



Prior encounters modulate subsequent choices in host acceptance behavior by the bark beetle *Ips pini*

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Abstract

Laboratory bioassays indicate that the bark beetle *Ips pini* employs flexible, rather than absolute, responses to phytochemicals in its host acceptance behavior. Each beetle's decision to enter substrate was influenced by the types and concentrations of monoterpenes present. However, previous rejection of a simulated host containing a moderate concentration of monoterpenes increased the likelihood that the same concentration would be accepted upon a second or third encounter. This flexibility more than offsets any loss of vigor due to starvation and age that accompanies a process of trial and error. Starvation decreased beetles' total lipid content, but beetles can recover some energetic losses by a small amount of feeding during each trial. In addition to its adaptive value, a flexible host acceptance strategy may yield population level consequences. That is, bark beetles preferentially enter trees having low concentrations of monoterpenes, but may modify their acceptance thresholds when cues associated with stressed trees are not available. This could partially explain how some tree-killing bark beetles colonize a broader physiological range of trees during outbreaks. The adaptive value of relating individual decisions to population density may arise from two ecological relationships: first, as populations rise, the pool of stressed trees is rapidly depleted; secondly, healthy trees are attainable through pheromone – mediated mass attacks when adequate numbers of beetles are present. Flexible host acceptance behaviors may also reduce the advantage of relying exclusively on pre-landing cues to distinguish between susceptible and non-susceptible trees.

Introduction

Host selection by insect herbivores consists of several sequential steps, leading to acceptance or rejection of individual plants (Futuyma, 1983). Insects respond to different types and magnitudes of stimuli, during different phases of this process, until they locate and accept a suitable host (Singer, 1982; Papaj & Rausher, 1983; Honda, 1995). Most insect herbivores encounter substantial variation in host suitability both among plant species and among individuals of appropriate species (Fox & Morrow, 1981). Intraspecific variation in hosts is especially critical for groups such as bark beetles (*Coleoptera*: *Scolytidae*) that enter host tissue, risk mortality to high concentrations of host phytochemical, particularly monoterpenes if they

attempt to colonizing resistant individuals (Nebeker et al., 1993; Raffa & Smalley, 1995), can experience high egg and larval mortality due to induced host defenses, and deposit all or nearly all of their egg clutch within one plant (Atkins, 1966; Wood, 1982). Decisions regarding oviposition by such insects are often highly discerning, and must incorporate not only host species recognition, but also 'diagnosis' (Safranyik et al., 1975) of each plant's defensive capacity. If a beetle lands on a tree that it subsequently perceives as not suitable, its host selection sequence is interrupted, and it resumes flight and repeats the process.

Host-seeking bark beetles, however, cannot search indefinitely for a suitable plant, as movement outside of their subcortical breeding habitat incurs substantial risks. For example, bark beetles can live only a

few days outside of trees (Coulson, 1979), and exposure to predation increases with the duration of host searching (Schmid, 1969; Amman, 1973; Dahlsten, 1982). Also, flight expends considerable energy (Kinn et al., 1994), which would otherwise be available for gallery formation and oviposition. Moreover, each decision to accept or reject is made against the background of scramble competition among intraspecific and closely related beetles for a limited resource of unknown availability (Anderbrandt et al., 1985; Denno et al., 1995; Reeve et al., 1998).

Bark beetles rely on complex host selection behaviors to locate and colonize trees (Elkinton & Wood, 1981). Initial landing on unattacked trees appears relatively nondiscriminating in terms of ultimate host mortality (Hynum & Berryman, 1980). Landing can be elicited by vertical silhouettes (Strom et al., 1999) and some host compounds (Byers et al., 1988; Borden, 1989; Miller & Borden, 2000), but usually occurs independent of tree susceptibility, as indicated by subsequent colonization or abandonment (Hynum & Berryman, 1980; Raffa & Berryman, 1980; Moeck et al., 1981; but see Gara et al., 1984). In contrast, landing on trees, which other beetles have already entered is usually directed by aggregation pheromones (Borden, 1982; Seybold, 1993).

Environmental factors such as disease (Nebeker et al., 1993; Klepzig et al., 1995), severe drought (Dunn & Lorio, 1993; Paine & Baker, 1993), and defoliation (Wallin & Raffa, 1999; 2001) can weaken tree defenses against bark beetles. Different species of beetles vary in the physiological condition of trees that they prefer (Wood, 1972). Among species that can colonize live trees, there is typically preference for stressed trees, and beetles can reproduce in dead trees also. Colonization of live trees is contingent on rapid response by many conspecifics to pheromones from beetles engaged in the early stages of colonization (Raffa & Berryman, 1983). Reproductive success is further complicated by a general trade-off between host susceptibility and suitability, i.e., those trees, which are weakest and easiest to kill generally have the lowest phloem quality, and are also most available to interspecific competitors (Amman, 1972; Robins & Reid, 1997).

The combined factors of an unpredictable resource, reliance on conspecifics to exhaust host defenses and progressive removal of stressed trees from the population, make host suitability a relative, not absolute condition (Raffa, 2001). Yet the outcome of colonization decisions is discrete. It may be advanta-

geous to resume searching after encountering a tree that is not sufficiently weakened, but conversely, a beetle has no chance of reproducing if it does not eventually enter some tree. The possibility of perceiving and joining a mass attack is likewise unpredictable and relative (Birgersson et al., 1988).

