Entomologia
Experimentalis et Applicata

DOI: 10.1111/eea.12161

The effects of α-pinene on the feeding performance and pheromone production of *Dendroctonus valens*

Bing-Bing Xu, Zhu-Dong Liu* & Jiang-Hua Sun*

State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beichen West Road 1-5, Chaoyang District, Beijing 100101, China

Accepted: 2 December 2013

Key words: bark beetle, GC-MS, feeding activity, red turpentine beetle, Coleoptera, Curculionidae, Scolytinae, Pinaceae, *Pinus tabuliformis*, monoterpene

Abstract

Herbivorous insects exploit multiple plant cues to detect and orient toward suitable hosts and, accordingly, hosts have evolved complex constitutive and inducible defenses in response. In China, the red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae), an invasive bark beetle from North America, attacks mainly *Pinus tabuliformis* Carrière (Pinaceae), which contains many monoterpenes. In this study, we explored how the monoterpene α-pinene affects the feeding performance and pheromone production of *D. valens*. First, the composition and quantities of monoterpenes of both *P. tabuliformis* healthy trees and fresh stumps were determined and the infestation of *D. valens* in healthy trees and fresh stumps was investigated, linking the amount of monoterpenes and *D. valens* infestation. Gas chromatography–mass spectrometry (GC-MS) analysis showed that *P. tabuliformis* mainly contained α-pinene, with concentrations of 0.1 and 0.5 mg g^{-1} in healthy pine phloem and stump phloem, respectively. Second, the monoterpene's influence on feeding performance was tested using phloem media with α-pinene concentrations ranging from 0 to 30 mg g^{-1}. The results showed that the percentages of beetles boring and the gallery lengths of both adult females and larvae were negatively correlated with the α-pinene concentration although body weight changes did not correlate with α-pinene concentration. Finally, pheromone analysis showed that the production of all pheromones increased with increasing α-pinene concentrations. This study showed the dual effects of α-pinene on *D. valens*: α-pinene inhibited the bark beetle's feeding activities and in turn the bark beetle made use of it to produce pheromones. Our study indicated the importance of promptly removing fresh stumps in the field for the management of the bark beetle.

Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae), a group of subcortical insects that feed as larvae and adults in the phloem of trees and woody shrubs (Wood & Bright, 1992), often exploit multiple plant cues to detect and orient toward suitable hosts (Seybold et al., 2006). After locating a possible host, bark beetles must assess host quality, as this can strongly affect reproductive success, particularly in species that use only one host for their entire larval development (Wallin & Raffa, 2000; Franceschi et al., 2005; Keeling & Bohlmann, 2006). Accordingly, host conifers have evolved complex constitutive and inducible defenses in response to subcortical insects (Raffa & Berryman, 1983; Werner & Illman, 1994; Franceschi et al., 2005; Keeling & Bohlmann, 2006). Trees' responses to invasion by bark beetles include the rapid exudation of resins containing monoterpenes and phenolics at the entry site, the accumulation of high concentrations and altered ratios of these compounds during induced localized reactions, and necrotic lesion formation in advance of beetle galleries (Paine et al., 1987; Wallin & Raffa, 1999; Erbilgin et al., 2003; Franceschi et al., 2005; Keeling & Bohlmann, 2006; López et al., 2011). Furthermore, most plant secondary metabolites involved in plant–insect interactions serve two functions. On one hand, some metabolites with antioxidant, antimicrobial, and antifungal defensive properties can protect plants from being attacked by

*Correspondence: E-mails: liuzd@ioz.ac.cn, sunjh@ioz.ac.cn

herbivores and attract their natural enemies when trees are attacked (Pettersson, 2001; Keeling & Bohlmann, 2006; Seybold et al., 2006; López et al., 2011). On the other hand, some chemicals also are the first attraction for insects in host selection and serve as precursors to pheromone biosynthesis (Seybold et al., 2006; Zhang & Sun, 2006; Liu et al., 2011).

The red turpentine beetle, *Dendroctonus valens* LeConte (Scolytinae), is a secondary pest in its native North America but a destructive pine killer in China, where it first appeared in large numbers in 1999, in Shanxi Province (Yan et al., 2005; Liu et al., 2006). Since the first outbreak in Shanxi, the bark beetle has spread rapidly to four adjacent provinces, Hebei, Henan, Shaanxi, and Inner Mongolia, and it has killed over 6 million Chinese pines, *Pinus tabuliformis* Carrière (Pinaceae) (Sun et al., 2004). Because *P. tabuliformis* is widely planted across a large portion of the country, the majority of Chinese pines is at risk from infestation by *D. valens*.

The beetle has been known to use host odors or kairomones to select and locate its host (Vité & Gara, 1962; Owen, 1985; Hobson et al., 1993; Sun et al., 2004; Erbilgin et al., 2007; Owen et al., 2010; Liu et al., 2011). *Dendroctonus valens* females are pioneers, locating and boring into trees, making galleries where they wait for males to join them (Liu et al., 2006). When enough males and females are attracted to the volatiles and pheromones released by the attacking beetles, they are able to overcome a tree's resistance (Smith, 1971; Owen et al., 2010; Liu et al., 2013). After a female starts boring into the bark, resin may begin to flow; the resin contains various kinds of monoterpenes, including α-pinene, β-pinene, myrcene, limonene, and 3-carene, which can injure or kill insects through their toxic effects (Shi & Sun, 2010; López et al., 2011). Pine bark beetles complete their life cycle entirely within the bark of host trees, and thus both adults and offspring are invariably exposed to high concentrations of these monoterpenes (Wood, 1982). Host monoterpenes are known to be closely related to *D. valens* feeding and pheromone synthesis (Shi & Sun, 2010; López et al., 2011, 2013). However, how *D. valens* deals with those monoterpenes is not known.

