J Insect Behav (2010) 23:251–267
DOI 10.1007/s10905-010-9211-2

Walking Response of the Mediterranean Pine Engraver, *Orthotomicus erosus*, to Novel Plant Odors in a Laboratory Olfactometer

Abigail J. Walter · Robert C. Venette · Stephen A. Kells · Steven J. Seybold

Revised: 21 December 2009 / Accepted: 19 March 2010 /
Published online: 24 April 2010
© US Government 2010

Abstract When an herbivorous insect enters a new geographic area, it will select host plants based on short and long distance cues. A conifer-feeding bark beetle that has been recently introduced to North America, the Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston), has a potentially wide host range, especially among members of the Pinaceae. The long-distance response of the beetles to tree odors may be a key feature of the mechanism of host recognition and selection. We used a laboratory olfactometer to study the walking response of 1,440 *O. erosus* to odor cues from the bark and phloem of six North American tree species. The beetle moved toward the angiosperm non-host *Betula papyrifera* more than would be expected by chance, but had a neutral response to odors of two tree species that support reproduction and three species that do not. These results suggest that tree odors alone may not be adequate for *O. erosus* to recognize novel hosts.

Keywords Bark beetle · host range testing · Pinaceae · Scolytidae · sequential no-choice olfactometer · balsam fir · *Abies balsamea* · eastern hemlock · *Tsuga canadensis* · paper birch · *Betula papyrifera* · red pine · *Pinus resinosa* · tamarack · *Larix laricina* · white spruce · *Picea glauca*

A. J. Walter · S. A. Kells
Department of Entomology, University of Minnesota, 219 Hodson Hall, 1980 Folwell Ave, St. Paul, MN 55108, USA

R. C. Venette (✉)
USDA Forest Service, Northern Research Station, 1561 Lindig St., St. Paul, MN 55108, USA
e-mail: rvenette@fs.fed.us

S. J. Seybold
Chemical Ecology of Forest Insects, USDA Forest Service, Pacific Southwest Research Station, 720 Olive Dr., Suite D, Davis, CA 95616, USA

Introduction

Many species of herbivorous insects are capable of feeding and developing on several plant species, but only a subset of those plant species are encountered by adults and accepted for oviposition (Janz 2002). Plant species that an insect accepts and that support insect development comprise the ecological host range for that insect (Schaffner 2001). When the geographic range of an herbivorous insect expands during a biological invasion, the ecological host range may also expand if the herbivore accepts novel plant species that support the development of its offspring (Jermy 1988). However, new plant-herbivore associations can also lead to "host confusion" (Larsson and Ekbom 1995; Stastny et al. 2006) when the insect fails to distinguish plants that are suitable for larval development from those that are unsuitable. An insect entering a new area may misinterpret the information it receives from formerly allopatric plants and deposit its eggs on a non-host. If this occurs, an invader may be unable to establish, even in an environment containing everything needed for a population to persist.

For bark beetles (Coleoptera: Scolytidae, sensu Wood 2007), mobile adults accept a host tree for their larvae. There has been debate about the nature and importance of environmental cues that adults of various bark beetle species might use to accept hosts, and at which point the final host selection decision is made. Species of bark beetles have been reported to locate hosts based on odor cues received during flight (Tunset et al. 1993; Brattli et al. 1998; Pureswaran and Borden 2005) or based on cues received after landing and sampling the outer bark (Elkinton and Wood 1980; Wallin and Raffa 2002) or phloem (Wood 1963; Elkinton and Wood 1980) of a tree. Several conceptual models to explain host selection behavior by bark beetles have been proposed (Graves et al. 2008).

The importance of plant odor as a cue for host selection varies among bark beetle species. Non-host volatiles are known to deter some bark beetle species from landing on unsuitable hosts (Schroeder 1992; Schlyter et al. 2000; Huber and Borden 2001; Jactel et al. 2001; Zhang and Schlyter 2004; Fettig et al. 2009). Some bark beetle species are attracted to host volatiles (Schroeder 1987; Brattli et al. 1998; Kohnle 2004; Erbilgin et al. 2007) alone or in concert with aggregation pheromones (reviewed in Seybold et al. 2006), but other species appear to land indiscriminately on objects that have the correct visual profile (Hynum and Berryman 1980; Moeck et al. 1981).

The Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston) (Coleoptera: Scolytidae, sensu Wood 2007), is a bark beetle that was recently introduced to North America, with an established population in California (Lee et al. 2005; Seybold and Downing 2009). The beetle has been reported in association with many conifer species, leading to concern that it may attack a wide range of North American conifers should its current geographic range expand (Eglitis 2000). The beetle feeds on a variety of pines (*Pinus*) and has been reported in association with other members of the Pinaceae, such as Douglas-fir (*Pseudotsuga*), spruce (*Picea*), fir (*Abies*), and cedar (*Cedrus*) (Wood and Bright 1992; Bright and Skidmore 1997; Lee et al. 2008), and cypress (*Cupressus*) (Cupressaceae) (Mendel and Halperin 1982) in the field.

Previous work with North American tree species suggests that *O. erosus* may not distinguish reproductively suitable tree species from unsuitable species when adults

are in contact with the outer bark (Walter et al. 2010). If this is the case, it lowers the probability that *O. erosus* could establish in new areas of North America. In previous physiological host-range testing, the beetle has been able to reproduce on pines, spruces, Douglas-fir, and, to a lesser extent, on tamarack (*Larix*) but not on balsam and white fir, eastern hemlock *(Tsuga*), paper birch *(Betula),* coast redwood *(Sequoia*), or incense cedar (*Calocedrus*) (Lee et al. 2008; Walter et al. 2010). In contrast to the previously documented physiological host range, the ecological host range of *O. erosus* may be determined by its host acceptance behavior. When placed in contact with the outer bark, the beetles bored into logs of red pine, *Pinus resinosa* Aiton; white spruce, *Picea glauca* (Moench) Voss; and balsam fir, *Abies balsamea* (L.) Mill, at the highest rates. Beetles bored into tamarack, *Larix laricina* (Du Roi) Koch, and eastern hemlock, *Tsuga canadensis* (L.) Carrière, logs at slightly lower rates; and into paper birch, *Betula papyrifera* Marsh, logs very infrequently (Walter et al. 2010). Although certain volatiles from non-host plants are known to interfere with the pheromone response of *O. erosus* (Zhang and Schlyter 2004), the beetles' use of plant odors in host selection has not been characterized. The long-range response to tree odor cues may be an important component of host acceptance by *O. erosus* because attraction to suitable species or repellence by unsuitable species may prevent beetle adults from coming into contact with trees that are unsuitable for reproduction but would otherwise elicit adult boring behavior.