Complex decisions based on trade-offs and uncertainties may be facilitated if beetles can use previous encounters with potential hosts to modify subsequent behavior. Such processes have been shown to improve reproductive success in other insects (Rausher, 1983). A process of trial and error may be even more advantageous if beetles can recover some energetic losses by feeding during each trial (Norris & Baker, 1967).

The pine engraver, *Ips pini* (Say), Coleoptera, Scolytidae, a moderately aggressive transcontinentally distributed species that colonizes almost all species of *Pinus* within its range. It can colonize and kill live trees, but is associated with trees that are under stress or were recently killed (Wood, 1982; Klepzig et al., 1991). This range of behavior appears to be reflected in its response to host phytochemicals, in that particular monoterpenes can either elicit or deter entry, depending on their concentrations. *I. pini* is the most common bark beetle colonizing red pine, *Pinus resinosa* Aiton, within the Great Lakes region (Klepzig et al., 1991). Males select suitable hosts and are subsequently joined by females in their entrance chambers. Male *I. pini* live approximately 3.6 days when held singly without food at room temperature, and approximately 3.9 days when held singly with outer bark of *P. resinosa* (Wallin, 2001).

The objective of this study was to determine if prior encounters influence subsequent choices in post-landing host acceptance behavior by *I. pini*. Secondly, how does such flexibility interact with internal physiological changes that accompany the time required for repeated trials?

Materials and methods

I. pini from a laboratory culture that was initiated from south-central Wisconsin populations in 1995 were used in assays conducted during 1996. We reared beetles by introducing 10 groups of one male and three females each into a 0.3-m by 20-cm section of red pine using the method of Dahlsten and Raffa (1995). Each colonized log was placed in a metal rearing can that had two clear glass emergence jars securely attached to the wall. The can was held at 12L:12D 24°C and 80%

r.h. Progeny development requires three to four weeks under these conditions. Adult progeny were collected daily from the jars at 07:00 h, 14:00 h and 18:00 h, with most beetles emerging between 14:00–18:00 h. The progeny were sorted according to gender and used for either colony maintenance or behavioral assays.

Each assay had a sample size of 30 beetles unless stated otherwise. Emerged male beetles were held separately in 15 × 45-mm glass vials at 18L:6D 24 °C prior to bioassay. The duration and conditions of this holding period varied with the objectives of each experiment. We used a common bioassay; in which defined concentrations of host monoterpenes were applied to a denatured phloem-based medium, which is not repellent by itself (Wallin & Raffa, 2001). Concentrations of these terpenes were selected based on previously determined responses by *I. pini* and known concentrations within local *Pinus* species (Wallin & Raffa, 2000). The predominant terpene in each of these host species is alpha-pinene.

Experiment 1: Do prior encounters with unacceptable hosts modulate subsequent host acceptability?

We quantified the effects of a beetle's previous experience on its subsequent host entrance behavior. This experience consisted of a beetle encountering, and rejecting an opportunity to enter, phloem-based medium amended with a moderate concentration of alpha-pinene. Our objective was to simulate a beetle in the field that lands on a tree, can either enter or reject that tree, and if the latter, lands on another tree and makes another decision. Beetles were assayed repeatedly to determine if the number of times they were given an opportunity to enter this medium influences their subsequent response to the same amended medium. A flow diagram of this bioassay is shown in Figure 1.

The arena for this bioassay consisted of a 9-cm plastic petri dish containing a mixture of agar, phloem, and water. Phloem from *P. resinosa* was freeze-dried, ground, and autoclaved to sterilize and remove volatile monoterpenes, as described by Wallin & Raffa (2000). Bacto-agar® (Difco, Detroit, MI) was mixed with boiling distilled water and ground phloem. A 2.0-mm layer of medium was poured into each petri dish and dried in a fume hood for 24 h. The medium was removed from the bottom of the petri dish and placed in the center of the 11-cm petri dish lid, leaving a 1-cm space around the medium. One hundred and fifty newly emerged male beetles were tested

using 1.4 mg racemic alpha-pinene/g phloem-based medium. At this concentration approximately 50% of male *I. pini* enter the medium (Wallin & Raffa, 2000). Enantiomeric composition of alpha-pinene does not influence post-landing entrance behavior of *I. pini* (Wallin & Raffa, 2000). The alpha-pinene (purity = 95.0%; Aldrich, Milwaukee, WI) was applied to the surface of the medium, which was then covered with a 9-cm disk of plastic transparency film (3 M, Inc., Austin, TX pp2500). Monoterpene concentrations remain stable in this medium for 48 h under these conditions (Wallin & Raffa, 2000).

A single beetle was placed on the transparency film within each arena, and allowed to enter or reject the medium. We considered a beetle as having rejected the medium if it remained on the film or walked around the perimeter of the arena for 15 minutes, and as entering if it began tunneling within this interval. Previous assays demonstrated that 15 minutes is adequate for beetles to make such decisions under these conditions (Wallin & Raffa, 2000). Beetles that rejected the medium were removed from the assay arena and held singly in 15 × 45-mm glass vial without food for 24 h at 18L:6D, 24 °C (Figure 1). Assay units in which beetles entered the medium were placed in an environmental chamber, and the beetles were allowed to construct galleries. Their galleries were traced onto the lid of the dish, using a different colored marker for each day. Gallery lengths were quantified using a map measurer (PECO, Jackson, MS).

Beetles that rejected the medium were held for 24 h, and then exposed to the same concentration of amended medium in a new arena. Beetles that did not enter the second time were held for an additional 24 h without food under the above conditions, and assayed again. As before, assay units containing beetles that entered the medium were placed in an environmental chamber, and gallery lengths were measured.