Monoterpenes in resin are considered to play important roles during pest infestations (Phillips & Croteau, 1999; Seybold et al., 2006). Myrcene and limonene are toxic to herbivores and sometimes lead to the death of bark beetles (Reddy & Guerrero, 2004). α-Pinene is one of the most abundant compounds in conifers' volatiles (Tingey et al., 1980; Chen et al., 2006) and also one compound of a standard *D. valens* lure that contains (+)-α-pinene, (-)-β-pinene, and (+)-3-carene (1:1:1) (Sun et al., 2004). Although α-pinene is attractive to bark beetles, it has been shown to be toxic (Seybold et al., 2006; López et al., 2011). It is also a precursor of the pheromone compounds *trans*-verbenol and verbenone; these compounds may mediate bark beetles' behavior during colonization, affecting how they attack and feed on trees (Sandstrom et al., 2006; Blomquist et al., 2010).

In the current study, we wanted to understand how α-pinene affects both the larvae and adults of *D. valens* with regard to their feeding behavior and ability to produce pheromone. First, the monoterpene levels in healthy *P. tabuliformis* trees and stumps were measured and infestations of *D. valens* in healthy trees and stumps were investigated in the field. Then, in the laboratory, the effects of various concentrations of α-pinene in artificial media on the beetles' feeding performance were evaluated. Finally, pheromone production in the midgut was analyzed to understand the relationship between α-pinene and pheromone synthesis in *D. valens*.

Materials and methods

Insect collection and rearing

Field trapping was conducted in a natural stand of *P. tabuliformis* at Beishe Mountain, located at the foot of the Luliang Mountains (37°48′N, 111°57′E; mean elevation 1 400 m), west of Gujiao City, Shanxi Province, China. Eight-unit Lindgren funnel traps baited with the kairomone attractant 3-carene (Lvzhou Bio-control, Taiyuan, Shanxi, China; Sun et al., 2004; Erbilgin et al., 2007) were used to catch adult *D. valens* in flight as they emerged from their overwintering sites during peak emergence. In 2010, this occurred from early May to early June. Traps were checked every other day; *D. valens* were collected alive, and the sexes were separated by listening for stridulation produced by males (McGhehey, 1968) and then stored in plastic boxes with holes for ventilation for later bioassays.

Some adult *D. valens* were introduced into fresh bolts (ca. 30 cm diameter × 80 cm long, and five pairs in each bolt) and kept at room temperature to obtain fourth instars for later bioassays. Three large trees of ca. 30 cm diameter at breast height (DBH) were randomly selected in the valley at Beishe Mountain, cut into bolts ca. 80 cm long from the bottom section of each tree and transported to the laboratory. Bolts were placed upright in a room with natural light, and their cut ends were coated with melted wax to retard moisture loss. Trapped beetles (one pair per hole) were inoculated into holes that had been drilled with a 1.0-cm-diameter cork borer (five holes were drilled in each of six bolts). Two months after beetles were inoculated into bolts, those bolts that had been successfully

bored into were dissected to collect fourth instars for bioassays.

Monoterpene composition and quantity in *Pinus tabuliformis* phloem and infestation of *Dendroctonus valens* in the field

As bark beetles prefer to attack large pine trees (Liu et al., 2006, 2011) and fresh stumps (Owen et al., 2010; López et al., 2013), 10 large, healthy *P. tabuliformis* trees (DBH about 30 cm) and seven fresh stumps (diameter about 30 cm, cut about 1 month earlier) were selected at Beishe Mountain in May 2010. Phloem samples (approximately 5 × 5 cm) were scraped and taken back to the laboratory where the composition and concentration of monoterpenes were determined. Phloem samples from healthy trees and stumps were sheared into strips. A small number of sheared strips (about 0.5 g) were weighed, extracted using 2-ml hexane (HPLC purity; J&K Scientific, Beijing, China), and stored at −20 °C for later analysis. Before gas chromatography–mass spectrometry (GC-MS) analysis, all samples were condensed by highly purified nitrogen gas (Hatfield, 2004; Jiang et al., 2006) and extracted with 500-μl hexane with 5 ng μl^{-1} heptyl acetate as an internal standard.

All extracts were analyzed using a DB-WAX column (60 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA, USA) on which quantitative and qualitative analysis using a GC-MS was performed (Agilent 6890N-5973N/MSD; Agilent Technologies, CA, USA). The temperature was programmed from 50 °C for 1 min, 3 °C per min to 240 °C, and held for 5 min. The flow of helium (carrier gas) was 1.0 ml per min. Aliquots of extracts (1 μl) were injected splitless at 220 °C. Detected components were identified by comparing retention times and mass spectra of standard chemicals, and the quantity of volatiles was calculated by using 5 ng μl^{-1} heptyl acetate as an internal standard.

