

# Influences of Host Chemicals and Internal Physiology on the Multiple Steps of Postlanding Host Acceptance Behavior of *Ips pini* (Coleoptera: Scolytidae)

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**ABSTRACT** A series of bioassays were performed to evaluate the effects of host tree monoterpenes on initial entry, within-tissue orientation, and gallery construction by the bark beetle *Ips pini* (Say). Monoterpenes at concentrations present in host trees affected all stages of postlanding host acceptance behavior. However, the effects of each monoterpene varied with the particular stage of the orientation sequence. The number of beetles entering a phloem-based medium decreased with increased concentrations of most monoterpenes. An exception occurred with  $\beta$ -pinene, which elicited increased beetle entry at moderate concentrations. Once beetles began to form galleries within phloem-based media, higher monoterpene concentrations increased the likelihood that they would move from amended to nonamended sections of the substrate. However, low to moderate concentrations of  $\beta$ -pinene arrested beetles within treated regions of the arena. The total gallery length generally decreased with increasing monoterpene concentrations. However, gallery lengths were consistently higher at intermediate concentrations of  $\alpha$ -pinene. The enantiomeric composition of  $\delta$ -pinene did not influence entrance or gallery formation by *I. pini*. Moreover, low to moderate concentrations of  $\beta$ -pinene arrested beetles within treated regions of the arena. The total gallery length generally decreased with increasing monoterpene concentrations. However, gallery lengths were consistently higher at intermediate concentrations of  $\alpha$ -pinene. The enantiomeric composition of monoterpenes appears to be more important than the type of monoterpene in affecting postlanding host selection behavior. There was a positive correlation between host entry and total percentage of lipids within beetles. We describe a new bioassay that evaluates initial host entry by bark beetles, a critical qualitative decision in their life history, and relates this decision to quantitative aspects of host chemistry. Our results indicate that postlanding host acceptance is a multistep process shaped by environmental conditions and internal physiological state of the insect.

**KEY WORDS** *Ips pini*, bark beetle, host selection behavior, lipids, monoterpene, postlanding behavior

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HERBIVOROUS INSECTS OFTEN exploit multiple plant cues to detect and orient toward suitable hosts. Their responses to these cues are often both discrete and continuous, and occur in sequences that denote plant habitat, plant species, and specific attributes of plant architecture and quality. After locating a possible host, herbivores must assess host quality, which can strongly affect reproductive success, particularity among species that use only one host for their entire larval development, or that deposit their entire egg complement within one host. Bark beetles (Coleoptera: Scolytidae) generally exhibit both of these life history traits. They use single host trees for mate attraction, oviposition, larval nutrition, and pupation, and spend all but a brief portion of their lives as endophytic parasites. Bark beetles usually deposit their entire egg clutch within single trees, although in some species a few individuals may reemerge to oviposit in additional trees (Coulson 1979).

Adult bark beetles emerge from brood trees, fly variable distances, and arrest on new hosts in response to visual (Shepherd 1966, Groberman and Borden 1982) and chemical (Mustaparta et al. 1979, Geiszler et al. 1984) cues. They either resume flight or enter the tree based on subsequent chemical and tactile stimuli

(Wood 1972, Elkinton and Wood 1981, Wood 1982, Raffa and Berryman 1982). Beetles mate beneath the bark and construct ovipositional galleries. The emerging larvae radiate from the gallery as they feed, develop, and pupate. The brood completes development within the phloem and bark, and exits the host as adults.

Bark beetles require dead tissue in which to reproduce, so they must either locate dead or dying trees, as do most scolytid species, or kill entire or large parts of living trees, as do the so-called "aggressive" species that colonize the main stems of living conifers. The gradient of host physiological conditions ranging from dead to vigorous trees presents colonizing beetles with ecological trade-offs between inter-specific competition and host resistance, respectively (Raffa and Berryman 1983a). The tree-killing species of bark beetles periodically undergo extensive population eruptions, during which they cause severe losses to North American forests (Rudinsky 1962, Wood 1972, Berryman 1973, Safranyik et al. 1975).

Conifers have evolved complex constitutive and inducible defenses in response to subcortical insects (Hain et al. 1983, Raffa and Berryman 1983b, Christiansen 1991, Werner and Illman 1994, Klepzig et al.

1996). Tree responses to invasion by bark beetles include rapid exudation of resins containing monoterpenes and phenolics at the entry site, accumulation of higher concentrations and altered ratios of these compounds during induced localized reactions, and necrotic lesion formation in advance of beetle galleries (Cates and Alexander 1982, Stephen et al. 1983, Paine et al. 1987, Nebeker et al. 1994, Werner and Illman 1994, Wallin and Raffa 1999). These defenses can preclude entry into, or kill beetles that enter resistant trees. Resistance varies greatly among trees within a host species population because of a variety of environmental and genetic factors (Cates and Alexander 1982, Paine et al. 1987, Nebeker et al. 1994, Wallin and Raffa 1999). Confronted with these defenses, bark beetle adults must select both the appropriate host species and a suitable individual within the host species population. Once inside host trees, beetles must also avoid tissue in which allelochemicals are concentrated, such as resin blisters or resin canals (Ferrell 1983).

Patterns of tree mortality have sometimes been correlated with specific proportions or lowered amounts of monoterpenes (Cates and Alexander 1982, Raffa and Berryman 1983a, 1983b, Dunn and Lorio 1993, Sturgeon and Mitton 1986, Nebeker et al. 1994, Wallin and Raffa 1999). However, these patterns are highly variable. Outcomes of conifer-bark beetle interactions are influenced by a wide array of factors, including multifaceted constitutive and inducible defenses, complex beetle behaviors, beetle population density, forest community structure, and weather (Birgersson and Bergstrom 1989, Bentz et al. 1993, 1996, Preisler and Mitchell 1993, Logan and Bentz 1999).

Quantifying relationships between concentrations of host compounds and specific phases of the orientation sequence is an important step toward understanding overall host selection by bark beetles. This understanding is needed to improve indices of tree resistance, and to relate specific host properties affecting beetle orientation to predisposing agents such as defoliation, root pathogens, and drought (Paine and Baker 1993, Nebeker et al. 1994). In addition, increased understanding of mechanisms involved in postlanding host acceptance behavior may help explain behavioral differences between endemic versus epidemic bark beetle populations.