To control for age, a separate 150 beetles were held without food and assayed at the same intervals (Figure 1). Equal numbers of naïve and repeatedly assayed beetles were used at each time period. Control beetles that did not enter the medium were discarded.

Entrance within each group was analyzed using Fisher's Exact test (SAS, 1996). Entrance among groups was analyzed using Student's-*t* comparisons between test and control beetles at each interval (SAS, 1996).

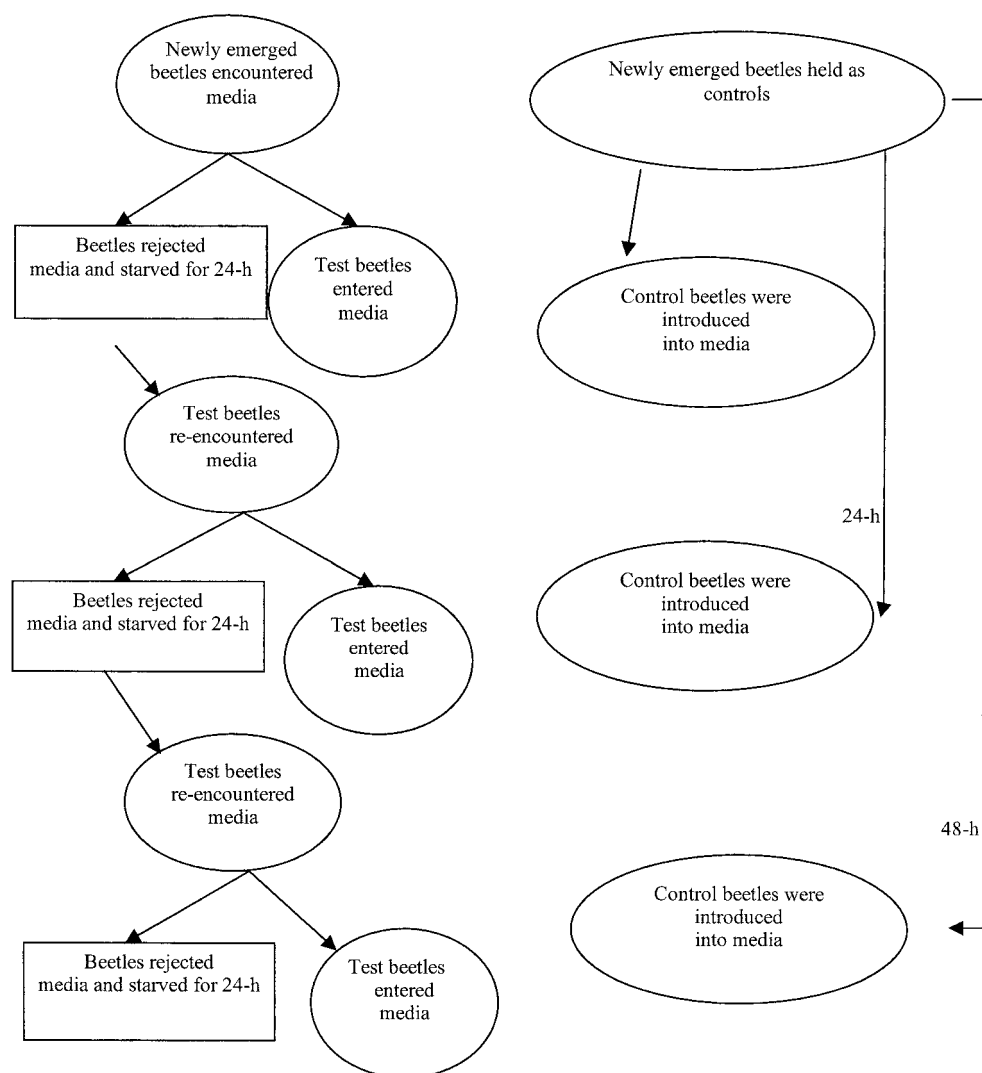


Figure 1. Flow diagram illustrating the sequence of assays used to assess the effects of previous encounters with simulated host tissue on subsequent choices.

Experiment 2: Do other factors that coincide with repeated no-entry decisions modulate subsequent assessments of host suitability?

Experiment 2a: Effects of starvation and age on host entry and subsequent gallery construction. We quantified the effects of starvation over the duration of the above repeated-decision assays (Exp. 1) on host entrance behavior. Beetles were held individually for approximately 24 h, 48 h, or 72 h in 15×45 -mm glass vials without food or water. To control for age without starvation, beetles were held in individual vials containing a 2×2 -cm piece of *P. resinosa* bark for 72 h. Assay arenas were prepared as described above. We

used three monoterpenes at various concentrations, plus controls. Concentrations of racemic alpha-pinene, racemic beta-pinene, and limonene (Aldrich, Milwaukee, WI) were calculated based on the weight of monoterpene per medium (mg/g) as described by Wallin & Raffa (2000). Entry was determined and analyzed as described above. Time until entry was regressed against type of monoterpene, concentration of monoterpenes and starvation treatment (SAS, 1996). Arenas were randomly arranged in stacks of 5, and stacks were randomly assigned to positions in dark environmental chambers at 24 °C. Total gallery lengths after 48 h were analyzed using 3-way ANOVA, with monoterpene type, monoterpene concentration, and

length of starvation and interactions as sources of variation (SAS, 1996). Contrasts were performed using Least Square Differences (SAS, 1996).

