

# Electrophysiological and behavioral responses of *Dendroctonus valens* to non-host volatiles

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**Abstract** – Non-host volatiles (NHVs) that are often reported as being disruptive to coniferophagous bark beetles were tested for both electrophysiological and behavioral effects on the red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae), which was accidentally introduced into China in the mid-1980's. All NHVs tested elicited dose-dependent antennal responses by *D. valens*. In Y-tube olfactometer trials, *D. valens* were repelled by NHVs tested. When NHVs were added to a kairomone blend, responses of *D. valens* were significantly inhibited. Further field trapping experiments showed that attraction of *D. valens* to kairomone baited traps was reduced by all individual NHVs, with reductions ranging from 26.3 to 70%. 1-Octen-3-ol, (Z)-3-hexen-1-ol, and (E)-2-hexen-1-ol were the three most effective NHVs, significantly reducing *D. valens* to kairomone-baited traps by 69.5, 68.3 and 66.0%, respectively. In the development and implementation of a semiochemical-based management programme for *D. valens*, NHVs may have considerable potential for disrupting the beetle's ability to locate suitable hosts.

**non-host volatiles / red turpentine beetle / electroantennograms / olfactory response / field trapping**

**Résumé** – Réponses électrophysiologiques et comportementales de *Dendroctonus valens* à des composés volatils non-hôtes. Au cours de cette étude, on a testé les effets de composés volatils non hôtes, souvent considérés dans la littérature comme pouvant affecter la reconnaissance de l'hôte chez les scolytes des conifères, sur les réponses électro-physiologiques et le comportement du scolyte *Dendroctonus valens* LeConte (Coleoptera : Curculionidae : Scolytinae), espèce introduite accidentellement en Chine depuis le milieu des années 1980. Tous les composés testés ont généré des réponses antennaires, dépendant de la dose. Dans des tests en olfactomètre en Y, on a observé une plus ou moins grande répulsion à certains composés. En particulier, les réponses au 1-octen-3-ol, au 1-hexanol, à l'hexanal et au 3-octanol ont été très faibles. Des expérimentations de terrain ont ensuite montré que l'attraction sur *D. valens* de pièges appâtés en kairomone a été réduite par l'adjonction de chacun des composés non-hôtes, cette réduction variant de 26.3 à 70 %. Parmi ces composés, le 1-octen-3-ol, le (Z)-3-hexen-1-ol et le (E)-2-hexen-1-ol ont été les plus efficaces, engendrant respectivement une réduction des captures de 69.5, 68.3 et 66.0 %. L'utilisation de composés volatils non-hôtes pour induire une confusion dans le choix de l'hôte représente donc un potentiel considérable pour le développement d'un programme de gestion des populations de *D. valens*, basé sur l'utilisation de produits semio-chimiques.

**scolyte / électro-antennogramme / réponse olfactive / composés volatils non-hôtes / piégeages de terrain**

## 1. INTRODUCTION

The red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae), an exotic pest in China, was first reported from China in 1983 in Shanxi Province [9, 26]. This exotic beetle was presumed to have been introduced via imported, unprocessed *Pinus ponderosa* Douglas ex P. and C. Lawson logs sent to Shanxi from the west coast of the United States [26]. *D. valens* is normally a non-aggressive pest of diseased and wounded conifers, principally in the genus *Pinus* (L.). In North America, *D. valens* is geographically widespread, ranging from Canada in the north to Central America in the south, and extending the breadth of

the continent in the northern part of its range [7]. It has a wide host range, attacking all pines within its geographic range [5]. Despite its abundance and wide distribution, outbreaks of *D. valens* have generally not been extensive or severe in North America, and its impact has been relatively minor [18]. In recent years, however, there have been outbreaks related to mechanical injury to tree roots [15, 19].

