Boring in Response to Bark and Phloem Extracts From North American Trees Does Not Explain Host Acceptance Behavior of *Orthotomicus erosus* (Coleoptera: Scolytidae)

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Environ. Entomol. 39(2): 661–669 (2010); DOI: 10.1603/EN09320

ABSTRACT When invasive herbivorous insects encounter novel plant species, they must determine whether the novel plants are hosts. The Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston), an exotic bark beetle poised to expand its range in North America, accepts hosts after contacting the bark. To test the hypothesis that *O. erosus* accepts hosts on the basis of gustatory cues, we prepared bark and phloem extracts from logs of four North American tree species that we had used in previous host acceptance experiments. Water, methanol, and hexane extracts of red pine, tamarack, balsam fir, and paper birch were presented alone and in combination on a neutral filter paper substrate in a section of a plastic drinking straw. Boring behavior in response to the three-extract combinations differed from the pattern of acceptance previously observed among species when the beetles were in contact with the bark surface. Only the aqueous extracts of tamarack, *Larix laricina*, increased the initiation and the extent of boring by *O. erosus* on the filter paper substrate. We conclude that the effects of extracted chemicals do not match the behavior of the beetles observed when penetrating excised bark and phloem discs, indicating that host selection by *O. erosus* may not be predictable from bark and phloem chemistry alone. Instead, host acceptance may be determined by nongustatory stimuli or by a combination of stimuli including gustatory and nongustatory cues.

KEY WORDS bark beetle, boring incitant, host-range prediction, invasive species, Pinaceae

When an herbivorous insect invades a new region, it forms host associations with novel plants in the new environment. For an insect to use a novel plant as a host, the plant must support the reproduction of the insect (host suitability) and the insect must undergo several behavioral steps that lead to sustained feeding or oviposition (host acceptance). Many insects rely on stimuli such as secondary plant compounds to make a host acceptance decision (Städler 2002). Some insects, particularly oligophagous and polyphagous species, may accept hosts based on difficult-to-predict information such as the absence of deterrent compounds (Thorsteinson 1960, Jermy 1966) or the presence of phylogenetically disjunct suites of traits (Becerra 1997, Agrawal and Fishbein 2006). In either case, in-depth understanding of the plant traits important for host acceptance and the distribution of those traits among plants that the insect will encounter in the new environment may increase the accuracy of host-range prediction in the new ecosystem (Payne et al. 2004). Predicting new insect–plant associations and their consequences is an important component of understanding the potential of an exotic species to cause environmental or economic damage (National Research Council 2002).

The Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston) (Coleoptera: Scolytidae, *sensu* Wood 2007), is a bark beetle species for which host-range prediction is important. The beetle invaded North America and presently exists as a small established population in California (Lee et al. 2005). *O. erosus* has been reported in association with a wide range of conifers from the Pinaceae and Cupressaceae in its native and adventive geographic ranges outside of North America (Mendel and Halperin 1982, Wood and Bright 1992, Bright and Skidmore 1997). The putatively broad host range contributes to the high risk *O. erosus* poses of occupying a large area in North America and causing damage to conifer species (Eglitis 2000). The beetle is capable of reproducing on logs cut from North American trees, including all pines tested; white and black spruce, *Picea glauca* (Moench) Voss and *P. mariana* (Mill.) Britton, Sterns, and Poggenb.; and Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco; a minor amount of reproduction can also take place on tamarack, *Larix laricina* (Du Roi) Koch (Lee et al. 2008, Walter et al. 2010a). Adult beetles will also accept bark and phloem samples of eastern hemlock, *Tsuga canadensis* L. Carrière, and

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balsam fir, *Abies balsamea* L. Mill., as hosts, but neither of these species support reproduction (Walter et al. 2010a). If the beetle accepts North American tree species that do not support its reproduction, it may be less likely to establish populations where those tree species are present. Therefore, prediction of which tree species will be accepted by *O. erosus* is critical to understanding the risk posed by this beetle.