The complexity and the lack of consistent correlations suggest that a single host parameter is unlikely to predict tree mortality. Thus, visual profiles and volatile phytochemicals mediate host species recognition and landing by bark beetles. For example, baiting traps with host material, monoterpenes, or ethanol can elicit beetle movement and landing (Angst and Lanier 1979, Volz 1988, Byers 1992). However, postlanding acceptance behavior is a critical but poorly understood phase of the overall host selection process (Schlyter and Lofqvist 1986). The importance of postlanding behavior is illustrated by Hynum and Berryman (1980), who demonstrated that the proportion of mountain pine beetles, *Dendroctonus ponderosae* Hopkins, that land on but subsequently leave trees, does

not vary from the preaggregation through aggregation phases.

Several approaches have been used to relate tree chemistry to postlanding host acceptance behavior. The most direct approach is to cage beetles onto trees, record entry rates, and compare the chemistry of entered versus rejected trees (e.g., Raffa and Berryman 1982). However, these experiments yield variable results because of uncontrolled environmental factors and do not control host chemistry. Applying defined concentrations of allelochemicals to an inert substrate such as filter paper provides some control (Raffa and Berryman 1982), but measurements of feeding area do not directly relate to locomotory behaviors involved in host entrance or gallery construction. Klepzig et al. (1996) incorporated locomotion into a tunneling assay by adding controlled quantities of host compounds to a phloem-based agar medium, and observed that gallery lengths decreased as monoterpene concentrations increased under no-choice conditions. However, total gallery length may reflect beetle vigor in addition to behavior. In two-way choice assays, beetles preferred control media over media amended with higher concentrations of monoterpenes, constitutive over induced tissue, and stem phloem from trees with root disease over healthy trees (Klepzig et al. 1996). However, these assays do not allow insects to elect whether or not to enter the medium, which occurs after a beetle lands on a tree. Physiological changes within an individual insect may also affect orientation to hosts and responses to external cues (Kennedy 1977 a, 1977b; Miller and Strickler 1984; Hoffman et al. 1997). For example, Douglas-fir beetles, *Dendroctonus pseudotsugae* Hopkins, and the California fivespined ips, *Ips paraconfusus* Lanier, become host positive as their fat reserves are depleted (Atkins 1966, Hagen and Atkins 1975). Response of flying pine engravers, *Ips pini* (Say), to host odors has been shown to be positively correlated to insect desiccation, but not related to visually rated fat content (Gast et al. 1993).

*Ips pini* is a widely distributed bark beetle that can colonize either stressed or dead trees of most species of *Pinus* within its range. Males determine the susceptibility of potential hosts, construct a nuptial chamber under the bark, and emit pheromones. Females enter the galleries created by the males, copulate, and construct ovipositional galleries. Males are polygamous and are typically joined by up to three females.

The objective of this study was to determine the effects of a common set of host compounds on different stages of postlanding host acceptance behavior by *I. pini*. We also evaluated the relationship between two internal physiological characteristics, moisture and total lipid content, and postlanding host acceptance behavior by *I. pini*.

## Materials and Methods

*Ips pini*, from a laboratory culture that was initiated with local beetles in 1994, and replenished with wild beetles in the spring and fall of each year, were used in all assays. Detailed rearing methods are described in

Raffa and Smalley (1995). Briefly, 12 males are introduced into newly cut log sections of *P. resinosa*. After 72 h, three females were added to each entry site and the log was placed into a metal rearing can (50 cm diameter by 40 cm tall). Adult progeny emerged 3–4 wk after the introduction of parental beetles and were collected in clear jars on the side of the rearing can. Emerging beetles were used either to maintain the colony or for behavioral assays. All beetles used for behavioral assays were adult males that had emerged within 24 h.

**General Bioassay Conditions.** The assay arena consisted of a plastic petri dish (9 cm diameter by 3 cm high) containing a mixture of agar, phloem, and water (Klepzig et al. 1996). Phloem was scraped from a felled mature *P. resinosa*, freeze-dried in a Vitis lyophilizer for 48–72 h, ground through a mill (0.5 mm screen), and autoclaved for 20 min at 104.4°C and 33.3 kg of pressure, which removes volatile monoterpenes (Raffa and Smalley 1995). Bacto-agar (Difco, Detroit, MI) (5 gm) was mixed into boiling distilled water (150 ml), and ground phloem (10 gm) was added to the agar-water mixture. Medium was poured into each petri dish to the level of 2.0 mm, and dried at 22°C in a fume hood for 24 h. Synthetic monoterpenes of various concentrations were dissolved in pentane and spread upon the dried surface of the medium. Controls were treated only with pentane. Each assay arena received 1 ml of solvent or solution.

The monoterpenes used were (+)- $\alpha$ -pinene, (-)- $\alpha$ -pinene, racemic- $\alpha$ -pinene, racemic  $\beta$ -pinene, and racemic limonene (Aldrich, Milwaukee, WI). Compounds were administered in units of milligrams of monoterpenes per gram of ground phloem medium, to simulate host subcortical tissue. Alpha-pinene and  $\beta$ -pinene account for 98% of the total monoterpenes in phloem tissue of *P. resinosa* (Raffa and Smalley 1995), the most common host of *I. pini* in Wisconsin. We used concentrations similar to those found in phloem tissue of *P. resinosa* (Raffa and Smalley, 1995, Klepzig et al. 1996) as our model. Test concentrations were selected to span the concentrations of monoterpenes in *P. resinosa* phloem: mean quantities of  $\alpha$ -pinene range from 8.0 mg/gm in constitutive tissue to 243.0 gm/mg (including accumulation of liquid resin) in induced reaction (post-attack) tissue (Raffa and Smalley 1995);  $\beta$ -pinene ranges from 1.6 mg/gm in constitutive tissue to 50.3 mg/gm in reaction tissue; limonene ranges from 0.2 mg/gm in constitutive tissue to 6.2 mg/gm in reaction tissue. All other monoterpenes in *P. resinosa* amount to  $\approx$ 2% in constitutive tissue and 5% in induced reaction tissue. Monoterpene concentrations were calculated using the following equation: monoterpene added to phloem media (ml/gm) = [concentration of monoterpene desired (mg/gm)\*phloem-diet weight (gm)]/[1,000\*density of monoterpene (gm/ml)\*purity of monoterpene]. There were 20 replications of each treatment unless otherwise stated.