In contrast with its role as a secondary pest in North America, *D. valens* has become a very destructive pest to its new host species *Pinus tabulaeformis* Carrière in China. Since its first large outbreak in 1999 in Shanxi Province, *D. valens* spread rapidly to the adjacent provinces of Hebei, Henan and Shaanxi, and has infested over 500 000 ha of Chinese pine stands, primarily those over 30 years old, killing more than 6 million trees to date [9, 10]. It is currently the second most

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damaging forest pest in the country [10]. Considering the wide host range of *D. valens* in North America [5], the majority of Chinese pines are probably at risk. Moreover, *P. tabuliformis*, the principal host of *D. valens* in China, is extremely important for reforestation and is widely planted across a large portion of the country [9]. The potential damage from *D. valens* is therefore enormous, and effective measures for monitoring and controlling *D. valens* are desperately needed [9, 19].

*D. valens* is known to use host odors or kairomones to locate and select its preferred hosts [8, 11, 23]. Hobson et al. [8] showed that the primary attractants in central California were (-)- $\beta$ -pinene and (+)- $\alpha$ -pinene, with 3-carene as a minor component of the attractive blend. Sun et al. [20] showed that 3-carene was the dominant attractant for Chinese populations of *D. valens*, and (-)- $\beta$ -pinene and (+)- $\alpha$ -pinene were behaviorally neutral, or nearly so.

Recent electrophysiological and behavioral studies clearly indicate that conifer bark beetles are not only able to recognize, but also to avoid, non-host habitats or trees by olfaction. Non-host volatiles (NHVs) may represent a key semiochemical signal in the discrimination between host and non-host species by conifer-infesting bark beetles [3]. NHVs comprise both green leaf volatiles (GLVs) and non-host bark volatiles. Green leaf volatiles are aliphatic 6-carbon primary alcohols, aldehydes and acetates that are ubiquitous in broad-leaf trees and herbaceous plants [21, 22], for example, 1-hexanol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol and hexanal. Non-host bark volatiles, including 3-octanol, 1-octen-3-ol, benzyl alcohol and *trans*-conophthorin, have also been found in the bark of several non-host angiosperm trees [29].

In this study, we tested several non-host volatiles that are often reported in the literature as being disruptive to conifer-attacking scolytids, using electroantennogram (EAG) assays and behavioral tests both in the laboratory and field. The goals of this study were: (1) to determine by EAG if the non-host volatiles are perceived by *D. valens* antennae; (2) to evaluate the behavioral response in a Y-tube olfactometer to the antennally active NHVs; (3) to find the potential disruptants for *D. valens* in a field trapping experiment in China.

## 2. MATERIALS AND METHODS

### 2.1. Insects

*D. valens* were collected from a *P. tabuliformis* plantation at Tunlanchuan Forest Station, west of the city of Gujiao, Shanxi Province, in 2004. Adults were sexed and maintained in an incubator at 25 °C and 55% RH, under a light-dark cycle of 14L:10D before being used in EAG and bioassay experiments.

### 2.2. Chemicals

NHVs used in electroantennogram tests and Y-tube olfactometer trials were obtained from Acros Organics (Geel, Belgium), and their chemical purities were as follows: 1-hexanol, 98%; (*Z*)-3-hexen-1-ol, 98%; (*E*)-2-hexen-1-ol, 96%; 3-octanol, > 99%; 1-octen-3-ol, 98%; hexanal, 96%; and benzyl alcohol, 99%. The components of

the kairomone blend, (+)- $\alpha$ -pinene, (-)- $\beta$ -pinene, and (+)-3-carene, were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin). Their chemical purities were 99%, 98%, and 97%, respectively.

### 2.3. Electroantennogram (EAG) response

The EAG technique was modified from the method used by White and Hobson [24] as follows: antennae were excised from the heads of *D. valens*, then mounted between two glass micropipettes filled with insect Kaissling saline. The recording electrode was carefully inserted into the distal edge of the antennal club and the indifferent electrode into the basal scape of the same antenna. Micro-electrodes were held with micromanipulators (style MP-15, Syntech, Hilversum, The Netherlands) and connected to a high input impedance AC/DC micro-amplifier (model Un-06, Syntech) via Ag/AgCl junctions.