Orthotomicus erosus bores into the outer bark of logs or bark and phloem discs from several North American tree species, although at different rates. Other aspects of host selection behavior such as olfactory attraction and gallery abandonment are similar among tree species (Walter et al. 2010a, b). Thus, it seems likely that the host acceptance decision by *O. erosus* takes place at the time of boring (i.e., during sustained bark penetration) into the outer and inner bark of a tree. A number of tree-derived compounds that promote (Doskotch et al. 1970, Levy et al. 1974, Meyer and Norris 1974, Raffa and Berryman 1982) or discourage (Gilbert et al. 1967; Norris and Baker 1967; Gilbert and Norris 1968; Klepzig et al. 1996; Wallin and Raffa 2000, 2002; Faccoli et al. 2005) boring are known from other bark beetles. When considering all phytophagous insects, Beck (1965) defined several terms to describe the behavioral impact of semiochemicals: incitants or suppressants are semiochemicals that increase or reduce feeding initiation, respectively, and stimulants or deterrents refer to compounds that affect the extent of feeding. We apply these same definitions to describe the boring behavior of bark beetles.

We hypothesized that *O. erosus* would accept trees on the basis of chemical compounds in the bark and phloem. Under this hypothesis, we expected that boring behavior on a neutral substrate treated with chemicals extracted from potential host species should be similar to the behavior observed when beetles were placed on the bark. Furthermore, we expected that extracts from the tree species that were accepted by *O. erosus* at the highest rates would contain incitants and/or stimulants, or extracts from the tree species that were the least accepted by *O. erosus* would contain suppressants and/or deterrents.

Materials and Methods

Beetles for Behavioral Assay. Work with live insects took place in the Minnesota Agricultural Experiment Station/Minnesota Department of Agriculture Biological Level 2 Containment Facility in St. Paul, MN. All handling procedures were approved by the USDA APHIS Plant Protection and Quarantine Division (Permit 74447).

Beetles used in the behavioral assay were from the field population in California or a laboratory colony. Cut logs of Italian stone pine, *Pinus pinea* L., and Aleppo pine, *Pinus halepensis* Mill., infested with *O. erosus* were collected from the established population in Fresno County, CA, in summer 2008 and held in emergence boxes (Browne 1972) at the Chemical Ecology of Forest Insects Laboratory, Davis, CA. Adults were allowed to emerge naturally and crawl or fly to a lighted exit, indicating they were ready to begin searching for a new host. Once emerged, they were kept in refrigerators (≈10°C) in jars with moist paper toweling until shipment. On 13 August and 7 October 2008, emerged beetles were shipped to St. Paul, MN, in insulated styrofoam boxes with ice packs. All beetles were held in a commercial refrigerator for 4–15 d at ≈10°C and deprived of food until used in the experiment.

A laboratory colony of *O. erosus* was maintained on cut logs of red pine, *Pinus resinosa* Aiton (≈50 cm long by 15–40 cm diameter), in a growth chamber at 25°C, 16:8 L:D as described previously (Walter et al. 2010a). Because beetles in the colony tended to continuously reinfest the same brood log (Walter et al. 2010a), beetles were extracted from the colony by peeling the bark and phloem of the red pine logs with a draw knife and manually removing the beetles. Beetles from the colony were used the day after they were extracted.

Several precautions were taken to ensure that healthy beetles were used in the assay. Beetles were sorted by sex according to the morphology of their elytra (Reitter 1913) on the day that the shipment was received or they were extracted from the colony. This helped to ensure that the beetles used in the experiments were intact and healthy because we observed that the beetles would bite and damage the tarsi and antennae of other individuals when males and females were stored together. We did not observe this behavior when male and female beetles were stored in different containers. In addition, beetles were held at ≈10°C in the dark from the time they were received by courier or extracted from the colony until they were used in the assay. This helped to ensure that the beetles did not deplete their energy stores. Only actively moving beetles were used in the assay. The mating status of the beetles was unknown. However, up to 97% of females of a population in Israel were already mated on emerging from the brood log (Mendel 1983).

Preparation of Bark and Phloem Extracts. Tree species used in this study were as follows: red pine, *P. resinosa;* tamarack, *L. laricina;* balsam fir, *A. balsamea;* and paper birch, *Betula papyrifera* Marsh. Two trees of each species were felled at the University of Minnesota North Central Research and Outreach Center (Grand Rapids, MN) between 16 and 19 June 2008 and cut immediately to ≈50-cm lengths. The diameter of the logs used in the experiment was 28–53 cm. Cut surfaces were sealed with paraffin wax (Candle Crafting Products Premium Candle Wax; Yaley Enterprises, Redding, CA) and stored in a greenhouse with a minimum temperature of 7–13°C during the day and 4–10°C at night.