Within 1 h of applying the monoterpene treatment or control, a single beetle was introduced into each arena (either adjacent to or directly into the medium,

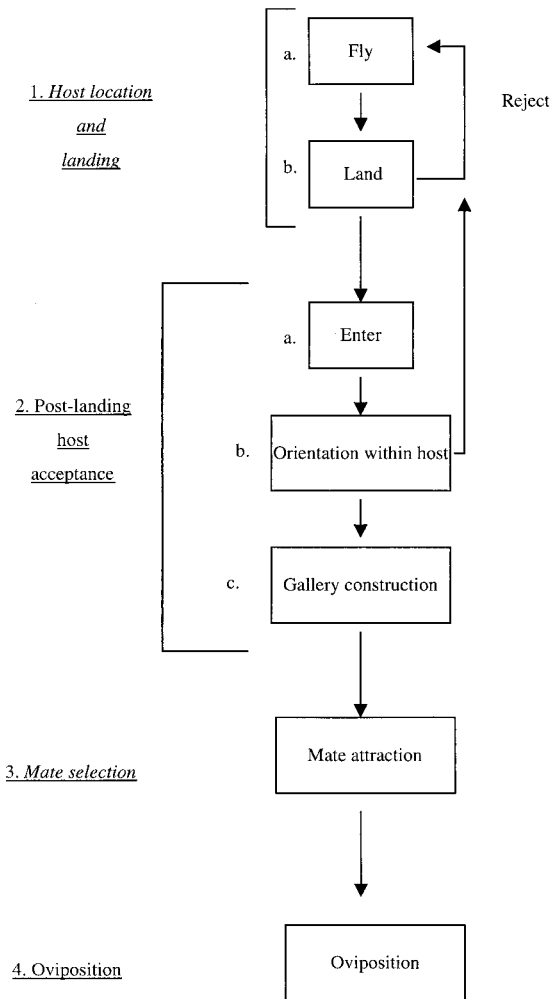
depending on the experiment; see below), and allowed to construct galleries for 72 h. At each 24-h interval, the gallery was traced onto the lid of the dish, using a different colored marker for each period, and the lengths of the galleries were recorded and quantified using a map measurer (PECO, Jackson, MS). Assay arenas were kept in darkness at 24°C. Arenas were randomly arranged in stacks of five, and stacks were randomly assigned to positions in environmental chambers.

Unless stated otherwise, statistical analyses were performed using analysis of variance (ANOVA, SAS Institute 1989), with appropriate transformations. When significant ( $P = 0.05$ ) treatment effects occurred, mean separations and contrasts were performed using least significant difference. We used two-way ANOVA to analyze total gallery length.

**Media Extraction.** As a quality control measure, we used gas chromatography to quantify the amount of monoterpene remaining in test media 24, 48, and 72 h after application (Wallin and Raffa 1999). Amended diet was extracted in 10 ml pentane. The pentane contained 0.1% *para*-cymene as an internal standard, to provide a check on retention times and a visual check on chromatograph performance. Extracts were separated from the phloem by vacuum filtration, and dried over calcium chloride for 1 h. Separations were performed on a 25 m by 0.25-mm bonded fused silica open tubular polyethylene glycol column (Alltech, Deerfield, IL) on a Shimadzu 9A (Shimadzu Scientific Instruments, Columbia, MD). Oven temperature was 60°C for 10 min, and increased to 160  $\times$  10°C per min. Helium served as the carrier gas at 30 cm/s, with a methane retention time of 83.8 s. Quantities of monoterpenes were determined using standard equations generated by regressing peak areas of pure monoterpenes on known quantities. Each standard equation generated an  $r^2$  of at least 0.96 (Wallin and Raffa 1999). After analysis, each sample was oven-dried and weighed. Repeated-measures ANOVA and linear regression (SAS Institute 1989) were used to analyze means of total monoterpenes per gram of media. A similar gas chromatography method was used to check that all ground phloem was devoid of natural monoterpenes before being amended with defined concentrations of monoterpenes.

**Experimental Overview.** A series of assays was conducted to simulate the major stages of postlanding host entrance behavior. The assays we conducted are illustrated within the overall process of host selection by bark beetles in Fig. 1. Our assays focused on section 2 of this sequence, and correspond to initial entry, within-host orientation, and gallery construction.

**Host Entrance.** In this behavioral choice assay, beetles could either enter or not enter the test medium (Fig. 1:2a). The arena was prepared as described above, except the 9-mm disk of phloem agar medium was placed in the 11-mm lid of the petri dish, creating a 1-mm wide circular channel. Monoterpenes were applied as described above, and a 9-mm disk of transparency film was placed over the amended media. One adult male beetle was placed on the center of the



**Fig. 1.** Multiple steps of host selection behavior by bark beetles. Host location and landing includes flight, arrival, and landing on a potential host tree. Postlanding host acceptance behavior begins with the individual either entering or rejecting the potential host. If the individual rejects the host, it resumes flight. If the individual enters the host it orients within the host and begins constructing galleries. The final step is mate selection behavior. The steps are sequential.

transparency film and observed for 15 min. Each beetle could either enter or reject the medium. A beetle was scored as rejecting the medium if it remained on the surface of the transparency or walked around the perimeter of the assay arena without entering during this period. A second reading was made at 24-h. Data on beetle entry were evaluated using chi-square and contingency analyses. The time (min) between when each beetle was placed into the arena and when it entered the medium was regressed against monoterpene concentration. After beetles entered the medium, the lengths of their galleries were recorded at 24-h intervals for 72-h.

**Orientation within Medium.** The phloem-based medium was prepared as above, and then divided in

half using a piece of transparency film. This plastic film served as a temporary barrier while treatments were applied to either section (Klepzig et al. 1996). The monoterpene solution or the pentane control was randomly applied to either side of the medium, the transparency film was removed, and the unit was sealed using transparency film and paraffin wax. One adult male beetle was gently inserted head first through a 2-mm-diameter hole that was predrilled at the point where the test medium was divided, and its exit was blocked with parafilm. Assay arenas were such that the beetle could construct a gallery in either the monoterpene-amended or control side, and also move freely between the two sides (Fig. 1:2b). We recorded gallery length and beetle location every 24 h for 72 h. We analyzed relative proportions of beetles found on either side of the assay arena using chi-square and, more conservatively, Fisher exact test analysis. Gallery lengths between the treated and control portions were compared using a paired-*t*-test.