To better understand the effects of tree odors on the behavior of *O. erosus*, this paper examines the walking response of adult *O. erosus* to the bark and phloem odors of six eastern North American tree species. These studies address two hypotheses: 1) Tree odors elicit behavioral responses from *O. erosus*, and 2) Odors from trees that differ in their suitability or short-range acceptability also differ in their attractiveness to *O. erosus*. We chose to include tree odors in this study from species that were acceptable and unacceptable to beetles in contact with the bark surface and suitable and unsuitable for reproduction to enable us to evaluate the ability of *O. erosus* to avoid non-hosts as well as its ability to locate suitable host plants.

Materials and Methods

This experiment took place in the Minnesota Agricultural Experiment Station/ Minnesota Department of Agriculture Biological Level 2 Containment Facility in St. Paul, MN, USA. Beetles from the established population in California and from a colony maintained in St. Paul were used for the experiments. All handling procedures were approved by the USDA APHIS Plant Protection and Quarantine Division (Permit 74447).

Field Collection of Beetles

Cut logs of Aleppo pine, *Pinus halepensis* Mill., and Italian stone pine, *Pinus pinea* L., infested with *O. erosus* were collected during the summer of 2008 in Fresno County, CA, USA and held in emergence cages in Davis, CA (Browne 1972). Adults were allowed to emerge naturally from brood logs, to crawl or fly towards a lighted

exit, and were kept in refrigerators (approx. 10°C) in jars with moist paper towel until shipment. On 24 June, 5 August, and 13 August 2008, emerged beetles were shipped to St. Paul, MN in insulated styrofoam boxes containing ice packs. All beetles were held in the dark at approximately 10°C and deprived of food until they were used in the experiment 1-20 d later.

Beetle Colony

A colony of *O. erosus* established from beetles collected in California in 2006 and refreshed with beetles from California in 2007 and 2008 was maintained on cut logs of red pine by using previously described methods (Walter et al. 2010). In our rearing conditions and in the field, *O. erosus* adults initiate new egg galleries in the brood log instead of emerging if the log is moist enough (Mendel and Halperin 1982). In our colony, adults did not respond to light traps, but continuously re-infested the brood log, resulting in severe competition and population decline. Therefore, beetles used for the experiments or to maintain the colony were obtained by peeling the bark and phloem of the colony logs and manually extracting adults. Between removal from the brood log and use in the experiment, beetles were kept in plastic Petri dishes at approximately 10°C for at least 24 h.

Collection of Logs for the Experiments

The tree species used in the experiment included: red pine, white spruce, tamarack, eastern hemlock, balsam fir, and paper birch. Two trees of each species were felled at the University of Minnesota North Central Research and Outreach Center (UMN-NCROC, Grand Rapids, MN) between 16 and 19 June 2008 and immediately cut to approximately 50 cm lengths. The cut logs (28–53 cm diameter) were sealed with paraffin wax and stored in a greenhouse for less than 8 weeks.

Laboratory Olfactometer Design

The experiment compared the response of individual *O. erosus* when exposed to clean air and to the odor of one of several North American tree species. Air was pushed by a pump through a filter (Big Hydrocarbon Trap, BHT-2, Agilent, Santa Clara, CA, USA), then humidified in a 250 ml Erlenmeyer flask filled with distilled deionized water with the air inlet below the water level (Fig. 1). After being humidified, the airstream was split, with each side passing through a 150 mm flowmeter (Size #3 Riteflow Flowmeter, Bel-Art Products, Pequannock, NJ, USA) and then into one of two 25 cm tall×15 cm diameter glass volatile collection chambers with one-port lids (ARS, Inc., Gainesville, FL, USA). Each volatile chamber contained a plastic stand to ensure air flow around the stimulus. After passing through the volatile chamber, each airstream was split again, and passed into four walking chambers, which were each composed of a 22-cm-long piece of Nalgene Premium Tubing (0.7 cm ID, Fisher Scientific, Pittsburgh, PA, USA). All connections prior to the walking chambers were made by using polytetrafluoro-ethylene (PTFE) tubing (Sigma-Aldrich, St. Louis, MO, USA). Assays were run concurrently in each of the four walking chambers, two for the clean control

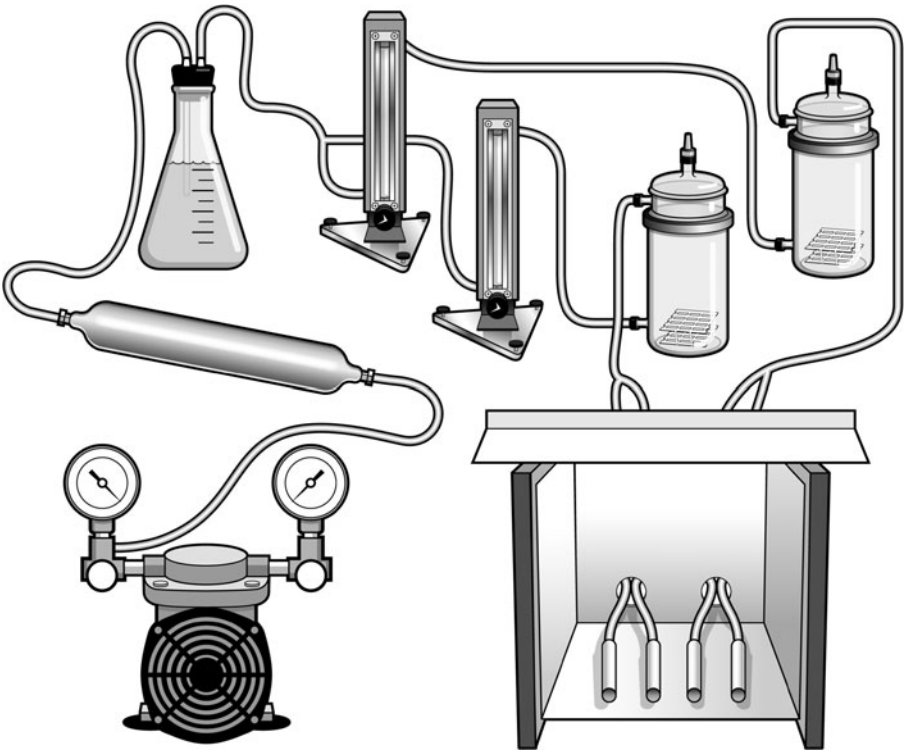

Fig. 1 Schematic of the laboratory olfactometer used to test the walking behavior of *Orthotomicus erosus*. Air flowed from the pump through the hydrocarbon filter and then through the humidifier. Next, the airstream was split, with each half going to a flowmeter set at 400 ml/min, then into an odor chamber, empty or with bark and phloem from one of the tested tree species. After leaving the odor chamber, each airstream was split again before entering walking chambers inside the white box. All connections prior to the walking chambers were made with PTFE tubing. After the airstream had been split, identical components of the olfactometer were connected by using tubing of the same length.

airstream and two for the odor-laden treatment airstream, in order to increase the throughput of the assay.