Potential semiochemicals were extracted from pieces of the cut logs on 4 August (two red pine, one balsam fir, one paper birch), 20 August (two tamarack, one balsam fir, one paper birch), and 23 September (one red pine, one tamarack, one balsam fir, one paper birch) 2008. The time between tree harvest and extraction corresponds to time since cutting for trap logs that have come under attack by *O. erosus* in Israel and

Spain (Amezaga and Rodriguez 1998). Red pine logs of this age were also readily accepted by beetles in our laboratory colony. In all, two sets of extracts were made from the first tree of each species, and one set of extracts was made from the second tree. For each extraction, three sets of two 5-cm-diameter discs were cut through the outer bark and phloem to the xylem surface of the cut logs with a hole saw attached to a power drill; the discs were removed with a draw knife. The discs were cut into strips a maximum of 1 cm wide and placed into 250-ml Erlenmeyer solvent flasks. We added 200 ml of solvent to each flask. The solvents were water (HPLC Grade; Fisher, Fair Lawn, NJ), methanol (HPLC Grade; Fisher), and *n*-hexane (Environmental Grade, 95%; Alfa Aesar, Ward Hill, MA). The flasks were shaken at 100 rpm at room temperature for 48 h, and the solvent and extracted chemicals were decanted into clean 500-ml amber glass jars. Between extraction and use in the experiment (36–132 d), the hexane and methanol extracts were stored in the dark in a flame-proof cabinet at room temperature, and the water extracts were stored in a dark growth chamber at 4°C to minimize decomposition by microbes.

Assay of Boring Behavior. The experiment followed a factorial design for each tree species with four factors and two levels per factor: (1) beetle sex (male or female); (2) water extract (present or absent); (3) methanol extract (present or absent); and (4) hexane extract (present or absent). In addition, four control treatments were presented to the beetles. These included a blank (nothing applied to the substrate) and pure water, pure methanol, and pure hexane. The "pure" solvents used for the controls were taken from the same lot as the solvents used for extraction.

Male and female beetles were presented with one of the four controls or one of the following extract combinations: water extract, methanol extract, hexane extract, water + methanol extracts, water + hexane extracts, methanol + hexane extracts, or water + methanol + hexane extracts for each of the four tree species (32 substrate treatments × 2 sexes = 64 treatments total, with controls shared among all four species). Treatments were presented on a neutral substrate (2.5 by 15 cm-strip of Fisherbrand P5 qualitative filter paper; Fisher, Pittsburgh, PA). Each combination was replicated three times in each of seven blocks. Four blocks of the experiment were conducted by using beetles from the laboratory colony, and three blocks were conducted using field-collected beetles. The source of beetles for each block was determined according to their availability. A total of 1,344 individuals were used in the experiment.

Bark and phloem extracts and pure solvents were applied to the substrate in 3-ml aliquots, so that the amount of extracted chemicals per unit mass in the experiment would be approximately equivalent to what the beetles would encounter from the bark and phloem of cut logs. (The filter paper strips used in this study weighed 0.28 g on average, which was ≈1.5% of the mass of the bark and phloem samples [6.5–24 g; mean, 14.7 g] used in the 200-ml extraction.) After an extract or pure solvent was applied to the paper, the paper was placed on clean aluminum foil in a fume hood while the solvent was evaporated. If more than one solvent was used in a treatment combination, one extract was placed on the paper, and the solvent was allowed to completely evaporate before the next extract was added. After all the solvents had evaporated, the paper strips were rolled into 2.5-cm-long cylinder by a technician wearing nitrile gloves, and each cylinder was inserted into 0.60-cm ID plastic drinking straws (Diamond flexible straws; Jarden Home Brands, Muncie, IN) cut to ≈8-cm lengths to create arenas that simulated bark beetle galleries. Some bark beetles, such as *Ips typographus* L., will not bore unless the substrate is presented in an artificial gallery (Schlyter et al. 2004). Our preliminary experiments showed this was also true for *O. erosus.* Inside the quarantine facility, one end of each arena was sealed with white laboratory tape (Timemed labeling tape; Fisher). The filter paper substrates in the plastic straws were moistened with 0.7 ml of distilled deionized water, so that the moisture content would be approximately equal to conifer phloem to encourage boring behavior by *O. erosus.* Approximations were based on the reported moisture content of the phloem of Norway spruce, *Picea abies* L. Karsten (1.4–2.6 mg/mg dry weight; Gall et al. 2002) because data for that species were readily available. The aliquot of water was placed in the open end of the arena, and the water was allowed to soak into the paper for at least 2 h. A small amount of water remained on top of the paper in some of the arenas, so excess water was shaken out of the straws before beetles were added. Magnitude of SEs from water extract treatments appeared similar to the SEs from treatments with other extracts, indicating that results were not affected by removal of water-soluble compounds with the excess water. A beetle was placed in the straw, and the open end was sealed with white laboratory tape. The straws were placed vertically in plastic cups in a growth chamber (25°C, 16:8 L:D, 70% RH) for ≈72 h.