**Gallery Formation.** The effects of monoterpenes on gallery construction (Fig. 1:2c) by *I. pini* were tested under no-choice conditions. Monoterpenes were applied uniformly to the surface of the phloem-agar medium. Phloem-agar media was covered with an 11-mm transparency disk and the parameter was then sealed with warm paraffin wax. A 2.0-mm-diameter entrance hole was drilled through the side of each assay arena, and one male beetle was gently inserted head first through the hole into the medium. The hole and petri dish lid were then sealed as described above, and the beetle was observed every 24 h for 72 h. Gallery length was measured and analyzed as above.

**Physiological Characteristics of Male *I. pini* in Relation to Host Selection Behavior.** To determine whether postlanding host acceptance behavior is associated with beetle condition, we repeated the entrance assay, and compared the water and lipid contents of entering versus rejecting beetles. The media were either amended with 1.4 mg  $\alpha$ -pinene/gm (based on the prior experiments which proved this concentration to be deterrent; see *Results*) or pentane controls. Beetles that entered the media were immediately removed from the assay arena, and the time to their entrance was recorded. If a beetle did not enter the medium within 15 min, it was scored as having rejected that treatment.

After each behavioral assay, all beetles were analyzed for water content and total lipid content. Each beetle was weighed, oven dried at 50–55°C for 24 h, and reweighed to determine its water content. A sulfophosphovanillin spectrophotometric method (Kinn et al. 1994) was used to determine lipid content. Each oven dried beetle was extracted in  $H_2SO_4$  and heated in a boiling water bath for 10 min. The mixture was cooled for 10 min, vortexed, filtered, and amended with color reagent. After a 30-min incubation period, 5  $\mu$ l of the solution absorbance was analyzed photometrically (530 nm) using a Beckman DU 640 spectrophotometer (Beckman Instruments, Fullerton, CA). We generated two standard curves daily, using controls prepared with known amounts of dry cho-

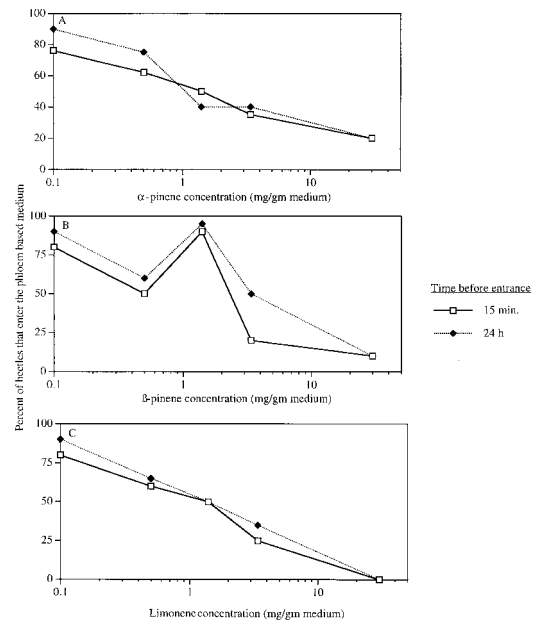
lesterol that replaced the beetle. Each standard curve had an  $r^2$  of at least 0.98. The relationships among entrance, moisture, and lipid content of individual insects were determined by standard linear, exponential, and quadratic curve-fitting procedures (SAS Institute 1989).

## Results

**Evaluation of Experimental Conditions.** Based on gas chromatography analysis, oven drying removed all but traceable amounts of naturally occurring monoterpenes from *P. resinosa* phloem before the bioassays. Amended monoterpene concentrations in media remained stable during the first 48 h ( $F = 0.9$ ,  $df = 3$ ,  $P = 0.52$ ) of the assay. During this period, concentrations of the extracted monoterpenes remained within 6.7% of the originally intended concentration. After 72 h there was some evidence that monoterpenes may have volatilized or degraded ( $F = 1.98$ ,  $df = 3$ ,  $P = 0.08$ ), because concentrations were 20.9% lower than the originally applied dosages. Gallery lengths in the control media of the no-choice assays stayed relatively constant during day 1 ( $1.75 \pm 0.5$  cm) and day 2 ( $2.4 \pm 0.4$  cm), but declined during day 3 ( $0.34 \pm 0.4$  cm). Based on these chemical and biological considerations, we present the data for the first and second daily increments, and use the 48-h period for evaluations of total gallery length.

**Effects of Monoterpenes on Host Entry.** Monoterpene concentrations significantly influenced the percentage of male *I. pini* that entered the media (Fig. 2,  $F = 2.9$ ,  $df = 1$ ,  $P = 0.04$ ). The chirality of  $\alpha$ -pinene did not affect entrance behavior ( $F = 1.09$ ,  $df = 1$ ,  $P = 0.29$ ), so these data were pooled. Increased concentrations of  $\alpha$ -pinene decreased beetle entry rates in a logarithmic fashion (Fig. 2A). Extending the duration of the assay from 15 min to 24 h had little effect on beetle entry, although there was a slight overall increase. Increased concentrations of  $\beta$ -pinene also affected beetle entry, but in a more complex fashion. The overall trend was for the percentage of beetles entering to decrease as  $\beta$ -pinene concentration increased, but entrance was highest at 1.4 mg  $\beta$ -pinene/gm phloem-based media (Fig. 2B). As with  $\alpha$ -pinene, increasing the duration of the assay to 24 h only slightly increased overall host entrance, with the greatest increase occurring at 3.4 mg  $\beta$ -pinene/gm phloem-based medium. Increased concentrations of limonene resulted in decreased percentage of beetles entering across all concentrations in a logarithmic fashion (Fig. 2C). Limonene generated the steepest negative slope of all five monoterpenes tested. No male *I. pini* entered medium amended with the highest concentration of limonene (30.0 mg/gm). As with the other monoterpenes, increasing the duration of the assay had little effect on the overall percentage of beetles entering the phloem-based media amended with limonene.