Experiment 2b: Effects of starvation and age on gallery construction. Because the same host phytochemicals can affect host entry and subsequent gallery construction behaviors differently (Wallin & Raffa, 2000), we also tested how starvation might modulate the latter behavior. In the first experiment (2bi), we evaluated within-host orientation using a two-way choice test. The second experiment (2bii) consisted of a no-choice gallery construction assay, which incorporates beetle vigor in addition to host selection behavior.

In the 2-way choice assay, the arena was prepared as described above, except an entrance hole was drilled through the side of the arena, and the medium was longitudinally divided in half, from the hole, with a vertical piece of transparency film (Klepzig et al., 1996; Wallin & Raffa, 2000). The monoterpene solution or pentane control was applied randomly to either side of the medium, the vertical transparency film was removed, and the lid was sealed using horizontally positioned transparency film and wax. One male beetle was inserted at the boundary between the amended and control sides, and could move freely between them. We recorded gallery length and beetle location at 24 h and 48 h. We analyzed the number of beetles on either side of the arena and gallery length, using paired-t comparisons (SAS, 1996).

We conducted no-choice tunneling assays using the methods of Klepzig et al. (1996) and Wallin & Raffa (2000). Monoterpenes were applied uniformly to the surface of the medium, which was then sealed with a 9 cm transparency disk and paraffin wax. Beetles that had been held without food were inserted through the hole into the medium, and the hole and petri dish lid were then sealed with parafilm. Each beetle was observed for two days. Total gallery lengths were measured and analyzed as described in Exp. 2a.

Experiment 2c: Changes in lipid content during the duration of behavioral assays. We analyzed 180 beetles for their total lipid content, with 60 beetles each starved for approximately 24, 48, or 72 h (Exp. 2ci). We used the sulfophosphovanillin spectrophotometric method of Kinn et al. (1994). Briefly, the beetles were weighed, oven dried at 50–55 °C for 24 h, re-weighed, extracted in H₂SO₄, and heated in boiling water bath for 10 min. The mixture was cooled

for 10 minutes, vortexed, filtered, amended with color reagent, and analyzed photometrically (530 nm) (Beckman). We generated two standard curves daily using controlled amounts of dry cholesterol (Aldrich, Milwaukee, WI), which yielded r^2 values ranging from 0.98 to 0.99, (Wallin & Raffa, 2000).

Since total lipid content changed with the lengths of time beetles were held without food (see Results), we also conducted a continual-observation assay (2cii). This provided a conservative control for the repeated-trials host-entry experiment (Exp. 1). That is, beetles that rejected medium amended with moderate concentrations of alpha-pinene could conceivably feed briefly on this phloem-based substrate, and thus not undergo the declining lipid content estimated from the above experiment (Exp. 2cii). Assay arenas were prepared as described above. We tested 80 beetles that had been starved for 48 h before entering 1.4 mg alpha-pinene/g phloem based medium. Each beetle was observed for 1 h, and its entry into, or rejection of, the medium was recorded. Half of the beetles that entered the medium were left to construct galleries for 24 h, and half were removed immediately. Total lipids were compared between beetles that entered and constructed galleries and those that entered and were removed from the medium, using a Student's-t comparison (SAS, 1996).

Results

Experiment 1: Do prior encounters with unacceptable hosts modulate subsequent host acceptability?

As anticipated, only 40% of the beetles accepted the simulated host tissue containing moderate levels of alpha-pinene during their first encounter. However, males that rejected the medium at their first encounter were 50% more likely to enter this substrate at their second opportunity (Fisher's Exact Test $P = 0.0021$) (Figure 2a). The percentage of beetles entering this substrate persisted at approximately the entry level of their second opportunity (58%) during their third opportunity.

The effects of prior rejection on subsequent choices were even stronger when the opposing effects of age and starvation that accompany the intervening periods were considered. As in the first assay, approximately 40% of naïve beetles entered the medium at their first opportunity. However, only 9 of the 90 naïve beetles that were held for 48 h, and none of the