At the end of the experiment, all beetles were removed from the straws, and straws were frozen for at least 24 h at −80°C before removal from the quarantine laboratory. The paper strips were removed from the straws and examined for boring activity. Initiation of boring was determined visually by examining the unrolled filter papers and establishing if any paper had been removed. For those straws where boring was initiated, the extent of boring was calculated by the area of paper removed by the beetle using a method analogous to O'Neal et al. (2002). Papers with a detectable area removed were scanned on a flatbed scanner (HP Scanjet 4890; Hewlett-Packard, Palo Alto, CA). A rectangle of the scanned black and white image was highlighted with the polygonal lasso in Adobe Photoshop 7.0 (Adobe, San Jose, CA); the percentage of black in the area was determined with the histogram tool, and the dimensions of the rectangle were determined with the cut-paste tool. From this information, the area of the filter paper that was consumed by the beetle was determined.

Various terms have been introduced to describe the proximal behavior of bark beetles as they pass through the outer bark and initiate their galleries in the phloem of a new host (e.g., penetration behavior, boring behavior, tunneling behavior, gallery initiation behavior, feeding behavior, or host acceptance behavior), and these terms have been applied based on the choice of assay by the investigators (Wood 1963, Gilbert et al. 1967, Doskotch et al. 1970, Elkinton and Wood 1980, Elkinton et al. 1981, Raffa and Berryman 1982, Wood et al. 1986, Klepzig et al. 1996, Wallin and Raffa 2000, Wallin et al. 2002, McNee et al. 2003, Faccoli et al. 2005). In our assay, because the paper substrate was placed in a simulated gallery (plastic straw) and because insects immerse themselves in the substrate as they construct the entrance to the gallery, we elected to use the term boring (=tunneling) behavior to describe the proximal response of *O. erosus.* We recognize that the removal of the paper from the substrate in the simulated gallery likely reflected biting, chewing, or feeding activity by the test beetles, but these behaviors were not verified directly. The term boring behavior accommodates both feeding and other types of physical damage to the substrate when a beetle attempts to create a gallery. In this simulated gallery (arena), thigmotaxis likely also contributed to the behavioral response of the beetles (Elkinton and Wood 1980, Wood et al. 1986).

Data Analyses

Among-Species Comparisons. We hypothesized that boring by *O. erosus* in response to the bark and phloem extracts would have the same qualitative patterns observed in our previous acceptance assay (red pine > tamarack, and all conifers > paper birch) (Walter et al. 2010a). To test our hypothesis, we compared boring on the methanol-hexane-water extract combination treatments among tree species. Boring initiation and extent of boring were analyzed separately because the presence of the bark and phloem extracts could affect both whether a beetle bored in the substrate and the extent of boring that took place.

The probability of boring initiation was analyzed with logistic regression (PROC LOGISTIC; SAS Institute 2008). The analysis began with a full model containing terms for extraction date, block, beetle sex, tree species, and all two-, three-, and four-way interactions of these variables. The model was reduced by stepwise selection with $\alpha = 0.05$. Contrasts between species were evaluated using a Bonferroni-adjusted P value of 0.0083 so that the experimentwise error was controlled at 0.05 (Kuehl 2000).

Differences in the extent of boring among species in those cases where boring was initiated were analyzed with a general linear model ANOVA. Because the data for this analysis did not meet assumptions of normality or homoscedasticity, the area removed by the beetles was taken to the power of 0.15 as suggested by a Box-Cox transformation (Arc version 1.06 software package, www.stat.umn.edu/arc/). After the transformation, which corrects for heteroscedasticity, data were retested for normality (PROC UNIVARIATE; SAS Institute 2008). The transformed data met the assumption of normality according to the Shapiro-Wilk statistic. The analysis of the extent of boring was performed on transformed data. However, data were back-transformed for the purposes of presentation in this article. The analysis began with a full model containing terms for extraction date, block, beetle sex, tree species, and all two-, three, and four-way interactions of these variables. The model was reduced with stepwise selection (PROC GLMSELECT with HIERARCHY = SINGLE option; SAS Institute 2008).