A 15-cm course was marked on each walking chamber beginning 1 cm from the open end of the tube. The walking chamber was straightened by fastening it to a plastic test tube rack with wire twist ties. The four walking chambers were placed in a 56×60×60 cm experimental arena made of veneered particle board with a white floor and white walls on three sides. The arena was illuminated by a fluorescent light fixture (two 40 W tubes, 60 cm long) placed 60 cm above the floor of the arena as described in Wyckhuys and Heimpel (2007) (Fig. 1). The airflow at both flowmeters was 400 ml/min and split downstream of the flowmeters, so that the airflow through the four individual walking chambers was approximately 200 ml/min.

Each beetle was exposed to two airstreams, first a control airstream, then a treatment airstream. Beetles were always exposed to the control airstream first in order to avoid any effect that moving out of an odor-laden airstream might have on beetle movement (i.e., casting behavior). To initiate each 5 min exposure to an airstream, beetles were placed in the open end of the walking chamber with soft

forceps. The time when the beetles passed the first mark of the 15 cm course, and the total distance that they moved in 5 min were recorded. If beetles turned and walked in the direction of the airflow and exited the chamber, the time that they left the chamber was recorded and the distance moved was considered -1.1 cm. If a beetle walked the entire 15 cm course, then the time when the beetle passed the end of the course was recorded and the distance was considered 15 cm. The same walking chamber was used for the control and treatment exposures. All ties were resolved by comparing the time required by the beetles to move the distance; a higher velocity was equivalent to a greater distance.

The stimulus chambers containing the controls and treatments were randomly assigned, as were the position of the control and treatment tubes within the white arena. Chambers and positions were reassigned before starting a new treatment. Each beetle was used only once in the experiment. Each walking chamber was used for one beetle before being cleaned; the stimulus chambers that contained bark and all downstream tubing were cleaned after each group of 20 beetles. The cleaning procedure involved washing the olfactometer components in soapy water, rinsing with hot water, and then rinsing again with 95% ethanol. Safety concerns precluded the use of other solvents within the quarantine facility. All parts of the olfactometer were thoroughly air dried before being reused. This was similar to the procedure used for other olfactometer experiments involving plant odors in the same quarantine facility (Wyckhuys and Heimpel 2007).

Determining Sample Size

We used a power analysis to determine the total number of beetles (N) needed to detect a 10% difference in the probability of moving further in the treatment airstream than in the control with 95% confidence by using the equation:

$$N = \frac{z_{\alpha/2}^2 \hat{p}\left(1-\hat{p}\right)}{E^2}$$

where $z_{\alpha/2}$ is 1.96 from the standard normal distribution with α=0.05, $\hat{p} = 0.5$, and E=0.10 represents the detectable difference in probability (Ott and Longnecker 2001). We determined that a minimum of 97 individuals would be needed. For the clean air assay, we elected to use six blocks (120 beetles total). For the attraction assay, we used six blocks with two trees for each tree species (120 female and 120 male beetles per species per block), allowing us to test the effects of block and beetle sex. The 10% difference was chosen arbitrarily based on the results that we observed from the first two blocks of the host attraction assay (see below), on our estimation of the magnitude of difference from random movement that would be biologically meaningful, and on a pragmatic estimate of how many beetles would be available for use in the experiment.

Clean Air Assay

This experiment involved 120 beetles and was designed to evaluate the tendency of *O. erosus* to move a longer or shorter distance the second time that they were placed

in the olfactometer. In this trial, termed the clean air assay, a total of 60 males and 60 females were exposed to airstreams without odors in the control and treatment exposures. Four blocks consisted of 10 male and 10 female beetles from the laboratory colony. Two blocks consisted of beetles from the CA field population. The order that individual beetles were used within each block was randomly determined, but the source of beetles (laboratory colony or field-collected) for each block was determined by the availability of beetles.

Host Attraction Assays

This experiment involved 1,440 beetles. The treatment stimulus was 200 g of fresh bark and phloem removed from the logs of one of the six test tree species with a draw knife (2 trees per species were used in the experiment). Six blocks of the experiment were run, each consisting of 10 male and 10 female beetles per test tree (20 male and 20 female beetles per tree species per block). Each block contained one bark/phloem sample from each of the 12 test trees (2 trees/tested species). Three blocks consisted of field-collected beetles, and three consisted of laboratory-reared beetles. The order that bark/phloem samples were presented in each block and the order in which individual beetles were used was randomly determined. However, the source of beetles used in each block (laboratory colony or field population) was determined according to availability of beetles.

Data Analysis

Because presenting treatments in the same order might lead to apparent differences based on treatment order rather than the treatments themselves, we confirmed our expected null probability with the results of the clean air assay. If each individual beetle moved a random distance every time it was exposed to clean air, the probability of moving a greater distance upstream during the second exposure to clean air than the first would be 0.5.

For the clean air assay, the effects of beetle sex, block, and their interaction on the probability of a beetle moving further during the second exposure to a clean airstream than during the first exposure during the clean air assay were analyzed by logistic regression. Model selection was carried out by forward, backward, and stepwise selection with an α of 0.05 (PROC LOGISTIC, SAS Institute Inc. 2004). In order to evaluate whether the results were equivalent to the expected value of 0.5, the 95% confidence interval of the probability of moving a greater distance during the second exposure was calculated. If the confidence interval did not include 0.5, the behavior of the beetles in response to the tested odor was considered different than would be expected by chance.

The probabilities that individual beetles would move a greater distance during the treatment exposure than during the control exposure in the host attraction assay were also evaluated by logistic regression (PROC LOGISTIC, SAS Institute Inc. 2004). The original model included tree species, beetle sex, block, and all two-way and three-way interactions. Significant effects (α=0.05) were selected by using forward, backward, and stepwise selection. The simultaneous 95% confidence intervals (calculated with the Bonferroni-adjusted α of 0.00833) (Kuehl 2000) of the

probability that a beetle moved a greater distance during exposure to each of the six tree odors were generated in order to evaluate whether the observed probabilities were different from the probability of 0.5 expected from random movement. The fifteen contrasts among individual tree species were also conducted and evaluated with a Bonferroni-adjusted α of 0.0033, resulting in an experimentwise α of 0.05 (Kuehl 2000).