Each 10  $\mu$ L dose of test material was applied to a filter paper strip (5 $\times$ 50 mm) and the solvent allowed to evaporate before use. The filter paper was then inserted into a Pasteur pipette. The tip of the pipette containing the sample dosage was positioned 1 cm upwind from the antennal preparation. Odor stimuli were delivered from the pipettes as 1.0-s pulses into a continuous airstream (124 mL/min). The EAG signal was amplified by a DC/AC amplifier. Amplified EAG responses were digitized using a Nelson 900 Series Interface and displayed and processed on a PC using Spike software (Syntech).

First, a single dose (1  $\mu$ g/ $\mu$ L) in hexane of the seven compounds was presented. Later, a log dilution series was made and tested for seven compounds, at doses of 0.0001, 0.001, 0.01, 0.1, 1, 10, and 100  $\mu$ g/ $\mu$ L, to better understand the response characteristics of *D. valens*. Seven compounds were presented to the antenna randomly, in order of concentration, with the lowest dose first. An interval of at least 1 min between puffs was utilized to ensure complete antennal recovery. At least 5 females and 5 males were tested with each compound. A hexane-only control and standard solution ((+)-3-carene at 1  $\mu$ g/ $\mu$ L in hexane) were presented before and after each test material. (+)-3-Carene has been shown to be the component of the kairomone blend which elicits the strongest electrophysiological and behavioral responses by *D. valens* in China [20].

### 2.4. Y-tube olfactometer trials

Bioassays were conducted in a glass Y-tube olfactometer (16 mm diam, main tube 20 cm long, arm length 25 cm), with a 120° inside angle. Incoming air was filtered through activated charcoal and humidified with doubly distilled, deionized water. The filtered air was split between two, 2-1 holding chambers [2]; one chamber, holding the kairomone blend (1:1:1 ratio of (+)- $\alpha$ -pinene:(-)- $\beta$ -pinene:(+)-3-carene), served as a positive control and the other chamber held the kairomone blend plus NHVs. The kairomone blend (100  $\mu$ g) and NHVs (100  $\mu$ g), in 10  $\mu$ L of hexane, were applied to a filter paper strip (5  $\times$  50 mm). The solvent was allowed to evaporate for 20 s, then the filter paper was placed into the chambers. From each holding chamber, the air passed into the respective arms of the Y tube. Airflow through the system was maintained at 200 mL/min by an inline flowmeter. A prior smoke test demonstrated laminar airflow in both arms and throughout the olfactometer. To eliminate visual cues, the Y-tube setup was surrounded by a 80  $\times$  60-cm black fabric enclosure. All experiments were conducted in the daytime between 08:00 AM and 17:00 PM. Temperature and RH in the olfactometer were maintained at 25° and 70%, respectively.

**Table I.** Description of semiochemicals employed in trapping experiments for *Dendroctonus valens*, Shanxi Province, 2004.

<sup>a</sup> Chemicals	Dispensers	<sup>b</sup> Release rate mg/d
Kairomone blend	15-mL Bottle	110.0
1-hexanol	Bubble cap 570 mg	5.2
(Z)-3-hexen-1-ol	Bubble cap 700 µL	25.6
(E)-2-hexen-1-ol	Bubble cap	16.0
3-octanol	Bubble cap	56.9
1-octen-3-ol	Bubble cap	29.3
Hexanal	Bubble cap 700 µL	29.2
Benzyl alcohol	Bubble cap	10.0

<sup>a</sup> The non-host volatiles (NHVs) used in field trials were obtained from Phero tech, while chemicals of the kairomone blend: [(+)- $\alpha$ -pinene: (-)- $\beta$ -pinene: (+)-3-carene (1:1:1)] were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin); their chemical purities were: (-)- $\beta$ -pinene, 99%, (+)- $\alpha$ -pinene, 98%, (+)-3-carene, 97%, respectively.

<sup>b</sup> Release rates for field trials determined under field conditions, (mean temperature > 27 °C). Note: the release rates of benzyl alcohol and (E)-2-hexen-1-ol were determined by Phero tech at 30°.