Within-Species Comparisons. To test for behaviorally active compounds in the bark extracts (incitants, suppressants, stimulants, and deterrents), we performed factorial analysis on the probability of boring initiation and the extent of boring on treatments from each tree species. We reported the number of individuals tested for the analyses of the extent of boring because only beetles that initiated boring were included in the analyses. For a given tree species, we were interested in the activity of each extract and synergistic effects that might occur as a result of presenting two or more extracts together. Each analysis included the boring response of male and female beetles to: the blank control; the three pure solvents; the three extracts; the three two-way combinations of extracts; and the three-way combination of extracts (11 substrate treatments × 2 sexes = 22 treatments total).

The data on boring initiation met all assumptions for logistic regression. The data on the extent of boring violated the assumptions of normality and homoscedasticity required for analysis of variance (ANOVA; PROC UNIVARIATE; SAS Institute 2008). We performed a Box-Cox transformation on each the dataset for each species (Arc 1.06). A transformation to the power of 0.15 was suggested for each dataset. The transformed data were normally distributed and met the assumption of homoscedasticity. The analysis of extent of boring was performed on transformed data; data were back-transformed for the purposes of presentation.

The probability of boring initiation was analyzed with logistic regression and the extent of boring was analyzed with a general linear model ANOVA. Each analysis began with a full model including the effects of block (1 term); beetle sex (1 term); the three pure solvents (3 terms); the extracts nested in the pure solvents (3 terms); two-way interactions among the nested extracts (3 terms); the three-way interaction among the nested extracts (1 term); and interactions between beetle sex and the pure solvents, nested extracts, and interactions among nested extracts (10 terms). The models were reduced with stepwise selection. Model reduction was carried out with PROC LOGISTIC for the probability of boring and PROC GLMSELECT with the HIERARCHY = SINGLE model option for the extent of boring (SAS Institute 2008). After the models of boring extent were selected, the F values of the model variables and their

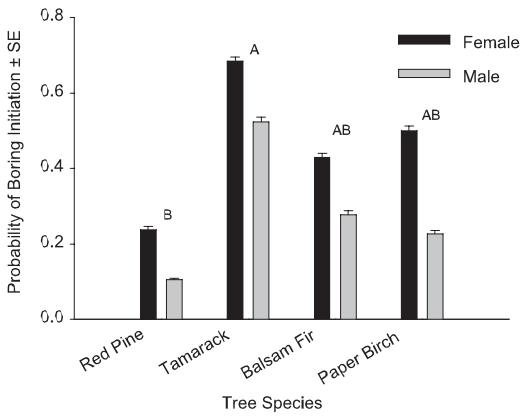

Fig. 1. Probability of boring initiation by the Mediterranean pine engraver, *O. erosus*, in response to the methanol-hexane-water extract combinations for each tree species ± binomial SE. Histogram bars for the tree species (pooled across both sexes) labeled with the same letter indicate probabilities that are not significantly different in contrasts performed with a logistic regression model. Beetle sex did not interact with the effect of tree species. Contrasts were evaluated with a Bonferroni-adjusted P value of 0.0083 (experiment-wise $\alpha = 0.05$).

associated probabilities were generated with PROC GLM (SAS Institute 2008).

The factorial analysis allowed us to separate the nested effects of the extracted chemicals presented in a solvent from the effects of the solvents alone. However, we were unable to evaluate the effects of interactions among the pure solvents because the pure solvents were not presented in combinations. If a significant interaction term had been discovered among the extracts, we could not determine whether it was caused by the combination of the pure solvents, the combination of the extracted chemicals, or a combination of extracted chemical(s) and the solvent from the other extract. Significant interactions did not occur in any of the eight selected models, so this shortcoming did not affect our conclusions.

Results

We present the results of our factorial analyses following the recommendations of Gómez and Gómez (1984). Simple summaries of the data are provided as supplementary material.