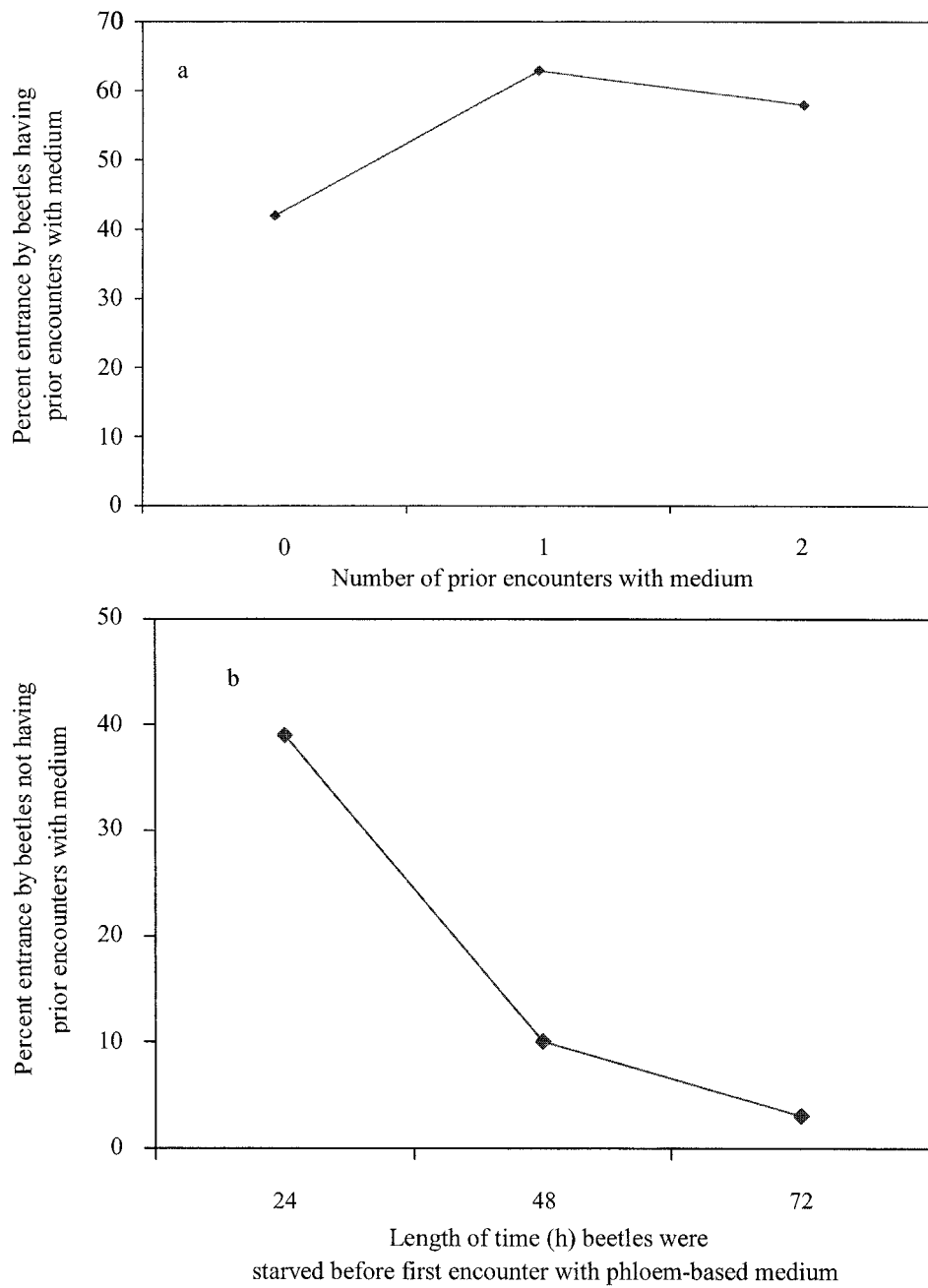


Figure 2. Effects of the number of previous opportunities to enter medium amended with 3.4mg alpha-pinene/g phloem on subsequent choices (a) Beetles given repeated choices; (b) Beetles controlled for age prior to their first choice.