Field investigations were conducted in May 2011 in a natural stand of *P. tabuliformis* at Beishe Mountain. We searched for *D. valens* infestations on large healthy *P. tabuliformis* trees and fresh large stumps. Before *D. valens* emerged in early May, 50 large healthy trees (DBH 30 cm) were selected randomly and marked with red rope. At the same time, 10 fresh large stumps (DBH 30 cm) cut by local people were selected. At the end of May, the selected trees were checked and attacks were identified by the presence of large, light-pink to reddish-brown pitch tubes, about 2.5–5 cm in diameter around the base of the tree (Smith, 1971; Liu et al., 2008). All pitch tubes of each infested tree were dissected and the beetles were captured and recorded. At the same time, stumps were checked and dissected, and the excavated beetles were counted.

The influence of α-pinene on bark beetles' feeding performance

Based on the quantity of α-pinene determined in the phloem of healthy pine trees and stumps mentioned above, artificial media with concentrations of α-pinene ranging from 0 to 30 mg g^{-1} were made using Wallin and Raffa's method (Wallin & Raffa, 2000). No-choice feeding bioassays were carried out to test the influence of α-pinene on the beetles' behavior. Six concentrations of α-pinene, i.e., 0, 0.1, 0.5, 1.0, 10.0, and 30.0 mg g^{-1}, were set up. Twenty g of dried phloem powder and 10 g agar mixed with 300 ml water were sterilized at 121 °C and 0.105 MPa pressure for 30 min. After the mixture of agar and water had cooled to about 60 °C, 0.88 g sorbic acid, 1.76 g methylparaben, and sterilized phloem powder were added to the mixture and shaken; then the corresponding amount of (1S)-(-)-α-pinene (98% purity; Sigma Aldrich, Milwaukee, WI, USA) dissolved in pentane (HPLC purity; J&K Scientific), 12 ml in total, was added and mixed. Finally, the mixture was poured into 10-ml plastic centrifuge tubes (10 cm length × 1.5 cm diameter) (each tube contained 7–8 ml medium). Once the medium solidified, tubes were stored at 4 °C overnight for the next day's bioassays.

In the no-choice feeding bioassay, adult females and larvae were starved for 24 h, then weighed and randomly assigned to six groups (seven replicates per group and 10 individuals per replicate), i.e., media with α-pinene at concentrations of 0, 0.1, 0.5, 1.0, 10.0, and 30.0 mg g^{-1}. Beetles – both adults and larvae – were inoculated individually into each kind of media (one beetle per larva per tube with a pressure-vent hole in the lid). After 72 h, the total boring percentage of each replicate was calculated by dividing the number that successfully bored into the media by the 10 individuals tested. All beetles were weighed again, and the lengths of their galleries were measured. Gallery lengths of those beetles that failed to enter the media were treated as zero.

The influence of α-pinene on *Dendroctonus valens* pheromone production

The hindguts of some beetle adults and larvae that bored into the media mentioned above were dissected (13, 13, 15, 12, 12, and 9 adults for α-pinene concentrations of 0, 0.1, 0.5, 1, 10, and 30 mg g^{-1}, respectively; 15, 14, 12, 11, 9, and 8 larvae for corresponding α-pinene concentrations, respectively), and each hindgut was extracted using 50-μl hexane with 5 ng μl^{-1} heptyl acetate, which was kept at −20 °C until analysis. One μl was injected for GC-MS analysis with a DB-WAX column (60 m × 0.25 mm × 0.25 μm; J&W Scientific). The temperature was set at 50 °C for 1 min, and then 5 °C per min to 200 °C, 10 °C per min to 230 °C and held for 10 min. The flow of

helium (carrier gas) was 1.0 ml per min. α-Pinene, *cis*-verbenol, verbenone, myrtenal, and myrtenol were identified by comparing retention times and mass spectra to those of synthetic standards. The detected volatiles were quantified using 5 ng μl^{-1} heptyl acetate as an internal standard.

Data analysis

Monoterpenes from healthy trees and stumps were analyzed by t-test, and those compounds that were not detected were treated as zeros (SPSS, 1999). Field investigations of *D. valens* attacking occurrence of healthy trees and stumps were compared by χ^2 test (SPSS, 1999). The number of *D. valens* excavated from healthy large trees and stumps was tested to ensure that it fits normal distribution before analysis. If it did, ANOVA was used to analyze differences between healthy trees and stumps. If it did not, non-parametric tests were carried out. The boring percentage of *D. valens* adults and larvae, gallery lengths, body weight changes, and identified pheromone compounds was regressed with variable α-pinene concentrations using SPSS (1999).

Results

Monoterpene composition and quantity in *Pinus tabulaeformis* phloem and infestations of *Dendroctonus valens* in the field

Monoterpenes α-pinene, camphene, β-pinene, 3-carene, limonene, β-phellandrene, and terpinolene were detected and quantified from the phloem samples. Levels of each compound were higher in the phloem of healthy pines than in the phloem of stumps and there were significant differences between the phloem of healthy trees and stumps in terms of the amounts of α-pinene, camphene, β-pinene, and β-phellandrene each contained (Table 1). As shown, α-pinene was the main compound, with significantly different concentrations of 0.5 and 0.1 mg g^{-1} fresh weight, respectively, in the phloem of healthy pines and stumps ($t = 2.254$, d.f. = 14, $P = 0.05$; Table 1).

Field investigations showed that *D. valens* preferred to attack fresh stumps over healthy trees: 10 of 10 stumps and 28 of 50 healthy large trees were attacked ($\chi^2 = 6.947$, d.f. = 1, $P = 0.008$; Figure 1A). Moreover, 25 beetles per stump were excavated on average from fresh stumps and only four were collected from standing large trees ($F_{1,36} = 30.922$, $P<0.001$; Figure 1B).