Approximately 30 min before trials were initiated, adult *D. valens* were introduced to a separate holding container, so they would not be exposed to test odors before their release. At the beginning of each trial, a beetle was released at the downwind end of the Y tube. It was given 10 min to respond to the treatment, and a choice for the left or right arm of the olfactometer was noted when the beetle went 5 cm past the Y junction. The beetle was scored as being attracted or being repelled by the experimental odor blend. The arms of the olfactometer were exchanged after each replicate.

## 2.5. Field trials

The field trapping experiments were conducted in 2004 in a 35-year-old plantation of *P. tabuliformis* in the Tunlanchuan Forest Station (N 37° 48', E 111° 44' average elevation 1400 m), west of the city of Gujiao, Shanxi Province. For the previous two years, stumps infested with *D. valens* had been treated with insecticide fumigation and sealed in plastic to prevent beetle emergence. Nevertheless, about 20% of standing trees were currently suffering *D. valens* attacks at the time of the study. All experiments employed 8-unit multiple funnel traps laid out between rows of *P. tabuliformis*. In order to suspend traps, 90 trees were randomly selected from all trees within the stand, with a minimum spacing of at least 50 m between selected trees. A trap was then suspended from each of those trees, with the collection cups ca. 20 cm above ground level. Traps were suspended at that height because that is the level at which *D. valens* attacks trees. A plastic insecticidal strip saturated with 2,2-dichlorovinyl dimethyl phosphate was put in each collection cup as a killing agent. The experiment was set up on 21 May 2004, and beetles were collected from traps every 5–7 days until 20 July 2004. A total of 9 treatments with 10 replicates each were applied in the study, with a totally randomized design.

The description of semiochemicals employed in trapping experiments for *D. valens* is presented in Table I. These seven NHVs were chosen because they are often reported in the literature as being disruptive to closely related conifer-attacking scolytids. In our experiment, the kairomone blend consisted of a 1:1:1 blend of three host

monoterpenes: (+)- $\alpha$ -pinene, (-)- $\beta$ -pinene, and (+)-3-carene, which has been used extensively over the last decade for monitoring of *D. valens* populations in North America [13], and was thus intended as a positive control against which the bioactivity of NHV treatments added to the kairomone blend could be assessed. Unbaited control traps served as negative control (blank) treatments. All *D. valens* adults captured in each trap on each date were counted. Voucher specimens were deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing.

## 2.6. Statistical Analysis

The net EAGs were calculated by subtracting the mean responses to the controls introduced before and after the sample. EAG data were standardized by calculating the percentages of the net EAGs relative to the standard solution. The difference of antennal responses by sex to NHVs at seven doses were analyzed using Mann-Whitney tests.

In Y-tube olfactometer trials, Chi-square tests were used to examine differences in response of *D. valens* to NHVs by sex. The null hypothesis that *D. valens* showed no preference for either olfactometer arm (a response equal to 50:50) was analyzed with a Binomial test. The numbers of insects captured were transformed by  $\log(x+1)$  to satisfy assumptions of normality and homoscedasticity. The transformed data were analyzed by analysis of variance (ANOVA) followed by the Ryan-Einot-Gabriel-Welsch range test. All data were analyzed with the program for Windows 11.0 (SPSS Inc., 2001).

## 3. RESULTS

### 3.1. Electroantennogram (EAG) response of *D. valens* to non-host volatiles (NHVs)

First, the antennal responses of *D. valens* to seven NHVs at a single dose (10 µg) of were recorded. Measurable antennal responses were elicited to all compounds tested. Subsequently, dose-response of EAG response of *D. valens* to dilutions of NHVs were tested. There were no significant differences in EAG responses to specific NHVs doses between the sexes, except to 1-octen-3-ol at dose of 0.1 µg ( $P = 0.031$ ), using Mann-Whitney tests.

*D. valens* antennae displayed a similar shape of dose-response curves for benzyl alcohol, 1-octen-3-ol, (Z)-3-hexen-1-ol, and 3-octanol (Fig. 1). With rising stimulus dose, the EAG amplitudes increased to a maximum level where they either reached a plateau or dropped off slightly. Most of the NHVs elicited the biggest EAG response at a stimulus of 10 µg or 100 µg, whereas the dose-response curves showed no evidence of saturation to (E)-2-hexen-1-ol, 1-hexanol and hexanal even at the highest dose tested.