Among-Species Comparisons. Tree species (df = 3, Wald $\chi^2 = 15.59$, $P = 0.0014$) and block (df = 6, Wald $\chi^2 = 17.05$, $P = 0.0091$) affected the probability that a beetle would initiate boring on the three-extract combination treatments. Among the tree species, *O. erosus* had a higher probability of boring in filter paper treated with the extracts from tamarack than with extracts from red pine (Fig. 1).

None of the factors affected the extent of boring among the four tree species; stepwise selection returned a model that included only the intercept (df = 1,53; $F = 2909.82$; $P < 0.001$).

Table 1. Probability and binomial SE of boring initiation by the Mediterranean pine engraver, *O. erosus*, in response to treatments from tamarack with and without pure water and the water extract and differences between pure water (column) and water extract (row) treatments

	Without water extract (SE)	With water extract (SE)	Difference
Without pure water (SE)	0.29 (0.03)	—	—
With pure water (SE)	0.26 (0.07)	0.52 (0.04)	0.26[a]
Difference	0.03	—	

[a] Cases where the row or column variable significantly affected the probability of boring initiation ($P < 0.05$).

Within-Species Comparisons

Probability of Boring Initiation (Incitants and Suppressants). Only one extract treatment (tamarack extracted with water) affected the probability that beetles would begin boring in a substrate. For treatments with red pine extracts, only the effect of block (df = 6, Wald $\chi^2 = 53.93$, $P < 0.001$) influenced the probability of boring initiation. For treatments with tamarack extracts, the probability of boring initiation was described by a model including the effects of block (df = 6, Wald $\chi^2 = 45.49$, $P < 0.001$), pure water (df = 1, Wald $\chi^2 = 3.31$, $P = 0.0690$), and the water extract nested in the effect of pure water (df = 1, Wald $\chi^2 = 9.24$, $P = 0.0024$). The water extract acted as a boring incitant, increasing the probability of boring initiation (Table 1). For treatments with balsam fir extracts, the effect of block influenced the probability of boring initiation (df = 6, Wald $\chi^2 = 51.56$, $P < 0.001$), but no other effects were significant. For treatments with paper birch extracts, block (df = 6, Wald $\chi^2 = 33.22$, $P < 0.001$) and the effect of pure methanol (df = 1, Wald $\chi^2 = 4.29$, $P = 0.0383$) affected the probability of boring initiation by *O. erosus.* Pure methanol acted as a boring suppressant, decreasing the probability that boring was initiated (Table 2).

Extent of Boring (Stimulants and Deterrents). There was one extract (tamarack extracted with water) that affected the extent of boring that took place by beetles that started boring. For treatments with red pine extracts, only block (df = 6, 125; $F = 9.17$, $P < 0.001$) affected the extent of boring. For treatments with tamarack extracts, block (df = 6, 155; $F = 7.88$; $P < 0.001$), pure water (df = 1, 155; $F = 0.92$; $P = 0.3397$), and the water extract nested in the effect of

Table 2. Probability and binomial SE of boring initiation by the Mediterranean pine engraver, *O. erosus*, in response to treatments from paper birch with and without pure methanol and differences between pure methanol treatments

	All treatments
Without pure methanol (SE)	0.29 (0.03)
With pure methanol (SE)	0.21 (0.03)
Difference	0.08[a]

[a] Cases where the variable significantly affected the probability of boring initiation ($P < 0.05$).

Table 3. Extent of boring (mm^2) and SE by the Mediterranean pine engraver, *O. erosus*, in response to treatments from tamarack where boring was initiated and differences between treatments with and without pure water (column) or treatments with pure water with and without water extract (row)

	Without water extract (SE)	With water extract (SE)	Difference
Without pure water (SE)	5.98 (5.12)	—	—
With pure water (SE)	3.87 (2.77)	10.07 (7.75)	6.20[a]
Difference	2.11	—	

Untransformed means and SEs are presented, but analysis took place on data transformed to correct for non-normality and heteroscedasticity.

[a] Cases where beetle sex significantly affected the probability of boring initiation ($P < 0.05$).

pure water (df = 1,155; $F = 8.65$; $P = 0.0038$) affected the extent of boring (Table 3). The water extract acted as a boring stimulant, increasing the extent of boring. For treatment with balsam fir extracts, none of the variables affected the extent of boring. For treatments with paper birch extracts, the only variable that affected the extent of boring was block (df = 6,107; $F = 5.41$; $P < 0.001$).