The influence of α-pinene on bark beetles' feeding performance

The boring percentages of adults were 67.0, 67.3, 59.6, 66.0, 17.4, and 10.0% on average at α-pinene concentrations of 0, 0.1, 0.5, 1.0, 10, and 30 mg g^{-1}, respectively; these values were significantly negatively correlated with α-pinene concentrations ($F_{5,35} = 45.73$, $P<0.0001$; Figure 2A). Correspondingly, the average larval boring percentages were 57.9, 30.8, 42.1, 39.2, 8.8, and 0% at α-pinene concentrations of 0, 0.1, 0.5, 1.0, 10, and 30 mg g^{-1}, respectively, which also significantly negatively correlated with α-pinene concentration ($F_{5,35} = 16.02$, $P<0.0001$; Figure 2B). For both adult females and larvae, media with concentrations of 10.0 and 30.0 mg g^{-1} of α-pinene deterred insects the most (Figure 2).

The gallery lengths made by adults were 4.8, 4.1, 2.1, 3.8, 0.7, and 0.1 cm on average at α-pinene concentrations of 0, 0.1, 0.5, 1.0, 10, and 30 mg g^{-1}, respectively, and the regression analysis showed that gallery length was significantly correlated with α-pinene concentration ($F_{5.427} = 71.86$, $P<0.0001$; Figure 3A). Gallery lengths made by larvae were 3.2, 1.6, 2.2, 1.5, 0.1, and 0 cm on average at the corresponding concentrations, and were also significantly correlated with α-pinene concentrations ($F_{5,331} = 37.96$, $P<0.0001$; Figure 3B).

Changes in body weight of all tested larvae were measured; neither adult females nor larvae significantly changed their weight despite the concentrations of α-pinene in the media (adult females: $F_{5,358} = 3.31$, $P = 0.07$; larvae: $F_{5,298} = 0.73$, $P = 0.40$; Figure 4).

Table 1 Monoterpene composition and quantity (mg g^{-1}) in *Pinus tabuliformis* healthy tree and fresh stump phloem

	Tree			Stump				
Compound	Concentration	Minimum	Maximum	Concentration	Minimum	Maximum	t	P
α-Pinene	0.5219 ± 0.1710	0.0769	1.6257	0.1264 ± 0.0393	0.0428	0.2968	2.254	0.050[1]
Camphene	0.0053 ± 0.0020	0.0000	0.0164	0.0003 ± 0.0003	0.0000	0.0016	2.483	0.037[1]
β-Pinene	0.0667 ± 0.0216	0.0019	0.2175	0.0035 ± 0.0014	0.0000	0.0081	2.353	0.035[1]
3-Carene	0.0001 ± 0.0001	0.0000	0.0008	0.0000 ± 0.0000	0.0000	0.0000	0.806	0.44
Limonene	0.0239 ± 0.0109	0.0016	0.1037	0.0027 ± 0.0013	0.0005	0.0091	1.924	0.090
β-Phellandrene	0.0088 ± 0.0021	0.0006	0.0194	0.0010 ± 0.0007	0.0000	0.0048	3.406	0.007[1]
Terpinolene	0.0284 ± 0.0134	0.0003	0.1114	0.0013 ± 0.0004	0.0003	0.0030	2.029	0.077

[1]Means significantly different in the phloem between tree and stump ($\alpha = 0.05$)

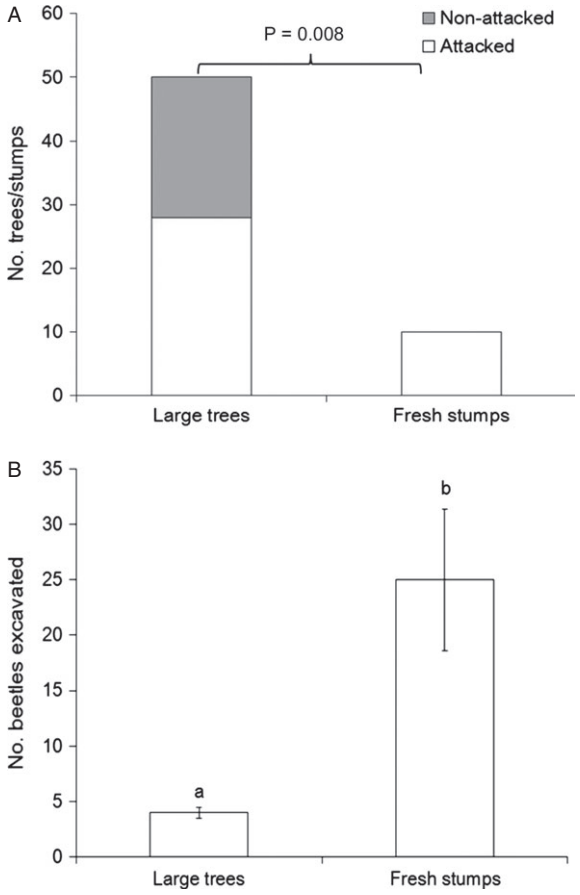

Figure 1 Infestation of *Dendroctonus valens* in healthy trees and fresh pine stumps in the field. Mean (± SE) numbers of (A) trees and stumps with and without *D. valens*, and (B) *D. valens* excavated from trees and stumps. Bars capped with different letters are significantly different at P<0.05 (χ^2 and ANOVA for A and B, respectively).