In whole, both sexes showed a similar EAG dose-curves, whereas there were also showed some differences between *D. valens* sexes to tested NHVs. For instance in the case of (E)-2-hexen-1-ol and 1-hexanol there is no increase in response from 10 µg in females whereas the amplitude still grows in males. For female EAG response to hexanal showed a clear dose-dependent effect, whereas in male responses plateau around 40 whatever the dose.

### 3.2. Y-tube behavioral bioassay

The walking behavioral responses to the tested compounds were similar for females and males of *D. valens* by Chi-square tests (1-hexanol,  $\chi^2 = 1.580$ ,  $P = 0.209$ ; (Z)-3-hexen-1-ol,  $\chi^2 = 0.418$ ,  $P = 0.518$ ; (E)-2-hexen-1-ol,  $\chi^2 = 0.170$ ,  $P = 0.680$ ; hexanal,  $\chi^2 = 0.170$ ,  $P = 0.680$ ; 3-octanol,  $\chi^2 = 1.360$ ,  $P = 0.244$ ; 1-octen-3-ol,  $\chi^2 = 0.257$ ,  $P = 0.612$ ; benzyl alcohol,  $\chi^2 = 0.224$ ,  $P = 0.636$ ), so data for males and females were pooled before further analysis. Several NHVs tested all reduced attraction of *D. valens* when added to the kairomone (Fig. 2).

### 3.3. Field trapping trials

A total of 3816 *D. valens* adults were captured in the field trapping experiments in 2004. All of the individual NHVs caused reductions (ranging from 26.3 to 69.5%) in trap catches, compared with the kairomone blend alone.

One-way ANOVA showed that traps releasing three NHVs (1-octen-3-ol, with reduction of 69.5%, (Z)-3-hexen-1-ol, with reduction of 68.3%, and (E)-2-hexen-1-ol, with reduction of 66.0%), all significantly disrupted the response of *D. valens* to the kairomone blend ( $F_{8,70} = 19.14$ ,  $P < 0.01$ ) (Fig. 3). Catches of traps releasing four other NHVs (hexanal, 1-hexanol, 3-octanol and benzyl alcohol) were somewhat lower than traps releasing the kairomone blend, with reductions of 52.9, 47.7, 35.2, and 26.3%, respectively, but not significantly so.

## 4. DISCUSSION

In identifying the volatiles that insects use to locate suitable host plants, electrophysiological recordings of olfactory responses to plant volatiles may give important information. Antennal responses to non-host leaf and bark volatiles have been found in more than 10 bark beetle species [29]. Previous research focused primarily on the use of host monoterpenes (kairomones) by *D. valens* to locate and select its preferred hosts. *D. valens* should employ a generalist type of olfactory receptor neuron on its antennae, which respond to a variety of host monoterpenes. Our results show that NHVs tested in this research elicit antennal responses in *D. valens*. Moreover, *D. valens* responded in an obviously dose-dependent fashion to all compounds tested. This result is consistent with previous studies showing that there are no significant differences between sexes in antennal responses to NHVs [29]. But the EAG dose-curves displayed slightly differences between sexes of *D. valens* to tested, especially hexanal and (E)-2-hexen-1-ol. Zhang et al. [31] suggested that there is a specialized type of NHV-sensitive receptor neuron on scolytid antennae, based on results from Europe and Canada. We hypothesize that *D. valens* have also such an NHV-sensitive receptor neuron. Such electrophysiological speculation can not be supported or rejected from EAG data, however, until individual cells (both receptor and antennal lobe neurons) are tested by using the

