done in 50:1 split ratio. The temperature program was 50 °C for 2 min, then increased to 200 °C at a rate of 5 °C/min, and finally increased and maintained at 220 °C for 5 min. The flame ionization detector temperature was 300 °C, with an injector temperature was 250 °C. The mass spectrometer was operated in the 70 eV ionisation mode (EI). Spectra were continuously scanned in a mass range from 30–300 amu.

2.3. Field trap experiment

The field trapping experiments were conducted in 2004 in the Jingtingshan Forestry Centre of Xuancheng, Anhui province. The attractive effects of the monoterpenes (+)-α-pinene, (-)-β-pinene, and (+)-3-carene were tested, as they were detected in the analyses of host volatiles. Terpinolene was included as it previously had been used in field trials to assess attraction of *M. alternatus* [11]. Each monoterpene was tested individually. An orthogonal design method also was employed to compare the attraction of different ratios of a blend of the four monoterpenes [15]. This design allows four factors (the monoterpenes) at three concentrations to be tested simultaneously, providing an easy assessment of optimal protocol conditions. Ethanol also was tested as a potential synergist. The treatments and ratios employed in the trapping experiments for *M. alternatus* are presented in Table II.

A cross vanes type trap was used for beetle collection [28]. The traps were suspended from a stick attached horizontally to two adjacent trees. The collection cups ca. 80 cm above ground level, the height at which *M. alternatus* attacks trees. The elution device for the chemicals was a closed 18 mL polyethylene release bottles, manufactured at the Hongzhi Plastic Plant in Taiyuan, Shanxi Province. Each bottle contained 15 mL of the specific monoterpene or semiochemical blend. The release rate of the lures was 300 μL/d, with replacement every 4 weeks [23].

Five replicates each of 15 treatments were randomly deployed at the Centre with a spacing of 20 m between traps. Ten traps with an empty release bottle served as controls. The experiment was established 1 June 2004, and beetles were collected from traps every 7 days through 1 September 2004. This time interval corresponds with the oviposition period of the beetles [6]. All *M. alternatus* adults captured in each trap on each date were counted and recorded. Female were dissected and the number of eggs counted.

2.4. 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 NIST Mass Spectral library. Data analyses were carried out using statistical software SPSS 11.0 for Windows [22]. Differences in absolute contents of volatile compounds between healthy and stressed pines were compared by Paired-Samples T Test. Differences of beetles attracted by different treatments were analyzed by One-Way ANOVA. Means were compared with Bonferroni multiple-comparison test. The optimal combination of the four compounds was analyzed by Univariate of General Linear Mode [31].

3. RESULTS

3.1. Volatiles analysis

There were significant differences between stressed and healthy trees for 7 of the 10 terpenes detected (Tab. I). Camphene, β-phellandrene, α-copaene, longifolene and β-caryophyllene were not detected in healthy trees. Moreover, α-pinene, β-pinene and D-limonene were more abundant in stressed trees than in healthy ones. Approximately 81% of the total volatiles emitted by stressed trees was α-pinene, and the level of α-pinene was more than 100 times higher than in healthy trees. β-Myrcene, 3-carene, and α-copaene were found in trace amounts and were not significantly different between stressed and healthy stems.

Table I. The quantitative analysis between the volatiles of stressed and healthy pines.

		Stressed pines (ng)	Healthy pines (ng)	*P*
1	α−pinene	89.65 ± 23.05 a	0.80 ± 0.22 b	0.031 < 0.05
2	Camphene	2.44 ± 0.86 a	0 b	0.038 < 0.05
3	β−pinene	5.41 ± 1.70 a	0.18 ± 0.08 b	0.039 < 0.05
4	β−myrcene	1.17 ± 0.32 a	0.18 ± 0.18 a	0.116
5	3-carene	0.65 ± 0.25 a	0.55 ± 0.22 a	0.800
6	D-limonene	3.75 ± 0.79 a	1.05 ± 0.35 b	0.040 < 0.05
7	β−phellandrene	1.18 ± 0.34 a	0 b	0.043 < 0.05
8	α−copaene	0.49 ± 0.32 a	0 a	0.191
9	Longifolene	3.79 ± 1.28 a	0 b	0.045 < 0.05
10	β−caryophyllene	0.68 ± 0.19 a	0 b	0.041 < 0.05

ab: Different letters mean terpene emission rates (Mean±SE, $n = 5$) are significantly different between stressed stem and healthy stem at $p = 0.05$ (paired-samples *T* test).

Table II. The catches of adult *M. alternatus* to monoterpene-baited traps.