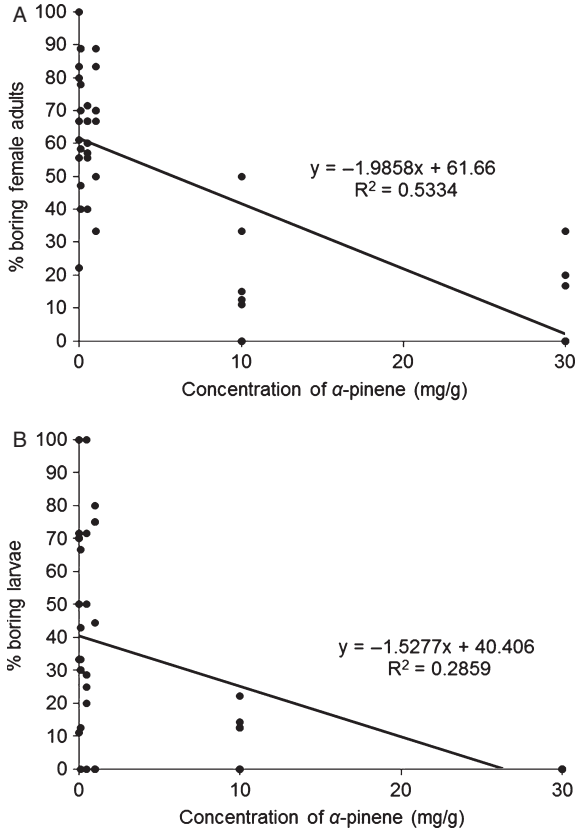

Figure 2 Boring percentages of *Dendroctonus valens* (A) adult females and (B) larvae, feeding on various concentrations of α-pinene in media.

The influence of α-pinene on bark beetles' pheromone production

α-Pinene and four common pheromone compounds, *cis*-verbenol, verbenone, myrtenal, and myrtenol, were detected and quantified from extracted hindguts in all female bark beetle adults (Figure 5). *Trans*-verbenol was identified in only one treatment and excluded from analysis. Clearly, the concentration of α-pinene significantly affected the amount of α-pinene and pheromone production in the hindguts (α-pinene: $F_{5,67} = 36.08$, P<0.001; *cis*-verbenol: $F_{5,67} = 10.89$, P = 0.002; verbenone: $F_{5,67} = 20.15$, P<0.001; myrtenal: $F_{5,67} = 43.25$, P<0.0001; myrtenol: $F_{5,67} = 16.08$, P<0.001; Figure 5), indicating that the amounts of these compounds increased with increasing concentrations of α-pinene in the media.

However, in larvae there were no obvious differences in hindgut α-pinene content among six concentrations of media ($F_{5,61} = 1.31$, P = 0.26; Figure 6A); *cis*-verbenol, verbenone, myrtenal, and myrtenol were significantly regressed with the α-pinene concentrations (cis-verbenol: $F_{5,62} = 72.14$; verbenone: $F_{5,62} = 38.40$; myrtenal: $F_{5,62} = 29.37$; myrtenol $F_{5,62} = 62.74$, all P<0.0001; Figure 6B–E).

Discussion

Our study confirmed that α-pinene is the main compound of the monoterpenes of *P. tabuliformis* and showed that the amounts of monoterpenes in healthy pine trees were higher than those in stumps. Field investigations showed that stumps were more easily attacked than healthy trees by the bark beetle *D. valens*, indicating host monoterpenes may affect *D. valens* beetle infestation. In the laboratory, the feeding behavior of *D. valens*, measured as boring percentage, gallery length, and body weight changes after 72 h of feeding, was determined for each of six concentrations of α-pinene in media. We found that boring percentages and gallery lengths were significantly related to α-pinene concentrations, that fewer beetles bored into the media,

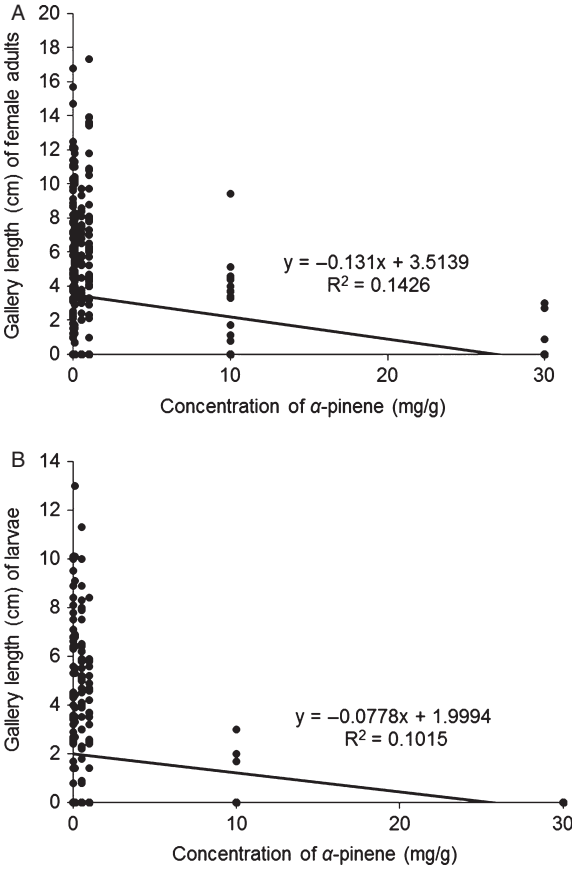

Figure 3 Gallery lengths of *Dendroctonus valens* (A) adult females and (B) larvae, feeding on various concentrations of α-pinene in media.