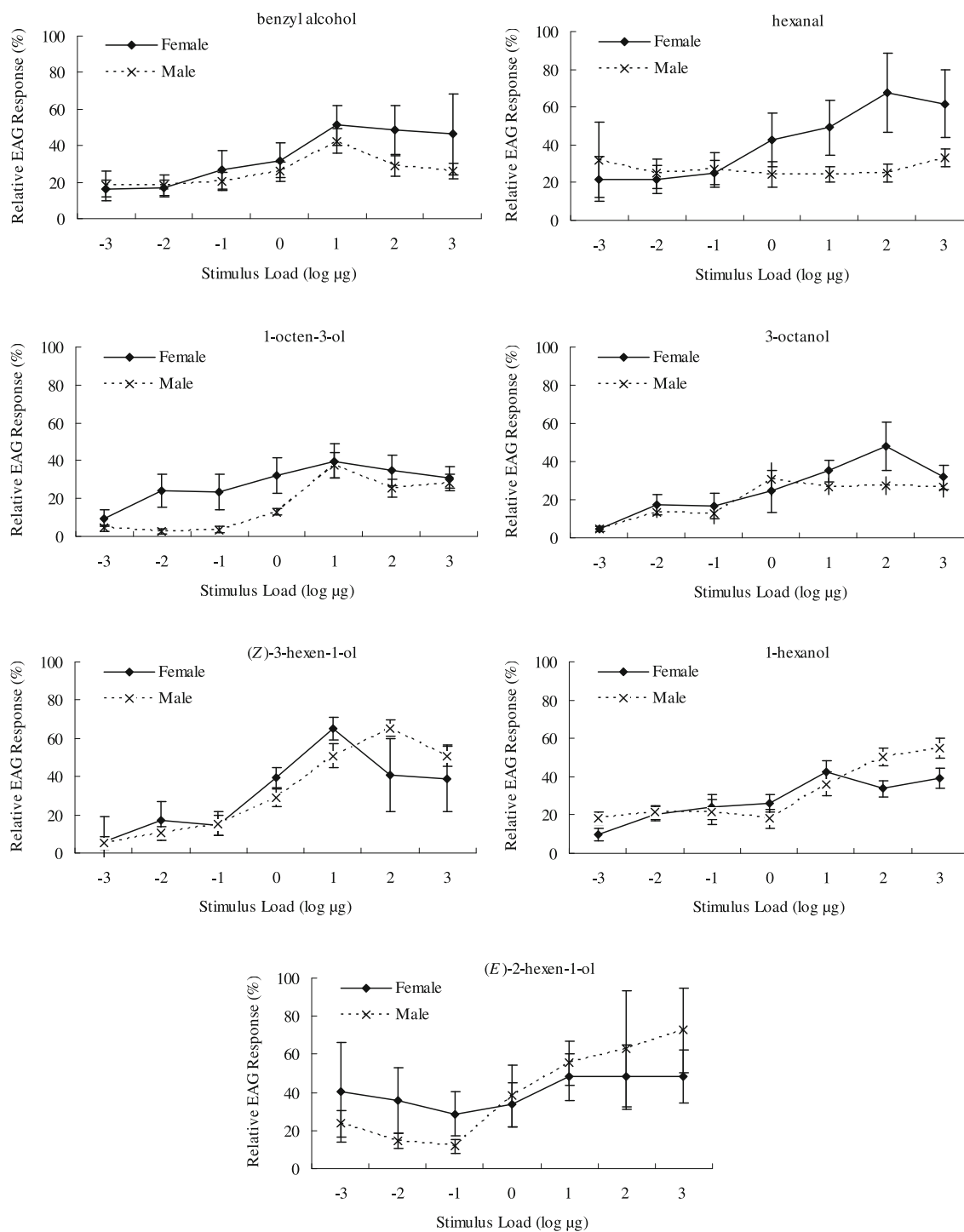
single-cell recording technique [28]. Further studies on diversity and specificity of olfactory receptor neurons responding to host and non-host volatiles will be helpful to answer this question and to understand the host-finding mechanism of *D. valens*.

The laboratory bioassays experiments demonstrated that the tested NHVs, individually, displayed more or less repellent effect to *D. valens*. The laboratory bioassays confirmed electrophysiological responses and the field trapping experiments gave further evidence that NHVs interrupt the host selection behavior of *D. valens*. The result was consistent with previous studies that antennally active NHVs disrupt the response of many conifer-infesting scolytids to the positive (pheromone/kairomone) signals [29].