Discussion

The goal of this experiment was to determine whether gustatory stimuli from the bark and phloem of logs from potential host trees dictate host acceptance by *O. erosus.* In previous no-choice experiments with bark and phloem discs, *O. erosus* bored into the bark of red pine more often than tamarack, and beetles bored into all three conifer species (red pine, balsam fir, and tamarack) more often than they bored into paper birch (Walter et al. 2010a). We focused on the response of *O. erosus* to semiochemical stimuli because the effect of chemical compounds can be separated from other host traits, such as bark texture or visual profile. Although nonchemical stimuli may be important to bark beetle host acceptance, they are more difficult to characterize.

If gustatory chemicals alone were the main determinants of boring behavior, a pattern similar to our previous host acceptance assay should have occurred when the three extract combination treatments from each species were compared. In this experiment, beetles had a higher probability of boring in substrates with the combined extracts of tamarack than red pine. The probabilities of boring initiation on balsam fir and paper birch were equivalent to both tamarack and red pine. There were no differences among the four tree species in the extent of boring. Therefore, extractable chemicals from the outer bark and phloem alone do not seem to be the main determinants of boring behavior for *O. erosus.* Another possibility is that behaviorally important chemicals may have degraded during extraction or storage. Because the boring response to the extracts was different from the pattern of acceptance that we had observed previously, we chose not to proceed with further fractionation or identification of the chemicals in our extracts. When the behavioral activity of the extracts was examined, only the water extract of tamarack incited and stimulated boring by *O. erosus.* The suppressant activity of the methanol solvent control in the assays with paper birch extracts was likely a spurious result because of type I error given the large number of effects that were all analyzed with an α of 0.05. Pure methanol did not have a statistically significant effect when tested in the context of any of the other three tree species. This methanol effect might also be caused by impurities in the solvent or an interaction between methanol and the filter paper, although it would be surprising because these results were not observed in all tree species.

Nonchemical stimuli are important for host acceptance in some bark beetles. For example, the texture of bark or artificial medium influences the probability that *Dendroctonus ponderosae* Hopkins, *Ips paraconfusus* Lanier, and *Scolytus multistriatus* (Marsham) will bore into the substrate (Shepherd 1965, Elkinton et al. 1981, Švihra and Volney 1983). In previous assays of boring behavior, tactile stimulation has been provided by presenting candidate compounds on elderberry pith disks with an insect pin (Norris and Baker 1967), a thumbtack (Doskotch et al. 1970), or notches cut in the disc perimeter (Thomas et al. 1981) to provide the thigmotactic stimulus; ground bark or phloem media packed into plastic dishes (Elkinton and Wood 1980, Wallin and Raffa 2000), or gelatin capsules (Elkinton et al. 1981); laboratory wipes packed into vials (Raffa and Berryman 1982); or agar-based media packed into glass tubes (Faccoli et al. 2005). *Dendroctonus pseudotsugae* Hopkins and *Trypodendron lineatum* (Olivier) are both attracted to blue and green light more than to other wavelengths in the absence of other stimuli (Groberman and Borden 1981), and silhouette color affects the catch of many species of bark beetles in baited (Dubbel et al. 1985; Fatzinger 1985; Strom et al. 1999, 2001; Strom and Goyer 2001; Campbell and Borden 2006) and unbaited (Niemeyer 1985; Campbell and Borden 2006) traps. Similar visual or tactile cues may be important in the host acceptance decision of *O. erosus* alone or in combination with other stimuli.

The length of our assay made it less likely that we would detect the presence of chemicals that reduced boring activity than those that increased it. Insects confined to a testing arena may become habituated to negative stimuli or more likely to feed on a substrate with a low level of stimulants because of hunger or thirst. The long assay time used in this experiment (72 h) increases the probability that increased boring will occur in response to even a slight positive (incitant or stimulant) stimulus but decreases the possibility that negative stimuli (suppressants or deterrents) will be detected. In a similar experiment with pales weevil, *Hylobius pales* (Herbst.), some compounds with statistically significant antiboring activity after the beetles had been exposed for 24 h were not active when the beetles had been exposed for 48 h (Salom et al. 1994). Long-duration no-choice tests are considered appropriate in host-range testing for biological control

agents, and by extension, for other insects entering new geographic areas. In these situations, the most preferred host of an insect might not be available in the field, but insects would encounter novel plants until they either accepted a novel plant or died (Tallamy 2000, Barton Browne and Withers 2002).