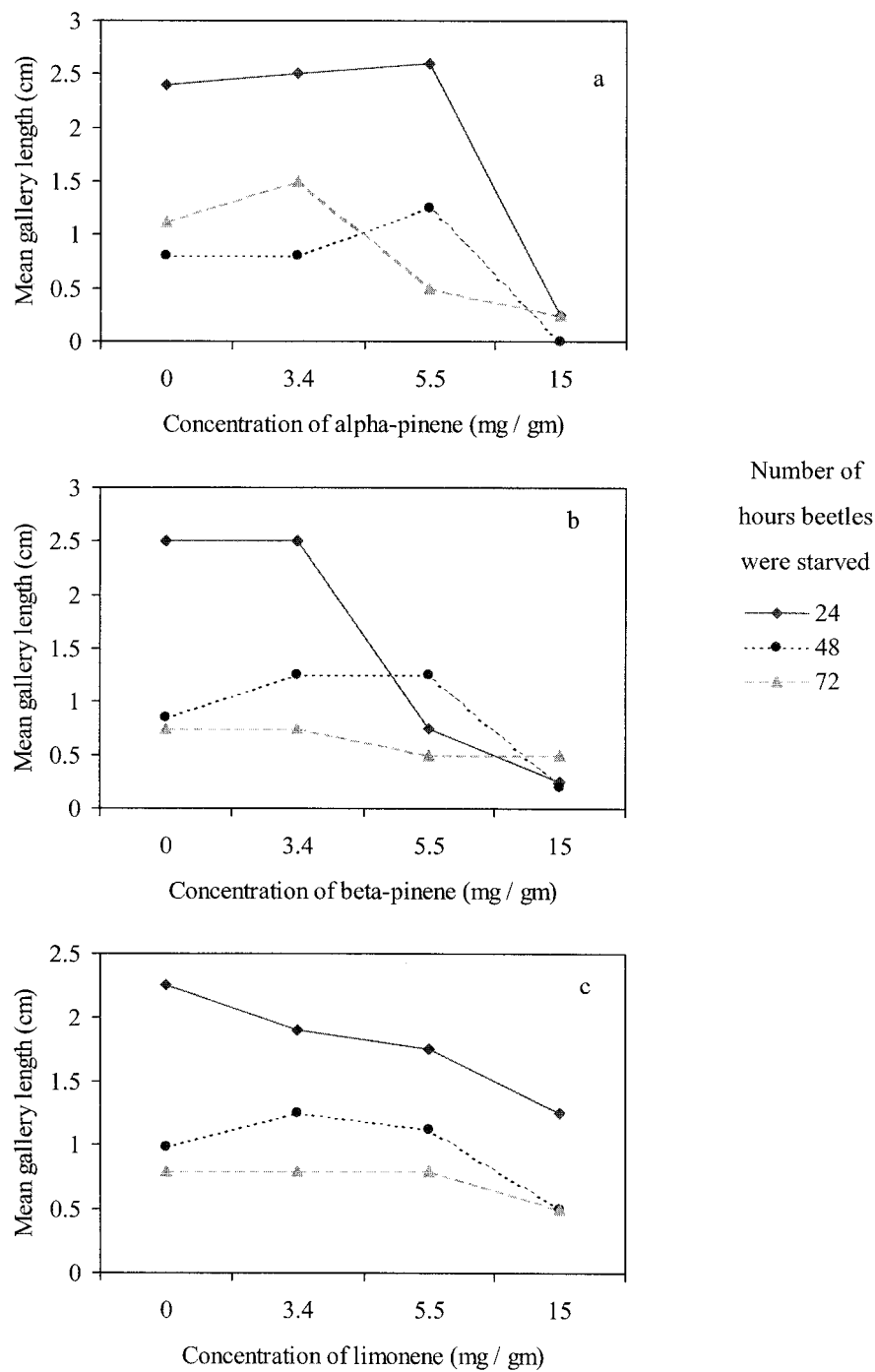


Figure 3. a–c: Gallery lengths (2-day mean \pm s.e.) constructed by *I. pini* starved for varying intervals. Phloem-based media were amended with various concentrations of synthetic monoterpenes (a) alpha-pinene, (b) beta-pinene and (c) limonene.

Table 1. Effects of length of starvation and beetle age on entry into medium with varying concentrations of monoterpenes. Percent of beetles entering within 15 min of first encounter with the medium. Capital letters indicate significant differences ($P < 0.05$) within a column within a monoterpene due to concentration, and lower case letters indicate differences within each concentration at different time periods. Asterisks indicate differences between entrance behavior of beetles held for 72 h with and without bark * $P = 0.05$, ** $P = 0.005$, *** $P = 0.001$

Monoterpene	Concentration (mg/g media)	Beetles held with bark for 72 h	Length of time beetles were starved (h)		
			24	48	72
Alpha-pinene	0	75***	60 Aa	40 Ab	0 Ac
	1.4	50***	50 Ba	10 Bb	0 Ac
	3.4	38**	75 Ca	0 Cb	10 Bc
	30	11	10 Da	0 Cb	10 Ba
Beta-pinene	0	75***	75 Aa	10 Ab	20 Ac
	1.4	39***	55 Ba	40 Bb	60 Ba
	3.4	85***	40 Ca	20 Ab	50 Ca
	30	8**	20 Da	0 Cb	20 Aa
Limonene	0	70*	65 Aa	0 Ab	60 Aa
	1.4	50***	50 Ba	10 Bb	90 Bc
	3.4	24**	40 Ca	100 Cb	50 Aa
	30	0	20 Da	0 Ab	0 Cb

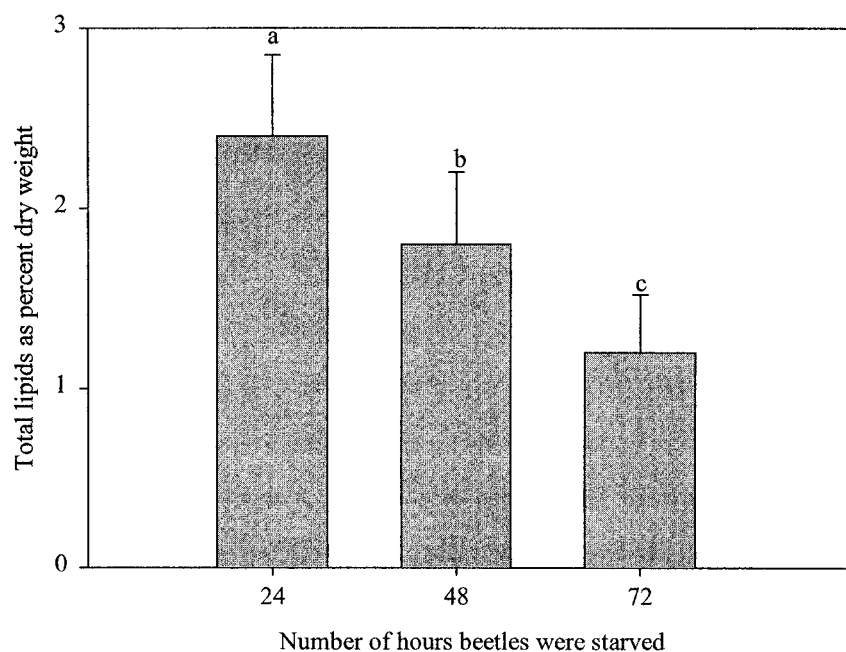


Figure 4. Effect of starvation on total lipid content of male *I. pini*. Mean lipid content and one standard error are given.

36 naïve beetles held for 72 h, entered the medium (Figure 2b). Regardless of the length of starvation, beetles that had previous experience were more likely to enter the medium compared to those without previous experience ($P = 0.007$) (Figures 2a & b). Thus, any reduction in entry that might accompany previous trials due to declining vigor (Figure 2b) was more than offset by accompanying behavioral changes (Figure 2a).

Experiment 2: Do other factors that coincide with repeated no-entry decisions modulate subsequent assessments of host suitability?