We hypothesized that the magnitude of the difference in the distance the beetles moved during the control and treatment exposures might contain information about the attractiveness of the odor. Therefore, an analysis of the magnitude of the difference between the distance beetles moved during the control and treatment exposures was carried out. Because the distribution of differences was non-normal (Kolmogorov-Smirnov D=0.15, $P<0.01$), a nonparametric ANOVA was carried out on the ranks of the differences of the distance traveled by each beetle in the treatment exposure versus the control exposure (PROC RANK, PROC MIXED, SAS Institute Inc. 2004). The fifteen contrasts among individual tree species were evaluated with a Bonferroni-adjusted α of 0.0033, corresponding to an experiment-wise α of 0.05 (Kuehl 2000).

Results

Clean Air Assay

In 13.3% of cases, the beetles moved an identical distance when exposed twice to a clear airstream. In 53.8% of those cases, the beetle moved out the back of the tube during both exposures. The beetles moved an average of 1.89 cm with a standard error of 0.21 cm during a 5 min exposure to a clean airstream.

The 95% confidence interval for the probability that a beetle moved further during the second exposure to a clean airstream contained the expected null value of 0.5 (actual probability=0.492, lower confidence limit=0.420, upper confidence limit=0.597). We concluded that beetle movement was not detectably different between the first and second exposure to clean airstreams. The effect of sex, block, and their interaction did not meet our criteria for inclusion in the logistic regression model by forward, backward, or stepwise selection. Therefore, these factors did not influence the probability. This result confirms our *a priori* expectation that the probability that a beetle would move a greater distance during the second exposure to an airstream was 0.5 if the treatment odor did not affect beetle behavior. Thus, in the analysis of the host attraction assays, 0.5 was used as the expected null probability that a beetle would move a greater distance in the treatment airstream than in the clean airstream.

Host Attraction Assay

Beetles moved an identical distance in the control and treated airstreams in 17.8% of the trials; 73.5% of these were instances where a beetle moved out the back of the tube during both exposures. Beetles moved a mean distance of 1.38–4.33 cm depending on treatment during a 5 min exposure (Table 1). However, the appropriate

Table 1 Mean distances moved (cm) by adult *Orthotomicus erosus* during a 5 min exposure to a clean airstream followed by an airstream carrying the odor of the bark and phloem of a tree species

Species	Clean airstream		Treatment airstream	
	Distance moved	SE	Distance moved	SE
Red pine	2.34	0.29	4.33	0.35
White spruce	1.84	0.28	3.21	0.33
Tamarack	1.72	0.26	2.91	0.31
Eastern hemlock	2.38	0.25	3.78	0.31
Balsam fir	1.38	0.20	1.96	0.27
Paper birch	1.86	0.22	3.30	0.28

response to examine is the difference between individual movements in the treatment and control airstream. The distributions of distances moved by individuals in the clean air and treatment airstreams appeared similar for all tree species (Fig. 2).

Tree species and block affected the probability that a beetle moved further in the treatment airstream than in the control airstream during the host attraction assay (tree species: df=5, Wald χ^2=18.96, P=0.0019; block: df=5, Wald χ^2=13.44, P=0.0196). The effect of sex or interactions among sex, block, or tree species did not meet our criteria for inclusion in the logistic regression model. Forward, backward and stepwise selection methods arrived at the same regression model. When the confidence intervals of the probability of moving further in the treatment airstream were compared to the expected value of 0.5, beetles had a higher than expected probability of moving towards the source only when the stimulus was from paper birch (Table 2). Thus, the odors from conifer bark/phloem samples in this experiment did not make *O. erosus* behave differently than it did in a clean airstream, but the odor from a paper birch bark/phloem sample made them more likely to move towards the source of the odor. The effect of block did not interact with the effect of tree species, and therefore did not affect our conclusions about the attractiveness of the tree odors. Beetles had a higher probability of moving a greater distance in the treatment airstream in blocks containing beetles from the CA field population than in blocks containing beetles from the laboratory colony (df=1, Wald χ^2=6.66, P=0.0098).

In the among-species comparison of the probability that a beetle would move a greater distance in the treatment airstream, the tree species fell into two groups (Fig. 3): one group consisting of balsam fir, tamarack, and white spruce and another consisting of paper birch (the attractive odor), tamarack, eastern hemlock, red pine, and white spruce. The probability of a positive walking response was numerically lowest when the odor stimulus was from balsam fir, and this probability was significantly lower than when the odor was from red pine, eastern hemlock, or paper birch (Fig. 3). Differences between individual species may occur even when neither is different from the null because the assay was designed to detect differences in probability of about 0.1. The difference between some treatments was greater than 10% even if neither treatment had a greater than 10% difference from the null. Therefore, the beetle responses to the odors of some tree species could be