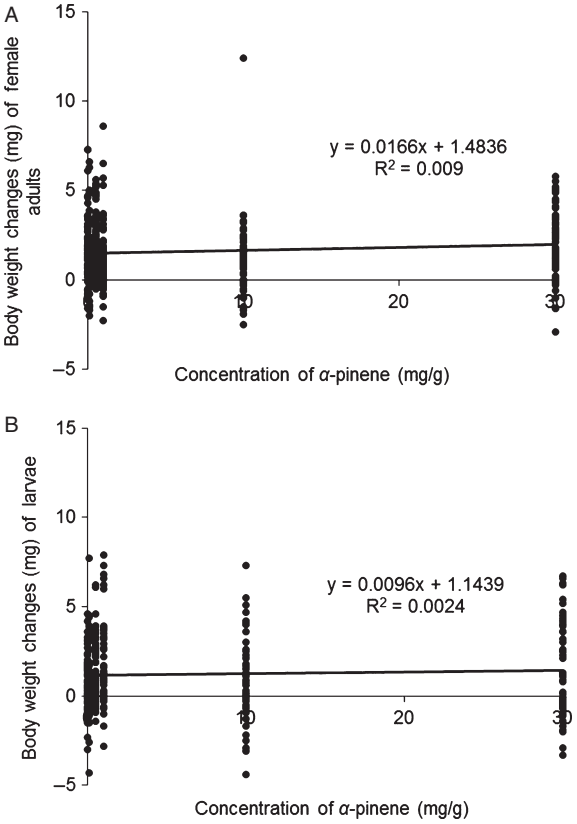

Figure 4 Body weight changes of *Dendroctonus valens* (A) adult females and (B) larvae, after feeding for 72 h on various concentrations of α-pinene in media.

and that gallery lengths were shorter as α-pinene concentrations increased in the media. However, after 72 h of feeding, the body weight of adult females and larvae did not significantly change. Moreover, we quantified the pheromone production of *D. valens* adult females and larvae fed on media containing α-pinene. Our results indicated that the amounts of pheromone compounds, i.e., *cis*-verbenol, verbenone, myrtenal, and myrtenol, increased when the concentrations of α-pinene in the media increased, suggesting that bark beetles may use α-pinene to synthesize components of their own pheromones.

The host acceptance behavior of bark beetles may be influenced by the composition and concentrations of host monoterpenes (Wallin & Raffa, 2002; Seybold et al., 2006). Conifer resin is known to contain monoterpenes, and both induced and constitutive monoterpenes are toxic to bark beetles (Raffa & Smalley, 1995; Franceschi et al., 2005; López et al., 2013). Interactions between conifer resistance and bark beetle colonization efforts were also associated with allelochemicals (Raffa & Berryman, 1983). In the current study, we found that the amounts of most monoterpene compounds, such as α-pinene, camphene, β-pinene, and β-phellandrene in healthy pine phloem were significantly higher than the amounts of these compounds in stump phloem. Do trees containing more monoterpenes better resist attacking beetles? In the field, *D. valens* was observed to attack large pine trees (Liu et al., 2011) as well as fresh stumps (Owen et al., 2010; López et al., 2013). When we carried out the field investigations, we found that fresh stumps incurred significantly more beetle infestation than did standing healthy pines and we often obtained dozens of adults in a stump but only a few adults in standing infested pine trees (Figure 1). We suggest that the serious *D. valens* infestation in stumps indicates that stumps are less resistant to beetles. As reported, the amounts of monoterpenes released may affect bark beetles' ability to colonize stumps (Seybold et al., 2006). In our research, the results suggest a causal relationship between the amount of monoterpenes and