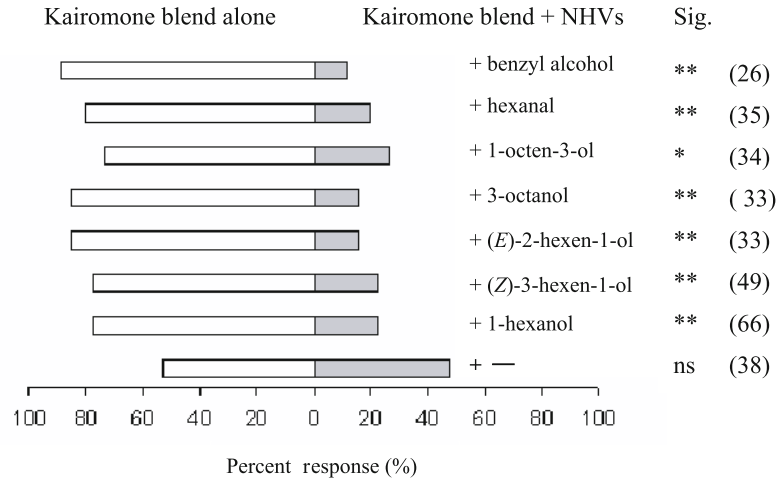
The life cycle of *D. valens* in its Chinese hosts appears to be more cryptic than in its native hosts, with deeper colonization of roots. Insecticidal and fumigation measures to control *D. valens* have had only limited success in China [9, 10]. For these reasons, current bark beetle management focuses on exploiting semiochemical signals that interrupt host selection by bark beetles [3, 16]. It would be adaptive for conifer-inhabiting bark beetles to recognize and avoid general volatile compounds that are commonly found in a wide variety of non-host deciduous and herbaceous volatiles rather than recognizing precise tree-specific volatiles for each non-host species [4]. In turn, we can make use of these properties to develop new management tools for controlling bark beetles. Overall, the results of our field trapping experiment show that 1-octen-3-ol, (Z)-3-hexen-1-ol, and (E)-2-hexen-1-ol were the three most effective NHVs in disrupting the location and selection of the preferred host of *D. valens* in China. Other reports showed similar disruption by alcohols, with the two most effective being (Z)-3-hexen-1-ol and (E)-2-hexen-1-ol [25]. 1-Octen-3-ol was not only identified in the bark volatiles of European birch and aspen [30, 31] but also identified in females of *D. ponderosae*, *D. rufipennis*, and *D. pseudotsugae* [14]. Further field tests found that 1-octen-3-ol was repellent to all three *Dendroctonus* spp. (*D. ponderosae*, *D. rufipennis*, and *D. pseudotsugae*), and 1-octen-3-ol can be potentially be classified as an antiaggregation pheromone, or serve as a kairomone, indicating unacceptable hosts or non-hosts [14]. In development and implementing a semiochemical-based management program for *D. valens*, NHVs may have considerable potential for disrupting the beetle's ability to locate suitable hosts.

Although NHV applications in pine stands showed interruption of the responses of *D. valens* to its preferred host odor, its efficacy is far from operational use. A possible means of improving attack disruption against bark beetles is to combine the use of antiaggregation pheromones with other repellent compounds, including beetle-produced synomones and NHVs [3]. Zhang and Schlyter (2003) reported that synergistic inhibitory effects appear to occur mostly between the negative signals from different levels of origin (nonhost habitats, unsuitable species, and unsuitable hosts) [27]. The general GLVs (e.g. 1-hexanol, (Z)-3-hexen-1-ol, and (E)-2-hexen-1-ol) may represent habitat-level signals, whereas specific bark volatiles (e.g. 3-octanol, and 1-octen-3-ol) and aromatic compounds (e.g. benzyl alcohol) may indicate non-hosts at the