Monoterpene concentration also influenced the time before beetles accepted simulated host tissue ( $F = 4.179$ ,  $df = 1$ ,  $P = 0.004$ ) (Table 1). There was no



**Fig. 2.** Effects of synthetic monoterpenes on the percentage of male *I. pini* that enter phloem-based media amended with various concentrations of monoterpenes. (A)  $\alpha$ -pinene, entrance within 15 min =  $-29.49 \log(x) + 58.04$   $r^2 = 0.91$ ; entrance within 24 h =  $-23.525 \log(x) + 52.617$   $r^2 = 0.98$ . (B)  $\beta$ -pinene, entrance within 15 min =  $0.22x^2 - 9.306x + 85.544$   $r^2 = 0.79$ ; entrance within 24 h =  $0.466x^2 - 16.328x + 80.455$   $r^2 = 0.68$ . (C) Limonene, entrance within 15 min =  $-33.282 \log(x) + 48.683$   $r^2 = 0.979$ ; entrance within 24 h =  $-36.331 \log(x) + 54.203$   $r^2 = 1.00$ .  $n = 30$  for each concentration of each synthetic monoterpene.

significant interaction between concentration and monoterpene ( $F = 1.29$ ,  $df = 1$ ,  $P = 0.175$ ). The relationship between the average time until host entry and concentration of  $\alpha$ -pinene was not linear. Rather, beetles more quickly entered media amended at 1.4 and 3.4 mg/gm concentration than the other concentrations (Table 1). At the highest concentration (30.0 mg/gm), the interval before entrance was 65% longer than in the control. Increasing the concentration of  $\beta$ -pinene from the control level did not increase the interval before host entry (Table 1). A concentration of 0.5 mg  $\beta$ -pinene/gm elicited more rapid host entry behavior, with beetles entering the medium nearly one-third sooner. Limonene had the strongest effect on the duration before host entry by male *I. pini*. At 1.4 and 3.4 mg limonene/gm phloem-based medium, this interval was 46.2 and 55.2% longer, respectively, than the control. This interval could not be estimated at the highest concentration, because no beetles entered the medium within 24 h.

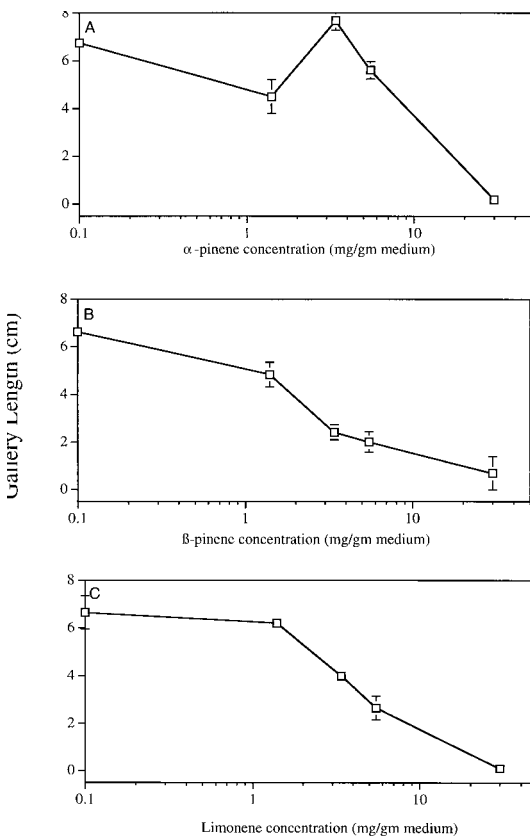
Once beetles entered the medium, the extent to which they constructed galleries was also influenced by monoterpene concentration (Fig. 3). However, the effects of these monoterpenes on gallery lengths differed from their effects on host entry. As the concentration of  $\alpha$ -pinene increased, total gallery length

**Table 1.** Effect of monoterpenes on the mean time (minutes) before male *I. pini* entered phloem-based media

Monoterpene	Concentration (mg monoterpene/gm phloem-based medium)				
	0	0.5	1.4	3.4	30
$\alpha$ -pinene	6.0 $\pm$ 0.7Aa	6.6 $\pm$ 0.5Aa	5.0 $\pm$ 0.9Ab	5.3 $\pm$ 0.7Aab	9.9 $\pm$ 0.6Ac
$\beta$ -pinene	7.2 $\pm$ 0.7Aa	4.9 $\pm$ 0.2Bb	6.6 $\pm$ 0.8Aa	7.0 $\pm$ 0.8Ba	7.1 $\pm$ 0.2Ba
Limonene	6.7 $\pm$ 0.1Aa	4.8 $\pm$ 0.3Bb	9.8 $\pm$ 0.3Bc	10.4 $\pm$ 0.4Cd	no entry

Lowercase letters indicate differences within a monoterpene treatment caused by concentration. Means within a row followed by the same lowercase letter are not significantly different at  $P \leq 0.05$ . Uppercase letters tested for possible differences within a concentration caused by monoterpene type. Means within a column followed by the same uppercase letter are not significantly different at  $P \leq 0.05$ .

dropped from  $\approx 7$  cm to almost 0 cm. However, there was a substantial increase in gallery length at 3.4 mg  $\delta$ -pinene/gm phloem-based medium (Fig. 3A). By contrast,  $\beta$ -pinene and limonene each caused reduction in gallery lengths with increasing concentrations. This decline was gradual until 1.4 mg monoterpene/gm phloem-based medium, but steepened thereafter, and eventually approached zero (Fig. 3B and 3C).



**Fig. 3.** Gallery lengths (mean  $\pm$  SE) by *I. pini* 2 d after entering media amended with various concentrations of monoterpenes. (A)  $\delta$ -pinene:  $y = -0.008 \times 2 + 0.058x + 6.075 r^2 = 0.832$ . (B)  $\beta$ -pinene:  $y = -2.54 \log(x) + 4.277 r^2 = 0.94$ . (C) Limonene:  $y = -2.69 \log(x) + 4.942 r^2 = 0.84$ .  $n = 30$  for each concentration of each synthetic monoterpene.

**Effects of monoterpenes on Within-Host Orientation.** In the within-medium orientation assay, male *I. pini* preferred the control amended medium to most monoterpene concentrations (Table 2). Within each monoterpene, this effect was most pronounced at the highest concentration, 30 mg/gm. Among monoterpenes,  $\beta$ -pinene had the lowest overall repellence. One exception to the general pattern of preference for control over monoterpene-amended medium again occurred with  $\alpha$ -pinene of 3.4 mg/gm, in which nearly three-quarters of the beetles formed galleries in the monoterpene-amended side.