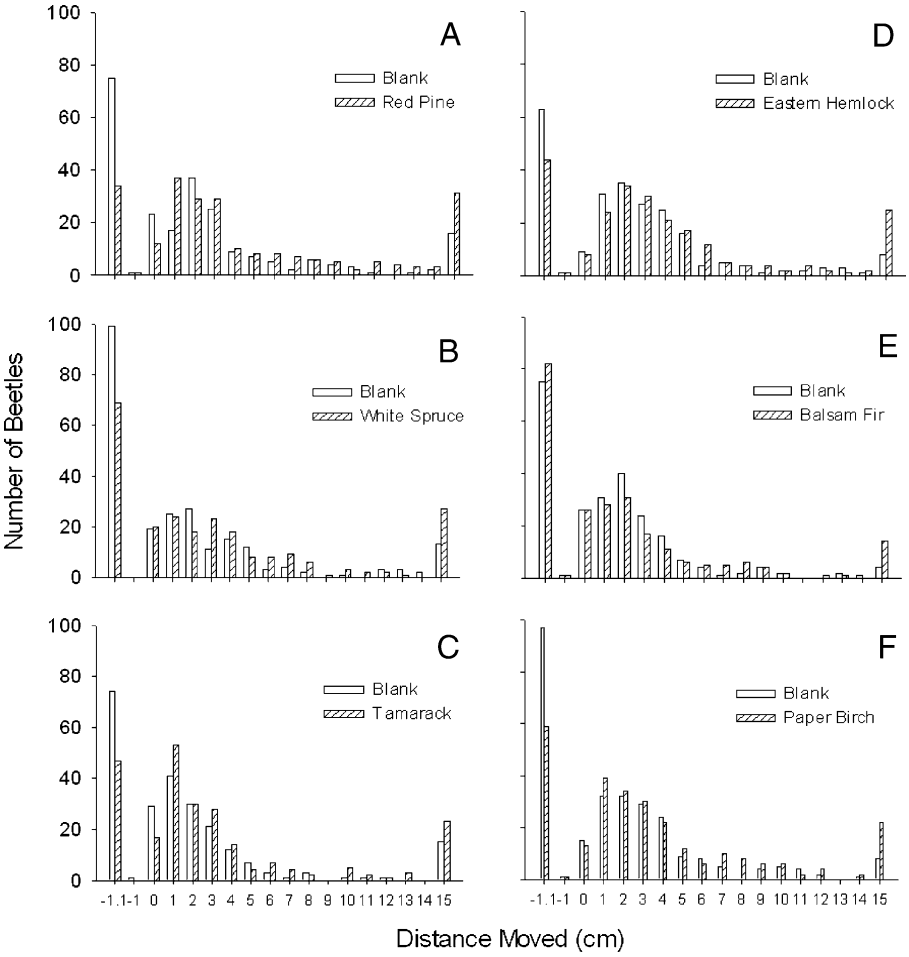

Fig. 2 Distributions of distances moved by individual *Orthotomicus erosus* in clean air and treatment airstreams for odors from **A** red pine, **B** white spruce, **C** tamarack, **D** eastern hemlock, **E** balsam fir, and **F** paper birch.

distinguished from each other, but the responses to all of the odors except paper birch were not distinguishable from random movement.

The qualitative results of the ANOVA on the ranks of differences between distances moved were similar to the results of the logistic regression. Tree species and block both affected the ranks of the distances (tree species: df=5, 1458, F=3.11, P=0.0084; block: df=5, 1458, F=2.25, P=0.0472). The tree species odors fell into two groups: one consisted of red pine, paper birch, eastern hemlock, white spruce, and tamarack. The other consisted of eastern hemlock, white spruce, tamarack, and balsam fir. Beetles from the CA field population tended to have a greater difference in distances moved than those from the laboratory colony (df=1, 1458, F=5.63, P= 0.0178). The only difference between the logistic regression and the nonparametric ANOVA was that eastern hemlock grouped with balsam fir in the ANOVA but not in the regression.

Table 2 Probability of a positive walking response by *Orthotomicus erosus* to an airstream with bark/phloem odor

Tree species	Probability of moving a greater distance when exposed to treatment odor[a]			
	Observed	LCI-95%	UCI-95%	*N*
Red pine	0.61	0.4908	0.7119	234
White spruce	0.57	0.4561	0.6800	239
Tamarack	0.59	0.4763	0.6977	240
Eastern hemlock	0.60	0.4848	0.7051	240
Balsam fir	0.46	0.3476	0.5732	240
Paper birch	0.63*	0.5207	0.7281	276

Probability that an individual would walk a greater distance in the olfactometer when presented with an airstream containing volatiles from the bark and phloem of the six tested tree species than in a clean airstream

*, Significantly different from 0.5 expected from random movement

[a] LCI-95%, Lower bound of the 95% confidence interval; UCI-95%, Upper bound of the 95% confidence interval; To control the experimentwise α at 0.05, a Bonferroni-adjusted α of 0.0083 was used for confidence interval calculations

Discussion

This study is part of a broader series of experiments aimed at predicting the establishment potential of a theoretically introduced population of *O. erosus* in eastern North America. The ability of pioneer beetles to locate a suitable host without the influence of conspecific aggregation pheromones is important for population establishment. Along with the behavior of beetles in contact with the bark, the long-range response of *O. erosus* to tree odors may be part of host location.

For establishment to occur, *O. erosus* must contact, bore into, and oviposit in material that can support the development of offspring. Although high populations have been reported to attack live trees, *O. erosus* usually utilizes recently killed trees

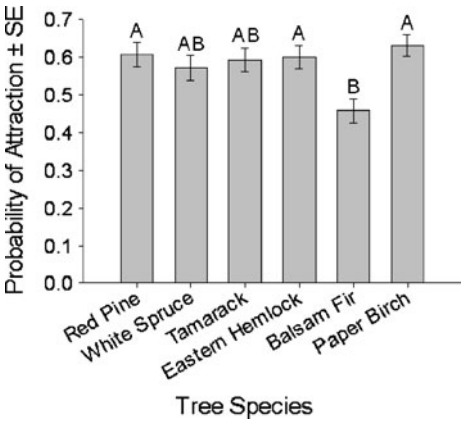

Fig. 3 Probability that individual *Orthotomicus erosus* moved further in a straight tube when exposed to an airstream containing odor from the bark and phloem of selected tree species than when exposed to a clean airstream. Bars labeled with the same letter were not significantly different according to a contrast among tree species in logistic regression.

or broken branches or stems for reproduction (Mendel et al. 1985). Therefore, the beetles can be expected to colonize dead host material during the establishment phase of a biological invasion, and bark and phloem from cut logs was an appropriate stimulus for our assay. In this olfactometer assay, none of the odors of conifer species stimulated beetle movement relative to the movement in a clean airstream. However, the odor of paper birch, an allopatric non-host angiosperm, elicited a positive response from the beetles. We found that wild beetles from the CA field population had a higher probability of responding to tree odors than the beetles from the lab colony, possibly because some of the beetles from the lab colony were not at the appropriate physiological state for host location. However, the lack of a significant interaction between block and treatment shows that the pattern of response of the two populations was the same. This might have been expected because the laboratory colony was derived originally from a wild population in California and only a limited number of generations had occurred while in culture. Since our goal was to determine whether there was a response that differed from random movement, it is appropriate to consider both populations together.

Male *O. erosus* is usually reported as the host-colonizing sex, but we did not observe a difference between males and females in the response to tree odors. We also did not find a difference between the sexes in bark boring behavior in a previous experiment (Walter et al. 2010). Since a large proportion of *O. erosus* females are mated when they emerge from the brood log (Mendel 1983), it is possible that females are capable of searching for a host if they are unable to detect a male.

Previous studies that assayed the behavior of walking bark beetles with olfactometers have demonstrated that the responses were indicative of responses obtained from flying beetles in field-trapping experiments (Wood 1970; Browne et al. 1974; Guerrero et al. 1997; Macías-Sámano et al. 1998; Hovorka et al. 2005), although exceptions do occur (Wood 1970; Zhang et al. 2009). We do not believe that our olfactometer results can be used to predict the exact percentage of beetles that will be attracted to the odors of potential host species in the field. However, we do assume that these results are representative of the behavior of flying beetles, i.e., an odor that elicits a random response from beetles walking in an olfactometer will elicit a random response from flying beetles.