Code	Monoterpenes*	Ratio	Mean ± SE beetles/week	Mean ± SE eggs/female
1	(+)-α-pinene	NA	7.42 ± 1.10 a	11.00 ± 1.29 a
2	(-)-β-pinene	NA	3.28 ± 0.77 bc	10.60 ± 1.38 a
3	(+)-3-carene	NA	2.42 ± 0.42 bc	10.31 ± 1.34 a
4	Terpinolene	NA	1.14 ± 0.55 bc	10.67 ± 1.18 a
5	(+)-α-pinene: (-)-β-pinene: (+)-3-carene:terpinolene	1:1:1:1	3.14 ± 1.22 bc	11.29 ± 1.32 a
6	(+)-α-pinene: (-)-β-pinene: (+)-3-carene:terpinolene	1:2:2:2	2.57 ± 0.68 bc	10.57 ± 1.08 a
7	(+)-α-pinene: (-)-β-pinene: (+)-3-carene:terpinolene	1:4:4:4	1.42 ± 0.61 bc	10.42 ± 1.11 a
8	(+)-α-pinene: (-)-β-pinene: (+)-3-carene:terpinolene	2:1:2:4	0.42 ± 0.20 c	10.32 ± 1.10 a
9	(+)-α-pinene: (-)-β-pinene: (+)-3-carene:terpinolene	2:2:4:1	2.71 ± 0.80 bc	11.11 ± 0.80 a
10	(+)-α-pinene: (-)-β-pinene: (+)-3-carene:terpinolene	2:4:1:2	3.85 ± 1.99 b	9.90 ± 1.08 a
11	(+)-α-pinene: (-)-β-pinene: (+)-3-carene:terpinolene	4:1:4:2	1.42 ± 0.78 bc	10.42 ± 0.78 a
12	(+)-α-pinene: (-)-β-pinene: (+)-3-carene:terpinolene	4:2:1:4	1.14 ± 0.50 bc	10.14 ± 1.20 a
13	(+)-α-pinene: (-)-β-pinene: (+)-3-carene:terpinolene	4:4:2:1	2.42 ± 0.71 bc	10.42 ± 0.91 a
14	(+)-α-pinene: (-)-β-pinene:(+)-3-carene:terpinolene: ethanol	1:1:1:1:1	7.14 ± 1.92 a	9.60 ± 1.38 a
15	Control (none)	NA	0 d	0 b

$N = 5$ for each treatment; Means in the same column followed by a different letter are significantly different at a = 0.05 using the Bonferroni approach.
* The release rate is about 300 μL/d.

3.2. Field trap experiment

Of the four terpenes tested alone, (+)-α-pinene was significantly more attractive to *M. alternatus* (Tab. II). There were no significant differences in trap catches between the other three terpenes, and they caught less than half the number of beetles as the (+)-α-pinene baited traps. None of the 10 control traps caught any beetles. All females collected were gravid, with a mean of 10.82 ± 0.31 eggs/female. There were no significant differences in eggs per female between different treatments.

The blends were less attractive than (+)-α-pinene alone. Only one significant difference in trap catch was detected between any of the terpene blends (Tab. II). In the univariate analysis of orthogonal design, the optimal monoterpene blend of (+)-α-pinene: (-)-β-pinene: (+)-3-carene: terpinolene was 2:4:1:2. Moreover, the amount of terpinolene was the most important factor in determining trap catch of the blend, and the optimal amount of terpinolene was two aliquots. The quantitative changes of (+)-α-pinene, (-)-β-pinene and (+)-3-carene had no significant effect on trap catch.

The lure with (+)-α-pinene + (-)-β-pinene + (+)-3-carene + terpinolene + ethanol in a 1:1:1:1:1 ratio also was attractive to *M. alternatus* (Tab. II). The trap catch with this blend was not significantly different from that of (+)-α-pinene alone. The addition of ethanol to a straight blend of the four terpenes resulted in a beetle catch almost double the number of the blend without ethanol.

4. DISCUSSION

Our results indicate that (+)-α-pinene can be considered as a primary attractant for *M. alternatus* in China. The amount of (+)-α-pinene greatly increased when the pines were stressed, suggesting that (+)-α-pinene is a critical element for *M. alternatus* in locating a preferred host. Ethanol may also be very important in host selection, as its addition to a monoterpene blend greatly enhanced attraction. Our results reaffirm the conclusion from a previous study that ethanol works as a synergist to host terpene [11]. Further research on the attractiveness of a combination of (+)-α-pinene and ethanol as well as the effects of different release rates of these attractants should assist in the development of effective lures.

All of the beetles attracted by these monoterpenes were adults in the oviposition period, as all female beetles were gravid. Moreover, all of the combinations of the four compounds failed to attract the adults in their preoviposition state. Adults of the subfamily Lamiinae in family Cerambycidae usually require a period of maturation feeding before mating [16]. The results suggest that the compounds tested are ineffective for attracting adults during maturation feeding and that other attractants are used by adults in this preoviposition period.

The addition of (-)-β-pinene, (+)-3-carene, and terpinolene decreased the attractiveness of (+)-α-pinene. (+)-3-carene was one of the compounds that elicited largest EAG responses in *M. alternatus* (unpublished data). However, the absolute abundance of (+)-3-carene was not significantly different between stressed and healthy stems (Tab. I). These results suggest that (-)-β-pinene, (+)-3-carene, and terpinolene may have value for *M. alternatus* in locating potential hosts (pines), but that (+)-α-pinene and ethanol may be utilized in landing and host acceptance for oviposition.

The importance of visual cues in host selection behavior has been reported in many phytophagous insects [2, 4, 10, 19, 24], and studies have demonstrated that visual cues are used in the host-finding behavior of Cerambycid species [8, 17, 18]. Further studies on how visual cues are utilized in combination with attraction to host volatiles should clarify the host selection precess of *M. alternatus*.

Acknowledgements: We greatly appreciate a thorough review and editing of the manuscript by Drs. Stephen Clarke and Daniel R. Miller, USDA Forest Service. We sincerely thank Drs. Liu Zhudong and Zhang Longwa (Institute of Zoology, Chinese Academy of Sciences) for their helpful comments on the manuscript. Thanks are also extended to Mr. Ma Shengan (Director of Xuancheng Forest protection Station, Anhui) for his assistance in fieldwork. This work was supported by the CAS Knowledge Innovation Program (KSCX1-SW-13) and National Natural Science Foundation of china (30525009).

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