Beetles that began gallery construction in the control side of the arena always remained there throughout the duration of this assay. However, beetles that began gallery construction in the monoterpene-amended side of the arena were more likely to move to the nonamended side during the first 24-h of the assay (Table 2). This effect was greatest in response to  $\alpha$ -pinene. Conversely, beetles that entered and began gallery construction in media amended with 1.4 mg limonene/gm media did not move to the control at any point during this assay (Table 2). This probably is not the result of a toxicity effect, because when beetles entered media with higher concentrations of limonene (e.g., 30.0 mg/gm), they rapidly exited the treated medium and began gallery construction in the control medium.

Beetles constructed longer galleries in the control than monoterpene-amended portions of the media (Fig. 4). However, the patterns differed among the monoterpenes. In the case of  $\alpha$ -pinene and limonene, the difference in gallery lengths between the control versus the treated medium increased until the moderate concentration (3.4 mg monoterpene/gm phloem-based media), and then decreased. However, in the case of  $\beta$ -pinene, this difference was not seen at the moderate concentration. Although gallery lengths are shown for the entire 48-h assay, differences among treatments were also apparent during the first 24 h ( $P = 0.01$ ).

**Effects of Monoterpenes on Gallery Construction Under No-Choice Conditions.** Monoterpene concentrations strongly affected total gallery lengths constructed by male *I. pini* under no-choice condition (Fig. 5). The relationships between monoterpene concentration and gallery length were similar to those in the postentry portion of the entrance assays (Fig. 3). That is,  $\alpha$ -pinene generated an overall decrease in

**Table 2. Effect of monoterpenes on male *I. pini* preference for amended versus control medium under two-way choice assay conditions**

Monoterpene	Concentration of monoterpene (mg/gm phloem-based media)		
	1.4	3.4	30
	% of male <i>I. pini</i> in the monoterpene amended side of arena		
$\alpha$ -pinene	26.0**	74.0*	5.0***
$\beta$ -pinene	47.5	48.5	22.0***
Limone	40.0**	45.0*	12.0***
Limone	40.0**	45.0*	12.0***
	% of male <i>I. pini</i> that moved from the monoterpene amended to control side of the assay arena		
$\alpha$ -pinene	50	90	80
$\beta$ -pinene	40	20	50
Limone	0	35	75

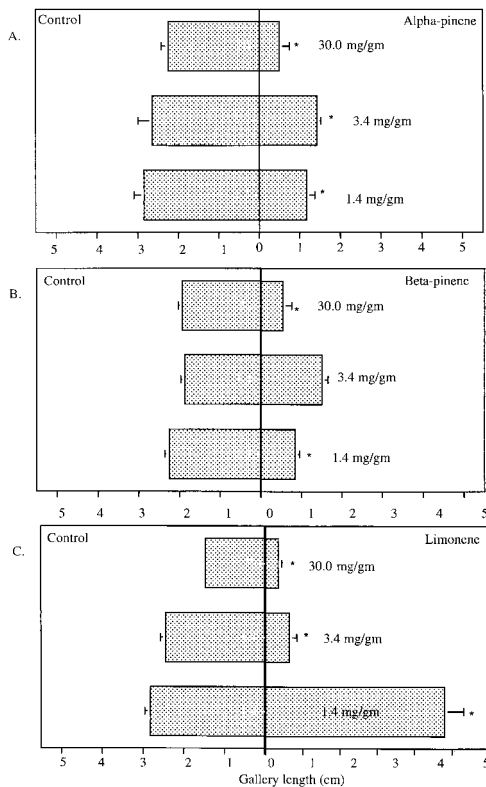
*P*-values for paired-*t* comparison between the numbers of beetles on the monoterpene amended versus control sides of the assay arena: \*, *P* = 0.05, \*\*, *P* = 0.005, \*\*\*, *P* = 0.0001.

<sup>a</sup> Percentage of males creating galleries in the monoterpene-amended side of the assay arena.

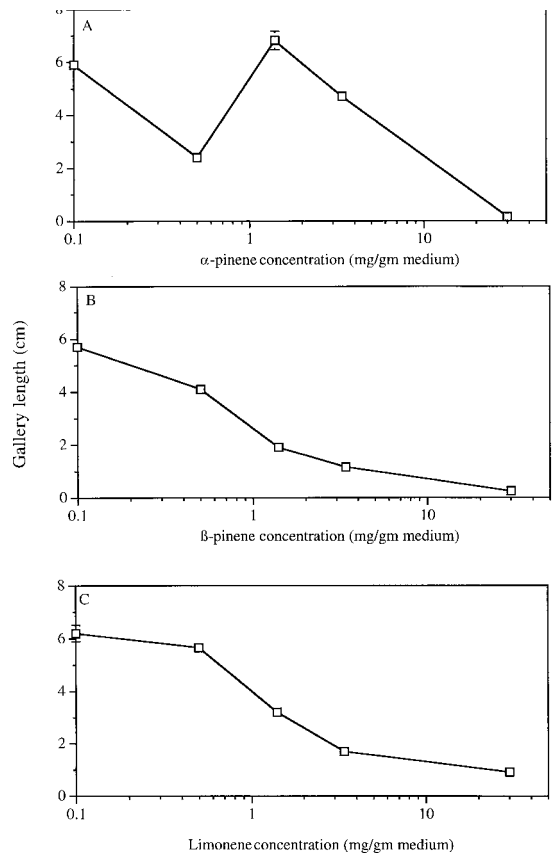
<sup>b</sup> Percentage of males that moved from the monoterpene-amended to the control side of the assay arena within 24 h after initiating the assay.

gallery lengths with increasing concentrations, but gallery lengths were longer than controls at moderate concentrations (Fig. 5A). Gallery lengths constructed

in amended media decreased with increased concentrations of  $\beta$ -pinene and limonene (Fig. 5B and 5C).



**Fig. 4.** Effects of synthetic monoterpenes on gallery lengths (mean  $\pm$  SE) constructed by male *I. pini* in two-way choice assay. Each set of bars shows the gallery lengths during 2 d in the in phloem-based medium amended with pentane or various concentrations of (A)  $\alpha$ -pinene, (B)  $\beta$ -pinene (C) limonene. Significantly different (*P* < 0.01) gallery lengths between control and monoterpene amended side are followed by an asterisk. *n* = 25 for each concentration for each synthetic monoterpene. Standard error bars are present for all treatments.