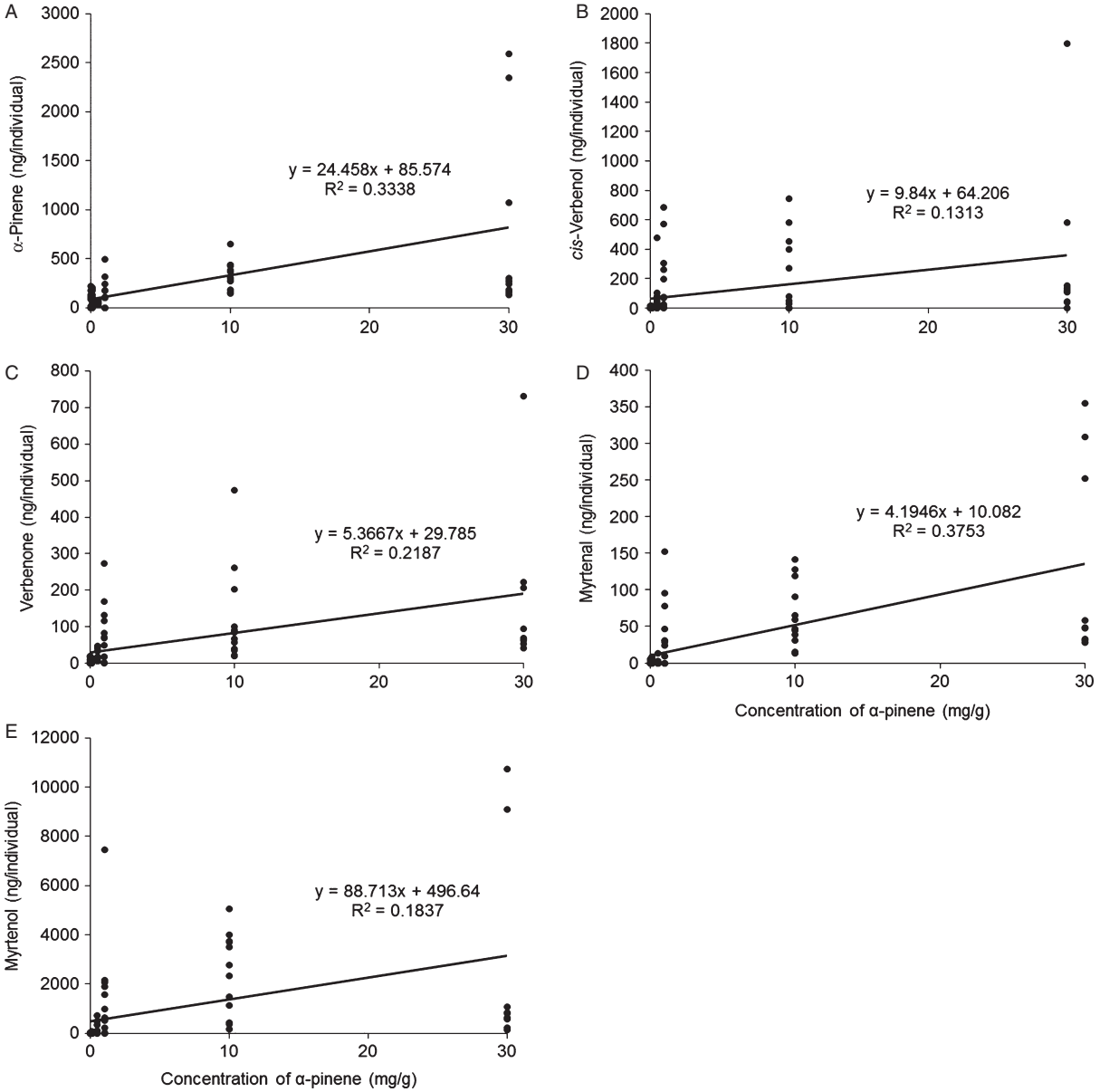

Figure 5 Amounts of α-pinene and pheromone compounds extracted from the hindguts of *Dendroctonus valens* adult females at various concentrations of α-pinene in media: (A) α-pinene, (B) *cis*-verbenol, (C) verbenone, (D) myrtenal, and (E) myrtenol.

D. valens infestation. As stumps are nearly useless to farmers, they are often discarded, creating the perfect habitat in which the pests can reproduce. We believe the main reservoir of *D. valens* in the field is most likely the stumps, which indicates that removing or fumigating stumps promptly to get rid of beetles is important for management of this pest.

α-Pinene was the most abundant monoterpene in *P. tabuliformis*, the main host of *D. valens* in China; its concentration was about 0.5 and 0.1 mg g^{-1} fresh weight in healthy pine phloem and stump phloem, respectively. We established α-pinene concentrations, based on the level found in host phloem, to test its influence on *D. valens*'s behavior and pheromone production in the laboratory. In our research, the boring percentages and gallery lengths were negatively correlated with α-pinene concentrations, supporting the idea that high monoterpene concentrations can inhibit beetle feeding (Wallin & Raffa, 2000; Reid & Purcell, 2011). When the level of α-pinene reached 10 mg g^{-1} for both