Experiment 2a: Effects of starvation and age on host entrance. Beetles were more likely to enter the control (un-amended) substrate if they had access to bark while they aged, than if they were starved an equivalent period (Table 1). The effects of starvation on entry into simulated hosts usually became manifested within 48 h. Entry rate decreased as the length of starvation increased ($y = 13.45 - 1.34x$, $r^2 = 0.76$). Beetles with access to bark for 72 h were more likely to enter media amended with all concentrations of alpha-pinene than beetles starved for 72 h (Table 1). In the case of beta-pinene and limonene, there was some tendency for entry rates to rise from the 48 h level after 72 h of starvation.

In addition to affecting whether a beetle entered, starvation and experience also influenced the interval before entering among those beetles that accepted medium. Overall, more time ensued before beetles entered the medium as the concentration of monoterpene increased ($y = 18.9 + 0.23x$, $r^2 = 0.66$). The type of monoterpene also affected this interval. Increased concentrations of alpha-pinene increased entry interval from 11.07 ± 0.2 to 14.9 ± 0.03 minutes ($y = 0.138 + 5.5$ (alpha-pinene), $r^2 = 0.841$ and beta-pinene yielded linear increases, with this interval ranging from 11.0 ± 0.2 minutes to 14.8 ± 0.75 minutes, $y = 0.134 + 6.35$ (beta-pinene), $r^2 = 0.818$. Increased concentrations of limonene also increased the time before beetle entry, but in a non-linear fashion, $y = 19.3 - 11.9$ (limonene) + 3.4 (limonene)², $r^2 = 0.728$), proceeding from 11.0 ± 0.24 minutes to 9.3 ± 0.4 minutes to 14.0 ± 0.5 minutes.

Once a beetle entered the medium, the length of gallery it constructed was influenced by the period of time it had been held without food ($df = 2,324$, $F = 6.78$, $p = 0.003$) (Figure 3). Gallery lengths constructed in control media decreased as the length of

starvation increased ($df = 2,324$, $F = 2.7$, $p = 0.05$). The galleries which beetles constructed in media amended with alpha-pinene after 24 h starvation were usually longer than those constructed after 48 h or 72 h starvation (Figure 3a). Increased starvation time decreased gallery length at 1.4-mg beta-pinene, but at higher concentrations of beta-pinene all galleries were short (Figure 3b). Gallery lengths in media amended with limonene likewise decreased as the duration of starvation increased (Figure 3c). Overall, limonene usually elicited shorter galleries than other monoterpenes.

Experiment 2b: Effects of starvation and age on gallery construction. In the 2-way choice assay (2bi), male *I. pini* usually preferred ($t = 3$, 72 , $p < 0.001$) (Table 2a) and remained within control ($t = 4.47$, $p < 0.001$) (Table 2b) versus monoterpene-amended media ($t = 3.72$ and 4.47 , respectively, $P < 0.001$) (Table 2a). Overall, gallery lengths were longer in the control than treated side of the assay arena. However, there were no consistent trends in how starvation affected this relationship.

The duration of starvation (2bi) and monoterpene concentration, but not the type of monoterpene (Table 3), affected gallery lengths constructed under no-choice conditions. Gallery lengths constructed in media not amended with monoterpenes became significantly shorter as the duration of starvation increased (Table 3).

Experiment 2c: Changes in lipid content during behavioral assays. Total lipid content declined with the duration of starvation ($n = 60$, $df = 2$, $F = 23.67$, $P = 0.0001$) (Figure 4). However, the mean time prior to beetle entrance was not related to total percent lipid content for either starved ($y = 3.22 + 0.017$ (lipid), $r^2 = 0.002$) or non-starved ($y = 3.1 + 0.03$ (lipid), $r^2 = 0.04$) beetles.

There was no difference in total lipid content between non-starved beetles that entered and constructed galleries versus those that entered and were removed immediately from the media ($n = 20$, $df = 1$, $t = 0.32$, $p > 0.4$).

Discussion

These results demonstrate flexibility in post-landing host acceptance behavior of bark beetles. An individual that rejects a simulated host containing a moderate

Table 2. Effect of starvation on male *I. pini* preference for amended versus control medium under 2-way choice assay conditions. (A) beetle location, and (b) gallery length. P-values for paired-t comparison between the number of beetles on the monoterpene amended versus control sides of the assay arena: *P = 0.05, **P = 0.005, ***P = 0.001

(a) Percentage constructing galleries in monoterpene-amended side of the assay arena.				
Monoterpene	Concentration of monoterpene (mg/g media)	Length of time beetles were starved (h)		
		24	48	72
alpha-pinene	1.4	40*	63*	50
	3.4	30*	40	70*
	30	3***	0***	0***
beta-pinene	1.4	10***	50	20***
	3.4	26**	40	50
	30	33**	10***	10***
limonene	1.4	10***	40	10***
	3.4	30*	43	50
	30	0**	10***	10***

(b) Length of galleries (cm) constructed in control and monoterpene-amended side of arena				
Monoterpene	Concentration of monoterpene (mg/g media)	Length of time beetles were starved (h)		
		24	48	72
alpha-pinene	0	1.5	1	1.75
	1.4	1.4	0.25**	2.2
	3.4	0.45**	0.5**	0.3***
	30	0.2**	0***	0
beta-pinene	0	1.5	1.1	1.5
	1.4	0.85**	0.8	0.7**
	3.4	1.4	0***	1.5
	30	0.6*	0***	0***
limonene	0	1	1.1	1.5
	1.4	1.9**	0.75	1.5
	3.4	0.2**	0.3	1.35
	30	0***	0***	0***

concentration of monoterpenes is more likely to accept and enter similarly amended substrate upon its second or third encounter (Figure 2). The intervening periods between encounters deplete total lipids and reduce vigor, which in the absence of experience decrease host entry. However, these vigor-related changes were more than offset by behavioral changes elicited by prior encounters that elicited host rejection.