Polar and nonpolar extracts of bark from normal host plants affect the boring behavior of a number of conifer- and angiosperm-feeding bark beetles. Methanol extracts from the host plants of *D. ponderosae*, *I. paraconfusus*, and *Coccotrypes dactyliperda* (Fabricius) incite boring (Elkinton et al. 1981, Raffa and Berryman 1982, Meisner et al. 1985, Wood et al. 1986, McNee et al. 2003). Boring behavior by *D. ponderosae*, *I. paraconfusus*, *Scolytus mediterraneus* (Eggers), and *S. multistriatus* is also incited by polar ether or nonpolar benzene extracts of their hosts (Norris and Baker 1967, Levy et al. 1974, Elkinton et al. 1981, Raffa and Berryman 1982, McNee et al. 2003). In addition to the effect of methanol and ether extracts, boring by *I. paraconfusus* is stimulated by water and the combined water-and-ether extracts of ponderosa pine (Elkinton et al. 1981, McNee et al. 2003). Negative effects on boring activity from host plant extracts were not reported in these studies, although extracts of diseased or damaged host trees have been reported to have antiboring activity compared with healthy host trees for *I. paraconfusus*, *I. pini* (Say), and *Hylastes porculus* Erichson (Klepzig et al. 1996, McNee et al. 2003).

A number of compounds known to be present in host plants, especially monoterpenes and phenolics, discourage boring when tested individually at high concentrations typical of induced plant defense (Klepzig et al. 1996; Wallin and Raffa 2000, 2002; Faccoli and Schlyter 2007). Our experiment was designed to assay compounds in uninduced, recently killed trees because we consider these trees to be the most likely hosts for a small population of invading bark beetles. This study would not show the effects of induced defenses in the trees because the trees used in the study did not seem to be under herbivore or pathogen attack when they were cut. For example, the tamarack trees used in this study had far less resin than similarly sized trees under attack by the eastern larch beetle, *Dendroctonus simplex* LeConte, when they were felled (A.J.W., unpublished data).

The extracts that promoted boring in this experiment were from logs of tamarack, a tree species that does not support reproduction by *O. erosus* (Lee et al. 2008, Walter et al. 2010a). Although various nonhost chemicals have been tested for gustatory activity individually for a variety of subcortical insect species (Gilbert and Norris 1968, Salom et al. 1994, Faccoli et al. 2005), only a few studies have examined bark extracts from nonhost trees. Four of 10 nonhosts of a weevil, the large brown trunk beetle, *Hylobius abietis* L., and four nonhosts of the smaller European elm bark beetle, *S. multistriatus*, had boring stimulants in nonpolar extracts (Gilbert et al. 1967, Eriksson et al. 2008). Polar extracts of 4 of the same 10 nonhosts of *H. abietis* and 1 nonhost of *S. multistriatus* had boring deterrent activity (Gilbert and Norris 1968, Eriksson et al. 2008).

When an insect encounters a plant, it decides whether to attempt to use the plant as a host based on a number of chemical and nonchemical stimuli. The boring behavior of *O. erosus* exposed to bark and phloem extracts in this study did not match the behavior of the beetle when exposed to the bark and phloem of the same tree species. This indicates that bark and phloem chemistry alone will not be a useful tool in making host range predictions for *O. erosus*. Other cues from the trees, alone or in combination with gustatory information, may be important in the host-use decision of this beetle.

Acknowledgments

We thank T. O'Brien (University of Minnesota North Central Research and Outreach Center, Grand Rapids, MN), B. Palik (USDA Forest Service Northern Research Station, Grand Rapids, MN), and B. Jones (Itasca Community College, Grand Rapids, MN) for assistance in felling trees; the MAES/MDA Containment Facility for laboratory space in their facility; B. Therens for assistance with the laboratory assays; J. C. Lee, D.-G. Liu, and S. M. Hamud (University of California, Davis and USDA Forest Service Pacific Southwest Research Station) for assistance with collecting, sorting, and shipping *O. erosus*; and R. A. Haack, K. F. Wallin, and two anonymous reviewers for reviewing the manuscript. 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 A.J.W., the USDA Forest Service Northern and Pacific Southwest Research Stations, and the Minnesota Agricultural Experiment Station.

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Received 23 October 2009; accepted 3 February 2010.