**Fig. 5.** Effects of synthetic monoterpenes on gallery lengths (mean  $\pm$  SE) constructed by male *I. pini* during 2 d in phloem-based medium under no-choice assay conditions. (A)  $\alpha$ -pinene:  $y = -0.165x + 5.158$   $r^2 = 0.62$ . (B)  $\beta$ -pinene:  $y = -2.306 \log(x) + 3.016$   $r^2 = 0.92$ . (C) Limonene:  $y = -2.39 \log(x) + 3.939$   $r^2 = 0.89$ . *n* = 25 for each concentration for each synthetic monoterpene.

**Table 3.** Effect of monoterpene type and concentration on length of galleries constructed by *I. pini* males at various times after entrance into the phloem-based media

	df	Interval (h) following entry into media							
		0-24		25-48		49-72		0-72	
		F	P	F	P	F	P	F	P
All monoterpenes									
Concentration	5	3.847	0.008	2.619	0.018	1.341	0.266	2.822	0.033
Monoterpene	2	1.313	0.277	0.366	0.695	1.230	0.300	0.379	0.686
Monoterpene * concentration	4	0.970	0.444	0.726	0.607	1.259	0.294	0.818	0.542
(+ and (-) stereoisomers of alpha-pinene									
Concentration	5	7.620	0.001	3.310	0.043	10.360	0.001	8.450	0.006
Enantiomeric composition (EC)	1	0.990	0.379	0.880	0.420	0.300	0.745	1.040	0.358
EC * concentration	3	1.380	0.258	1.010	0.370	0.090	0.912	0.850	0.433

Monoterpene concentration affected gallery construction during most time intervals of the bioassay (Table 3). Gallery lengths were similar during each 24-h interval of the assay within each treatment (i.e., each concentration of each monoterpene). There was no significant concentration by day interaction for any monoterpene: racemic  $\alpha$ -pinene ( $F = 1.07$ ,  $df = 4$ ,  $P = 0.38$ ),  $\beta$ -pinene ( $F = 1.06$ ,  $df = 4$ ,  $P = 0.38$ ), limonene ( $F = 1.6$ ,  $df = 4$ ,  $P = 0.76$ ), (+)  $\alpha$ -pinene ( $F = 2.18$ ,  $df = 4$ ,  $P = 0.12$ ) or (-)  $\alpha$ -pinene ( $F = 0.11$ ,  $df = 4$ ,  $P = 0.89$ ). There was no significant concentration by monoterpene interactions (Table 3), which suggests that the quantity of each monoterpene more strongly influences beetle gallery construction than the type of the monoterpene.

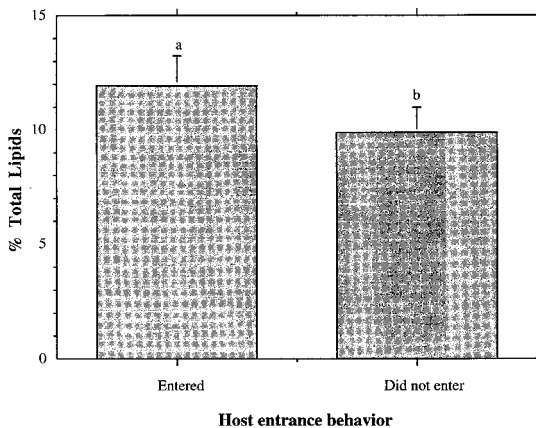
**Relationship of Lipid and Water Content of Male *Ips pini* to Host Entry.** The likelihood of beetle entry into media amended with 1.4 mg  $\alpha$ -pinene/gm phloem-based media (which is sufficient to reduce entry by approximately half; Fig. 2A), was significantly related to the total percent lipid content of individual beetles (Fig. 6). Beetles that entered amended medium had 21% more total lipids than those that re-

jected the medium ( $n = 60$ ,  $F = 5.513$ ,  $df = 1$ ,  $P = 0.023$ ). Conversely, the mean time before beetle entry ( $x = 3.75 \pm 6.0$  min) was not related to total percentage lipid content ( $n = 60$ ,  $F = 0.9$ ,  $df = 1$ ,  $P = 0.89$ ). Beetle entrance behavior was not significantly related to water content of individual beetles, which averaged  $0.3 \pm 0.008$  mg or 5-10% of beetle body weight ( $n = 60$ ,  $F = 2.195$ ,  $df = 1$ ,  $P = 0.1454$ ).

**Discussion**

Monoterpenes affected all stages of postlanding host acceptance behavior by *I. pini* in assays with phloem-based media. However, each stage of this behavioral sequence was affected differently by various host monoterpenes (Table 4). For example,  $\beta$ -pinene is the only monoterpene that elicited host entry at any concentration, but it had no effect on within-host orientation, and had the strongest inhibitory effect on gallery construction (Table 4).  $\alpha$ -pinene was the only monoterpene to elicit both positive within-host orientation, and also elicited extensive gallery construction. However,  $\alpha$ -pinene inhibited all phases of host entrance at higher concentrations. Limonene was not attractive during any step of postlanding acceptance behavior, but rather had relatively strong inhibitory effects on each stage (Table 4).

The interval between a beetle's contacting and entering the substrate generally increased with increasing monoterpene concentrations. An exception occurs with moderate concentrations of  $\beta$ -pinene, which elicited increased beetle entry. This concentration of  $\beta$ -pinene corresponds with levels seen in root-diseased *P. resinosa* (Raffa and Smalley 1995, Klepzig et al. 1996). Once in the substrate, beetles can orient to regions of relatively low monoterpene concentrations (Table 2). This may partially explain how individual bark beetles can avoid resin glands (Ferrell 1983) and also how beetles can survive the period between initial host entry, when induced reactions begin to raise monoterpene concentration to toxic levels, and when numerous responding beetles exhaust tree defenses (Raffa and Berryman 1983a). As with entry and preference behaviors, varying monoterpene concentration can either elicit or inhibit gallery construction by *I. pini* (Fig. 5). Complex relationships between alle-



**Fig. 6.** Relationship of host entry behavior to the total percentage of lipids in male *I. pini*. Adult males given choice to enter or reject phloem-based media amended with 1.4 mg  $\alpha$ -pinene/gm. Different letters indicate significant differences ( $P = 0.0001$ ).