The response of *O. erosus* to the odors of North American trees differed from the reproductive potential of the beetles on the same set of trees and their boring behavior when in contact with the bark surface. Lee et al. (2008) and Walter et al. (2010) showed that red pine and white spruce support beetle reproduction equal to or greater than the replacement rate. Very few offspring were produced on tamarack. Eastern hemlock, balsam fir, and paper birch did not support any reproduction. Adults in contact with the bark surface readily bored into red pine, white spruce, and balsam fir; boring in tamarack and eastern hemlock occurred with moderate frequency (Walter et al. 2010). The response of *O. erosus* to bark and phloem odors may not increase encounters with coniferous hosts and non-hosts beyond what would be expected through random chance; encounters with paper birch might be higher than predicted by chance if the beetle only uses odors to locate hosts. If the beetles arrived at all of the conifer species at the same rates, the suitable species red pine and white spruce and the unsuitable species balsam fir would be accepted at the highest rates, with tamarack and eastern hemlock accepted at lower rates.

Our experiment was designed to circumvent some of the more common problems with laboratory assays of host odors. The large sample size employed in this study (>1,400 individual beetles) ensures that we would be able to detect a proportion of the population as small as 10% that responded to a tree odor; as many as 50% of *Tomicus piniperda* respond to volatile components of the odor of Scots pine (Byers et al. 1985). Few olfactometer experiments have quantified the magnitude of the detectable response. In addition, the use of bark and phloem rather than compounds released from a headspace sampling device ensure that the concentrations used and representation of compounds in the assay should be reflective of the conditions the beetles experience in the field. Most significantly, the direct use of bark and phloem also ensures that we would detect effects due to minor odor components or synergy between compounds.

Because odor-plume dynamics in a forest can be very complicated, and odor concentration might not be indicative of distance to the source (reviewed in Riffell et al. 2008), we chose to use an enclosed olfactometer with no difference in odor concentration. During preliminary experiments, we observed a large variation in the tendency of *O. erosus* individuals to move in several different types of olfactometer, regardless of the stimulus presented (AJW, personal observations). We examined each individual's response to clean and odor-laden airstreams in order to remove the influence of individual tendency to move long or short distances. Although we did not find a repellent treatment in this experiment, another advantage of this olfactometer design is that repellency can be detected if beetles tend to move shorter distances in odor-laden versus clean airstreams. The assay results with balsam fir might suggest a negative tactic response of around 4%, but a larger sample size (N=603) would be required to detect this effect with 95% confidence.

The positive response of *O. erosus* to the angiosperm non-host paper birch was unexpected. Volatiles such as *trans*-conophthorin are found in paper birch and interfere with the pheromone response of *O. erosus* (Zhang and Schlyter 2004) and other bark beetles (Huber et al. 1999, 2001; Byers et al. 2000; Jactel et al. 2001; Zhang and Schlyter 2003; Graves et al. 2008). However, bark extracts from other *Betula* species have been reported to contain 2-methyl-3-buten-2-ol (Zhang et al. 2000), which is a component of the aggregation pheromone of *O. erosus* (Giesen et al. 1984). Other coniferophagous bark beetles employ olfactory (Schroeder 1992; Guerrero et al. 1997; Huber and Borden 2001; Jactel et al. 2001; Byers et al. 2004) and visual (Campbell and Borden 2006) non-host cues when selecting hosts, and it is possible that non-chemical cues would prompt *O. erosus* to avoid birch in the field while in flight. Alternatively, the response of *O. erosus* to paper birch, as well as the absence of a strong response to other tree species in this experiment, may arise because the beetle and the tree are naturally allopatric.

Host selection studies in quarantine conditions do not include the full complement of stimuli that insects might use to select hosts in the field, which may lead to false positive and false negative responses (Zwolfer and Harris 1971). Other stimuli such as visual profiles, both color and shape, and odors from parts of the plant other than the bark and phloem are known to be used for host location by other bark beetles (Person 1931; Schroeder 1992; Campbell and Borden 2006; Zhang et al. 2007). Nevertheless, the results of this study and our previous work (Walter et al. 2010) suggest that beetles would move towards and accept red pine, white spruce, and

balsam fir with similar frequency; similar results were obtained in a field cage experiment where *O. erosus* accepted several pine, spruce, and fir species at approximately equal rates in choice assays (Chararas et al. 1982).

Many bark beetle larvae are capable of developing on a wider variety of hosts than are actually selected by adults for oviposition in the field, and host selection rather than developmental ability is considered the major determinant of the ecological host range of bark beetles (Sauvard 2004). A similar pattern has been recorded for other insects (Courtney and Kibota 1990; Bernays and Chapman 1994). Thus, we had expected that the set of allopatric tree species that elicited a positive response in the olfactometer would be a subset of the species that the support larval development (Lee et al. 2008; Walter et al. 2010). However, it appears that more tree species are acceptable to *O. erosus* in contact with the bark than can support larval development, and odors from tree species suitable for the reproduction of the beetle do not elicit a strong response from the *O. erosus* even though at least one unsuitable species is attractive.

Invasive insects are a source of major environmental and economic damage in North America and throughout the world, but most species that are introduced do not become damaging invaders (Williamson 1996). *O. erosus* has established a small population in North America. Although the beetle has become a damaging invader in other parts of the world (Lee et al. 2005), its potential to cause damage in North America is poorly understood. Studying the walking response of this beetle to allopatric potential host plants under quarantine conditions helps to clarify the risk posed by the beetle. *O. erosus* responded positively to the odor of an allopatric angiosperm non-host but did not respond to the odors of several allopatric conifers in a way that can be distinguished from random movement. If the beetle's geographic range expanded to coincide with these tree species, it does not appear that the beetle could use bark and phloem odors alone to recognize novel tree species that are suitable for reproduction. If *O. erosus* accepts unsuitable tree species as hosts, the risk of establishment in large areas of North America may be less than previously estimated.

Acknowledgements We would like to thank T. O'Brien (University of Minnesota NC-ROC) for assistance in felling trees, and the MAES/MDA Containment Facility. We also thank J. C. Lee, D.–G. Liu, and S. M. Hamud (UC-Davis and USDA FS PSW Station) for assistance with collecting, sorting, and shipping *O. erosus*. B. Therens assisted with the laboratory assays. The manuscript was improved by the suggestions of E. Clark, D. Huber, W. Meyer, D. Pureswaran, and two anonymous reviewers. This research was funded by a University of Minnesota Graduate School Doctoral Dissertation Fellowship, and a University of Minnesota Department of Entomology Marion Brooks-Wallace Fellowship to AJW, the USDA Forest Service Northern and Pacific Southwest Research Stations, and the Minnesota Agricultural Experiment Station.