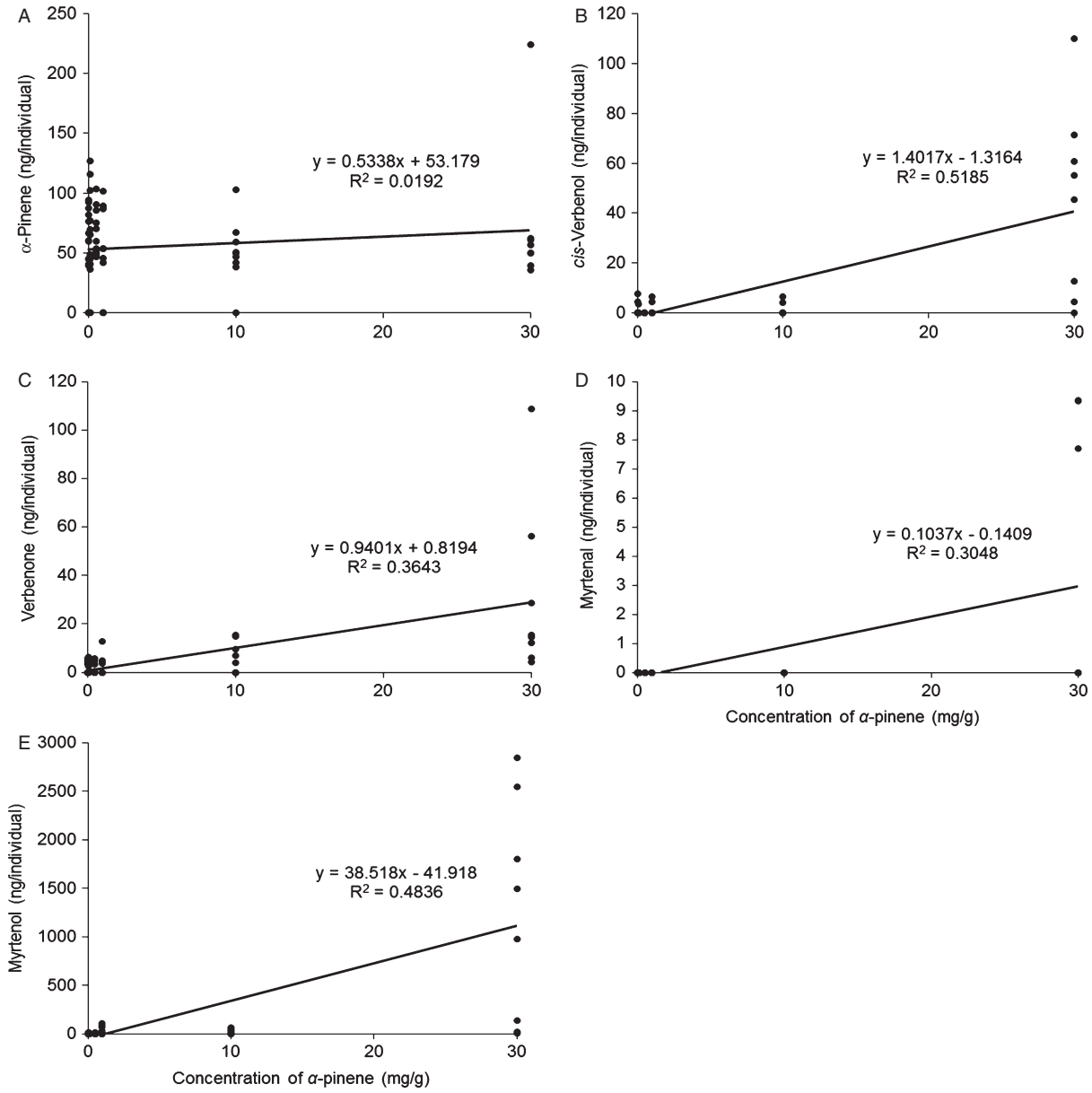

Figure 6 Amounts of α-pinene and pheromone components extracted from the hindguts of *Dendroctonus valens* larvae at various concentrations of α-pinene in media: (A) α-pinene, (B) *cis*-verbenol, (C) verbenone, (D) myrtenal, and (E) myrtenol.

adult females and larvae, few beetles were able to bore into the media and they made shorter galleries. However, the trends of boring rates and gallery lengths of adult *D. valens* fed on media made with a gradient of α-pinene concentrations were not identical with the trends of adult *Ips pini* (Wallin & Raffa, 2000). In Wallin & Raffa's experiment, *I. pini* often bored the most and had the longest galleries in media containing an intermediate α-pinene concentration (Wallin & Raffa, 2000), which suggests that *I. pini* has evolved an optimal strategy for the host α-pinene. In our experiment, we did not find such a peak but a linear relationship instead; the tested parameters decreased with increasing α-pinene concentration.

As mentioned above, α-pinene concentrations negatively affected bark beetle feeding and boring behavior, and the length of galleries. The negative effects of host monoterpenes could influence body size and fat content of

the beetles (Wallin & Raffa, 2004; Reid & Purcell, 2011). In this study, body weight changes of *D. valens* are expected to reflect the insects' physiological responses to α-pinene concentrations. However, our analysis showed that the body weights of adults and larvae did not change with the varying α-pinene concentrations after 72 h of feeding. We cannot exclude the possibility that α-pinene affects the body size of *D. valens*, a relationship that deserves further exploration. Moreover, *D. valens* grows slowly, taking about 2 months to reach the pupal stage (Smith, 1971). In our experiment, we let beetles feed on media for only 3 days, which may have been too brief a time to result in differences in body size.

Because monoterpenes are toxic to bark beetles (Seybold et al., 2006; López et al., 2011), we wondered how they dealt with these compounds. To survive in galleries filled with volatile monoterpenes, these beetles must possess effective detoxification mechanisms and three metabolic pathways have been proposed to account for the distribution of host monoterpenes (Pierce et al., 1987). One of these pathways is the use of plant monoterpenes to synthesize their pheromones, attracting more bark beetle individuals to conquer host resistance (Sandstrom et al., 2006; Blomquist et al., 2010), which is one of those three metabolic pathways. In this study, we investigated only the relationship between pheromone production and α-pinene. We found that common pheromone compounds – *cis*-verbenol, verbenone, myrtenal, myrtenol – were detected in the hindguts of *D. valens*, and their contents normally changed with the concentration of α-pinene in media. For both adults and larvae, pheromone content increased as the concentration of α-pinene in media increased, which supports the idea that both *D. valens* adults and larvae are able to transform α-pinene to *cis*-verbenol, verbenone, myrtenal, and myrtenol (Shi & Sun, 2010). In addition, although *trans*-verbenol was barely detected, it has been identified in previous experiments in our laboratory (Zhang & Sun, 2006; Shi & Sun, 2010). R-(+)-α-pinene was oxidized to *trans*-verbenol, and S-(-)-α-pinene was oxidized to *cis*-verbenol (Shi & Sun, 2010). However, in the current experiment, S-(-)-α-pinene with a purity of 98% was used; as a result *cis*-verbenol was transformed and *trans*-verbenol was absent, which indicates that the process of pheromone production is enantioselective (Byers, 1983).

Acknowledgments

We thank Mr. Gangyi Dong and Mr. Haihe Wang from the Tunlanchuan Forest Farm for their technical assistance in the field work, and Emily Wheeler for her editorial assistance. This work was supported by Beijing Natural Science Foundation (grant no. 6112019), the National Basic Research Program (2009CB119204), and the Scientific Research Foundation for Returning Overseas Chinese Scholars.

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