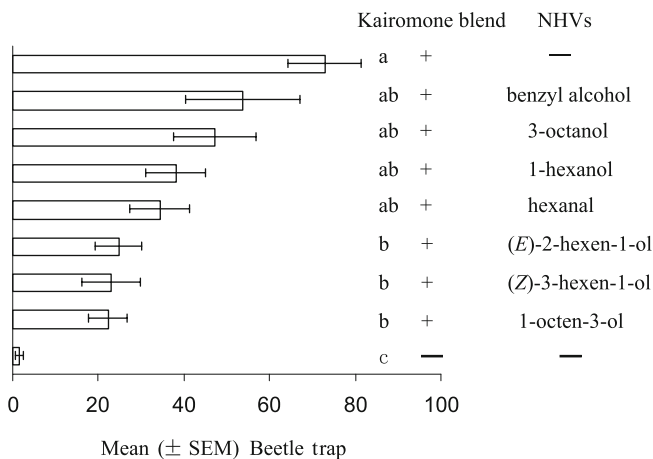




**Figure 1.** EAG dose-responses of *Dendroctonus valens* to dilutions of non-host volatiles (NHVs). Mean responses of *D. valens* to the standard kairomone solution (+)-3-carene ( $1 \mu\text{g}/\mu\text{L}$ ) was  $0.84 \pm 0.10$  mV. No significant differences were found between male  $0.87 \pm 0.15$  mV ( $N = 12$ ) and female  $0.81 \pm 0.15$  mV ( $N = 12$ ) antennal responses ( $P = 0.76$ ) to the (+)-3-carene by Mann-Whitney tests.



**Figure 2.** Response percentages of *Dendroctonus valens* to the non-host volatiles (NHVs) added to kairomone blend in Y-tube olfactometer trials. “ns” “\*” and “\*\*” indicate no significance and significant differences at  $P < 0.05$  and  $P < 0.01$ , respectively. Numbers in parentheses represent number of beetles responding.



**Figure 3.** The result of trapping experiments for *Dendroctonus valens*, Shanxi Province, 2004. Means followed by the same super-script are not significantly different ( $P > 0.05$ ), by ANOVA followed by Ryan-Einot-Gabriel-Welsh (REGW) range test.

species level [29]. In addition, there is another negative signal, verbenone, which is both a signal indicative of unsuitable or fully occupied older host trees [17] and an antiaggregation pheromone that has repeatedly been confirmed to interrupt the attraction of *D. valens* to its kairomones [15, 19]. Paine and Hanlon (1991) reported that verbenone disrupts the response of *D. valens* to traps baited with exo-brevicomin, frontalin, and myrcene [12]. In combining two or three types (habitat, species, and individual tree level) of negative stimuli, we may create an unnatural message indicative of a conspecific population that has mistakenly mass attacked an unsuitable and potentially lethal non-host habitat or species, and may be strong enough to deter bark beetles from entering treated areas [4, 29].

Poland et al. [12] found that verbenone combined with NHVs is more effective in disrupting the pine shoot beetle, *Tomicus piniperda*; a recommended operational disruptant consisted of four NHVs: 1-hexanol, (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, 3-octanol, and verbenone. Moreover, further studies on different NHV mixtures combined with verbenone will be helpful to understand the functional levels of negative stimuli to obtain economically and/or behaviorally optimal blends for tree protection [29].

Recently, Fetting et al. [6] found that the aggregation pheromone (racemic ipsenol, (+)-ipsdienol and *cis*-verbenol) inhibited the response of *D. valens* to attractant-baited traps. So the combination of verbenone, NHVs and allomones will increase the inhibitory effect associated with these semiochemicals.

Decisions regarding optimal pheromone-based pest control strategies must integrate several factors, including relative efficacies, costs, and unintended side effects of the use of these chemicals. Some semiochemicals pose a risk of inducing outbreaks of non-target species, and some, such as *trans*-conophthorin, are prohibitively expensive. Others show promise for pest control but have unacceptable effects on natural enemy complexes [1]. The optimum blend of semiochemicals should be composed of the simplest blend of the least expensive components with the broadest spectrum of behavioral activity for pest insects, while having the least detrimental impact on non-target biota. Future field tests will incorporate these factors in assessing behavioral chemicals for control of *D. valens*.

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