**Table 4.** Descriptive summary of differential effects of monoterpenes on various stages of postlanding host acceptance behavior by male *I. pini*

Stages of orientation	$\alpha$ -Pinene		$\beta$ -Pinene		Limonene	
	Inhibit	Elicit	Inhibit	Elicit	Inhibit	Elicit
Host entry	Logarithmic Moderate effect	None	Logarithmic Weak effect	Low concentration	Logarithmic Strong effect	None
Within host orientation	Asymptotic	Moderate concentration	None	None	Exponential	None
Gallery excavation	Logarithmic Weak effect	Moderate concentration	Logarithmic Strong effect	None	Logarithmic Moderate effect	None

lochemical concentration and insect orientation, such as those described here, have been reported in other plant-herbivore systems (Miller and Strickler 1984, Byers 1992, Honda 1995, Rossi et al. 1997).

The concentrations of monoterpenes that affect various stages of postlanding behavior in male *I. pini* correspond to those present in a major host species in Wisconsin, *P. resinosa*. The concentrations of  $\alpha$ -pinene (8.0 mg/gm) and limonene (0.2 mg/gm) present in constitutive tissue are sufficient to reduce beetle entry rate by 63 and 16%, respectively (Fig. 2). However, the concentration of  $\beta$ -pinene present in the reaction tissue of a host would be repellent to nearly all beetles (Fig. 2; Table 1). The concentrations of  $\alpha$ -pinene in constitutive tissue, 8.0 mg/gm, would likely elicit continued gallery construction (Table 2). This would be especially likely in trees with diminished phloem monoterpene concentrations caused by biotic agents such as defoliators (Wright et al. 1984, Wallin and Raffa 1999). Conversely, induced reaction levels, particularly of  $\alpha$ -pinene, could orient beetles away from localized host responses (Table 2). Gallery construction could be elicited by the concentrations of  $\alpha$ -pinene present in constitutive phloem (Figs. 3A and 5A) and would be inhibited only slightly by the concentration of  $\beta$ -pinene and limonene present. However, the concentrations of each of the monoterpenes present in the reaction tissue of healthy trees are high enough to cause nearly all gallery construction to cease. Beetles could only avoid this effect by either selecting trees that had very poor induced responses, or by colonizing trees at sufficient densities to prevent the potential monoterpene concentrations of these induced reactions from being realized (Christiansen 1991).

Despite some differences in how various monoterpenes affect orientation, concentration appears to be the most important factor. This is somewhat surprising, because previous workers observed correlations between the percent composition of certain monoterpenes and patterns of tree mortality or insect toxicity (Raffa and Berryman 1982, 1983b; Gara et al. 1984; Sturgeon and Mitton 1986), or have indicated differences among various monoterpenes in their toxicity to beetles (Raffa and Berryman 1982, 1983b). However, the relative importance of concentration is supported by observations that toxicity to *I. pini* varies only slightly among various monoterpenes, and relates more strongly to total monoterpene content (Raffa and Smalley 1995). Alpha-pinene and  $\beta$ -pinene are typically the most abundant allelochemicals in phloem

tissue of pine trees (Cook and Hain 1988, Nebeker et al. 1994, Klepzig et al. 1996, Wallin and Raffa 1999). Hence, for a bark beetle with a relatively broad host species range but narrow host physiological range, such as *I. pini*, perhaps several terpenes can serve as cues in host species recognition, but absolute concentrations are better predictors of host defensive capacity. This may partially explain why total monoterpenes is often a better predictor of tree mortality than is a particular monoterpene (Raffa and Berryman 1982, Wallin and Raffa 1999). We did not address potential synergistic or antagonistic interactions among combinations of monoterpenes, although prior work by Klepzig et al. (1996) suggests that such interactions are additive only.

Our observations that the enantiomeric composition of  $\alpha$ -pinene does not influence postlanding host entrance by *I. pini* is somewhat surprising, as stereochemistry is often critical to insect behavior (Gries et al. 1993, Camacho et al. 1994, Warthen et al. 1996). Responses by *I. pini* to its pheromone ipsdienol (Lanier et al. 1980, Raffa and Klepzig 1989, Seybold 1993), *Dendroctonus valens* (LeConte) to  $\alpha$ -pinene (Hobson et al. 1993), and *Hylastes porculus* Erichson to  $\alpha$ -pinene (unpublished data) are strongly influenced by chirality. One difference is that each of the above studies measured landing behavior rather than the postlanding host entrance behaviors we evaluated (Fig. 1). This supports the view that there are substantial differences between prelanding and postlanding behaviors of bark beetles, and that the same compounds can affect these components of host selection differently.

Postlanding responses to host allelochemicals are modified by the internal physiology of the insect, as has been demonstrated in several other systems (Rausher 1983, Miller and Strickler 1984, Fitt 1986, Thiery-Denis 1998). However, the direction of the relationship between lipid content and host entry was different from what we would have predicted. Several authors have suggested that an inverse relationship between lipid stores and response to arresting cues might assure some dispersal by bark beetles before they colonize new trees (Rudinsky 1962). It also seems reasonable that as their energy reserves become depleted, beetles might become less discriminating in their host acceptance behaviors (Gast et al. 1993). The positive correlation we observed between host entry and stored lipids suggests additional functions of lipids in host colonization. For example, lipids play a central role in detoxification of plant allelochemicals (Aucoin

et al. 1995). In the case of bark beetles, the relationship between host entry and lipid content could conceivably orient those beetles with relatively lower detoxification abilities to trees with lower levels of monoterpenes. This relationship could also provide a linkage between host entry behaviors and pheromone synthesis in bark beetles, which can be mediated by P-450 pathways acting on lipophilic substrates (Vanderwel 1994, Seybold et al. 1995). Because lipids represent only one form of energy stores, our results suggest that other forms of energy storage, such as carbohydrates, could also influence postlanding host entrance behavior.

Our results support the view that the overall process of host selection by bark beetles needs to be viewed as a sequence of behavioral events, and cannot be inferred from single factors such as landing, individual components of feeding behavior, or patterns of tree mortality. The host entrance bioassay we described provides a new tool for accurately and rapidly relating key processes of postlanding host selection behavior to monoterpene concentrations under conditions that simulate beetle's entry into trees. Components of host selection behavior are also context-dependent, and can be modulated by internal insect physiology.

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