References

Bernays EA, Chapman RF (1994) Host-plant selection by phytophagous insects. Chapman and Hall, New York

Brattli JG, Andersen J, Nilssen AC (1998) Primary attraction and host tree selection in deciduous and conifer living Coleoptera: Scolytidae, Curculionidae, Cerambycidae and Lymexylidae. J Appl Entomol 122:345–352

Bright DE, Skidmore RE (1997) A catalog of Scolytidae and Platypodidae (Coleoptera), supplement 1 (1990–1994). NRC, Ottawa

Browne LE (1972) An emergence cage and refrigerated collector for wood-boring insects and their associates. J Econ Entomol 65:1499–1501

Browne LE, Birch MC, Wood DL (1974) Novel trapping and delivery systems for airborne insect pheromones. J Insect Physiol 20:183–193

Byers JA, Lanne BS, Löfqvist J, Schlyter F, Bergström G (1985) Olfactory recognition of host-tree susceptibility by pine shoot beetles. Naturwissenschaften 72:324–326

Byers JA, Zhang Q-H, Birgersson G (2000) Strategies of a bark beetle, *Pityogenes bidentatus*, in an olfactory landscape. Naturwissenschaften 87:503–507

Byers JA, Zhang Q-H, Birgersson G (2004) Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors. Naturwissenschaften 91:215–219

Campbell SA, Borden JH (2006) Close-range, in-flight integration of olfactory and visual information by a host-seeking bark beetle. Entomol Exp Appl 120:91–98

Chararas C, Revolon C, Feinberg M, Ducauze C (1982) Preference of certain Scolytidae for different conifers: a statistical approach. J Chem Ecol 8:1093–1109

Courtney SP, Kibota TT (1990) Mother doesn't know best: selection of hosts by ovipositing insects. In: Bernays E (ed) Insect-plant interactions, vol 2. CRC, Boca Raton, pp 161–188

Eglitis A (2000) EXFOR Database pest reports: *Orthotomicus erosus*, USDA Forest Service, available on-line at: http://spfnic.fs.fed.us/exfor/data/pestreports.cfm?pestidval=9&langdisplay=english, accessed 21 January 2010

Elkinton JS, Wood DL (1980) Feeding and boring behavior of the bark beetle *Ips paraconfusus* (Coleoptera: Scolytidae) on the bark of a host and non-host tree species. Can Entomol 112:797–809

Erbilgin N, Mori SR, Sun JH, Stein JD, Owen DR, Merrill LD, Bolanos RC, Raffa KF, Montiel TM, Wood DL, Gillette NE (2007) Response to host volatiles by native and introduced populations of *Dendroctonus valens* (Coleoptera: Curculionidae, Scolytinae) in North America and China. J Chem Ecol 33:131–146

Fettig CJ, McKelvey SR, Dabney CP, Borys RR, Huber DPW (2009) Response of *Dendroctonus brevicomis* to different release rates of nonhost angiosperm volatiles and verbenone in trapping and tree protection studies. J Appl Entomol 133:143–154

Giesen VH, Kohnle U, Vité JP, Pan ML, Francke W (1984) Das Aggregationspheromon des mediterranen Kiefernborken-käfers *Ips* (*Orthotomicus*) *erosus*. Z Ang Ent 98:95–97

Graves AD, Holsten EH, Ascerno ME, Zogas KP, Hard JS, Huber DPW, Blanchette RA, Seybold SJ (2008) Protection of spruce from colonization by the bark beetle, *Ips perturbatus*, in Alaska. For Ecol Manag 256:1825–1839

Guerrero A, Feixas J, Pajares J, Wadhams LJ, Pickett JA, Woodcock CM (1997) Semiochemically induced inhibition of behaviour of *Tomicus destruens* (Woll.) (Coleoptera: Scolytidae). Naturwissenschaften 84:155–157

Hovorka O, Kindl J, Kalinová B, Knížek M, Vrkočová P, Koutek B (2005) The role of beetle and host volatiles in host colonization in the European oak bark beetle, *Scolytus intricatus* (Ratzeburg) (Col., Scolytidae). J Appl Entomol 129:221–226

Huber DPW, Borden JH (2001) Angiosperm bark volatiles disrupt response of Douglas-fir beetle, *Dendroctonus pseudotsugae*, to attractant-baited traps. J Chem Ecol 27:217–233

Huber DPW, Gries R, Borden JH, Pierce HD Jr (1999) Two pheromones of coniferophagous bark beetles found in the bark of nonhost angiosperms. J Chem Ecol 25:805–816

Huber DPW, Borden JH, Stastny M (2001) Response of the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae), to conophthorin and other angiosperm bark volatiles in the avoidance of non-hosts. Agr Forest Entomol 3:225–232

Hynum BG, Berryman AA (1980) *Dendroctonus ponderosae* (Coleoptera: Scolytidae): pre-aggregation landing and gallery initiation on lodgepole pine. Can Entomol 112:185–191

Jactel H, Van Halder I, Menassieu P, Zhang Q-H, Schlyter F (2001) Non-host volatiles disrupt the response of the stenographer bark beetle, *Ips sexdentatus* (Coleoptera: Scolytidae), to pheromone-baited traps and maritime pine logs. Int Pest Manag Rev 6:197–207

Janz N (2002) Evolutionary ecology of oviposition strategies. In: Hilker M, Meiners T (eds) Chemoecology of insect eggs and egg deposition. Blackwell Publishing Company, Berlin, pp 349–376

Jermy T (1988) Can predation lead to narrow food specialization in phytophagous insects? Ecology 69:902–904

Kohnle U (2004) Host and non-host odour signals governing host selection by the pine shoot beetle, *Tomicus piniperda* and the spruce bark beetle, *Hylurgops palliatus* (Col., Scolytidae). J Appl Entomol 128:588–592