Behavioral repellants and inhibitors have been categorized as 'absolute' or 'relative', depending on whether an organism's responses remain fixed or vary with experience, internal physiology and time (Papaj & Rauscher, 1983; Fitt, 1986). Both categories have been demonstrated in interactions between plants and insect herbivores (Wiklund, 1981; Futuyma, 1983;

Thomas & Pellmyr, 1991). The interaction between *I. pini* and conifer monoterpenes appears to be relative, in that prior rejection of moderate concentrations may raise the threshold required for subsequent rejection.

A number of studies have shown herbivores can more optimally exploit an unpredictable resource by employing flexible oviposition decisions (Papaj & Rauscher, 1983; Fitt, 1986). In the case of bark beetles, stressed trees within a particular species range comprise the preferred resource, and entry into healthy trees can be lethal. This pool varies dramatically between years and is linked to the population dynamics of the beetles. For example, suitable host trees are progressively removed as other beetles locate and colonize hosts, a situation that intensifies as populations

Table 3. Effect of duration of starvation on gallery length constructed by male *I. pini* in no-choice assay. Within each monoterpene, different letters indicate significant differences ($P < 0.05$) in gallery length. Capital letters indicate differences due to concentration and lower case letters indicate differences within each concentration at different time periods

Monoterpene	Concentration of monoterpene (mg/g media)	Length of galleries (cm)		
		24	48	72
alpha-pinene	0	4.5 Aa	1.5 Ab	0.75 Ac
	1.4	5.2 Ba	1.2 Ab	3.2 Bc
	3.4	3.4 Ca	1.5 Ab	0.5 Ac
	30	0.2 Da	0.1 Ba	0.1 Ca
beta-pinene	0	4.3 Aa	1.7 Ab	0.4 Ac
	1.4	6.2 Ba	1.7 Ab	0.4 Ac
	3.4	0.5 Ca	1.8 Ab	0.4 Aa
	30	0 Da	0.4 Bb	0.3 Ab
limonene	0	4.5 Aa	1.5 Ab	0.7 Ab
	1.4	4 Aa	3.2 Ba	0 Bb
	3.4	1 Ba	1.6 Ab	1.2 Ca
	30	0.4 Ba	0.5 Ca	0.4 Aa

rise. Conversely, high beetle populations can expand the number of available hosts by collectively overcoming the defenses of relatively non-stressed trees. Therefore, 'sampling' hosts and incorporating prior encounters into acceptance behavior can benefit individual beetles. This process imposes costs, however, such as decreased lipid content and increased risk of mortality (Figure 4) (Hagen & Atkins, 1975; Kinn et al., 1994; Zhang et al., 1995). An extended period before selecting a host also results in reduced gallery length, which is closely correlated with oviposition in *I. pini* and other bark beetles (Coulson, 1979; Sahota et al., 1987). Some energetic losses may be recovered by feeding during trial encounters, however. For example, beetles that land on and assess a potential host may consume outer bark, and thus remain more vigorous than beetles starved for a similar period (Table 1).

Flexibility in host selection behaviors may partially explain the relative lack of discrimination exhibited during landing by bark beetles that colonize live trees (Rudinsky, 1962). Although it has been argued from first principles, and perhaps may seem intuitive, that the ability to discriminate between susceptible and resistant trees while in flight should be an optimal behavior (Pearson, 1931; Heikenen, 1977), direct tests of this hypothesis have demonstrated that landing on trees that were subsequently killed or rejected

are equivalent (Berryman & Ashraf, 1970; Hymun & Berryman, 1980; Raffa & Berryman, 1980; Moeck et al., 1981; but see Gara et al., 1984). The act of landing incurs little cost, feeding during host assessment may partially replenish stores, and secondary attraction mediated by pheromones provides opportunities to aggregate. Furthermore, short-range cues may improve beetles' ability to detect subtle distinctions in host susceptibility, which may be particularly important given the enormous background emission of terpenoids in coniferous forests (Litvak et al., 1999).

Although selection operates at the level of individuals (Alcock, 1982; Raffa & Berryman 1983; Birgersson et al., 1988), flexible responses may yield population-level consequences. It has long been observed that some tree killing bark beetles, such as *Dendroctonus brevicomis* Le Conte and *Dendroctonus ponderosae* Hopkins, are restricted to highly stressed trees when populations are low, but colonize healthy trees when populations are high (Rudinsky, 1962; Safranyik et al., 1975). Although pheromone-mediated aggregations provide a physiological explanation for how a large number of entries can collectively exhaust host defenses (Christiansen & Horntvedt, 1983; Raffa & Berryman, 1983), they do not provide a behavioral explanation for how individuals accept trees they would otherwise reject. For example, Hynum & Berryman (1980) reported that a

substantial proportion of *D. ponderosae* that land on trees during peak aggregation resume flight, apparently after having made independent host-entry decisions. Our results suggest that one component of this process may include behavioral flexibility within individuals. As populations rise and the pool of stressed trees become depleted, some individuals may subsequently enter more vigorous trees. Those that enter relatively vigorous trees may experience success in colonization, due to the arrival of other beetles that would not occur if fewer beetles were available. This would suggest a genetic component to the process, if selection pressures varied with population density (Raffa & Berryman, 1983). Thus, behavioral flexibility could contribute positive feedback to unstable population eruptions.

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