Kuehl RO (2000) Design of experiments: statistical principles of research design and analysis, 2nd edn. Thompson, Pacific Grove
Larsson S, Ekbom B (1995) Oviposition mistakes in herbivorous insects: confusion or a step towards a new host plant? Oikos 72:155–160
Lee JC, Smith SL, Seybold SJ (2005) *Mediterranean pine engraver*, USDA Forest Service Pest Alert R5-PR-016 (pp 4)
Lee JC, Flint ML, Seybold SJ (2008) Suitability of pines and other conifers as hosts for the invasive Mediterranean pine engraver (Coleoptera: Scolytidae) in North America. J Econ Entomol 101:829–837
Macías-Sámano JE, Borden JH, Gries R, Pierce HD Jr, Gries G, King GGS (1998) Primary attraction of the fir engraver, *Scolytus ventralis*. J Chem Ecol 24:1049–1075
Mendel Z (1983) Seasonal history of *Orthotomicus erosus* (Coleoptera: Scolytidae) in Israel. Phytoparasitica 11:13–24
Mendel Z, Halperin J (1982) The biology and behavior of *Orthotomicus erosus* in Israel. Phytoparasitica 10:169–181
Mendel Z, Madar Z, Golan Y (1985) Comparison of the seasonal occurrence and behavior of seven pine bark beetles (Coleoptera: Scolytidae) in Israel. Phytoparasitica 13:21–32
Moeck HA, Wood DL, Lindahl KQ Jr (1981) Host selection behavior of bark beetles (Coleoptera: Scolytidae) attacking *Pinus ponderosa*, with special emphasis on the western pine beetle, *Dendroctonus brevicomis*. J Chem Ecol 7:49–83
Ott RL, Longnecker M (2001) An introduction to statistical methods and data analysis, 5th edn. Duxbury, Pacific Grove
Person HL (1931) Theory in explanation of the selection of certain trees by the western pine beetle. J Forest 29:696–699
Pureswaran DS, Borden JH (2005) Primary attraction and kairomonal host discrimination in three species of *Dendroctonus* (Coleoptera: Scolytidae). Agr Forest Entomol 7:219–230
Riffell J, Abrell L, Hildebrand J (2008) Physical processes and real-time chemical measurement of the insect olfactory environment. J Chem Ecol 34:837–843
SAS Institute Inc (2004) SAS Version 9.1. SAS, Cary
Sauvard D (2004) General biology of bark beetles. In: Lieutier F, Day KR, Battisti A, Gregoire JC, Evans HF (eds) Bark and wood boring insects in living trees in Europe, a synthesis. Kluwer Academic, Dordrecht, pp 63–88
Schaffner U (2001) Host range testing of insects for biological weed control: how can it be better interpreted? Bioscience 51:951–959
Schlyter F, Zhang Q-H, Anderson P, Byers JA, Wadhams LJ, Löfqvist J, Birgersson G (2000) Electrophysiological and behavioural responses of *Tomicus piniperda* and *Tomicus minor* (Coleoptera: Scolytidae) to non-host leaf and bark volatiles. Can Entomol 132:965–981
Schroeder LM (1987) Attraction of the bark beetle *Tomicus piniperda* to Scots pine trees in relation to tree vigor and attack density. Entomol Exp Appl 44:53–58
Schroeder LM (1992) Olfactory recognition of nonhosts aspen and birch by conifer bark beetles *Tomicus piniperda* and *Hylurgops palliatus*. J Chem Ecol 18:1583–1593
Seybold SJ, Downing M (2009) What risk do invasive bark beetles and woodborers pose to forests of the western U.S.?: A case study of the Mediterranean pine engraver, *Orthotomicus erosus*. In Hayes JL, Lundquist J E (eds) The Western Bark Beetle Research Group: A unique collaboration with Forest Health Protection: A workshop at the 2007 Society of American Foresters national convention, USDA Forest Service Gen Tech Rep GTR-PNW-784, Portland, OR, pp 111–134
Seybold SJ, Huber DPW, Lee JC, Graves AD, Bohlmann J (2006) Pine monoterpenes and pine bark beetles: a marriage of convenience for defense and chemical communication. Phytochem Rev 5:143–178
Stastny M, Battisti A, Petrucco-Toffolo E, Schlyter F, Larsson S (2006) Host-plant use in the range expansion of the pine processionary moth, *Thaumetopoea pityocampa*. Ecol Entomol 31:481–490
Tunset K, Nilssen AC, Andersen J (1993) Primary attraction in host recognition of coniferous bark beetles and bark weevils (Col., Scolytidae and Curculionidae). J Appl Entomol 115:155–169
Wallin KF, Raffa KF (2002) Prior encounters modulate subsequent choices in host acceptance behaviour by the bark beetle *Ips pini*. Entomol Exp Appl 103:205–218
Walter AJ, Venette RC, Kells SA (2010) Acceptance and suitability of novel trees for *Orthotomicus erosus*, an exotic bark beetle in North America. Biol Inv 12:1133–1144
Williamson M (1996) Biological invasions. Chapman and Hall, London
Wood DL (1963) Studies on host selection by *Ips confusus* (LeConte) (Coleoptera: Scolytidae) with special reference to Hopkins' host selection principle. Univ Calif Publ Entomol 27:241–282

Wood DL (1970) Pheromones of bark beetles. In: Wood DL, Silverstein RM, Nakajima M (eds) Control of insect behavior by natural products. Academic, New York, pp 301–316

Wood SL (2007) Bark and ambrosia beetles of South America (Coleoptera, Scolytidae). Brigham Young University, M.L. Bean Life Science Museum, Provo

Wood SL, Bright DE (1992) A catalog of Scolytidae and Platypodidae, Part 2, taxonomic index, volumes A and B. Great Basin Nat Mem 13:1–833

Wyckhuys KAG, Heimpel GE (2007) Response of the soybean aphid parasitoid *Binodoxys communis* to olfactory cues from target and non-target host-plant complexes. Entomol Exp Appl 123:149–158

Zhang Q-H, Schlyter F (2003) Redundancy, synergism, and active inhibitory range of non-host volatiles in reducing pheromone attraction in European spruce bark beetle *Ips typographus*. Oikos 101:299–310

Zhang Q-H, Schlyter F (2004) Olfactory recognition and behavioral avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles. Agr Forest Entomol 6:1–19

Zhang Q-H, Schlyter F, Birgersson G (2000) Bark volatiles from nonhost angiosperm trees of spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae): Chemical and electrophysiological analysis. Chemoecology 10:69–80

Zhang L-W, Gillette NE, Sun J-H (2007) Electrophysiological and behavioral responses of *Dendroctonus valens* to non-host volatiles. Ann Forest Sci 64:267–273

Zhang L-W, Clarke SR, Sun J (2009) Electrophysiological and behavioral responses of *Dendroctonus valens* (Coleoptera: Curculionidae: Scolytinae) to four bark beetle pheromones. Environ Entomol 38:472–477

Zwolfer H, Harris P (1971) Host specificity determination of insects for biological control of weeds. Annu Rev Entomol 16:159–178