

Density-mediated responses of bark beetles to host allelochemicals: a link between individual behaviour and population dynamics

KIMBERLY F. WALLIN and KENNETH F. RAFFA University of Wisconsin–Madison, Department of Entomology, U.S.A.

Abstract. 1. Bark beetles (Coleoptera: Scolytidae) accept or reject host conifers based partly on concentrations of phloem monoterpenes. They colonise trees in aggregations, in response to pheromones that attract flying beetles to trees undergoing colonisation. A series of entry and gallery construction assays was conducted to determine whether responses by individual beetles to monoterpenes are altered by pheromones and/or the presence of other beetles.

2. Entry into the amended media by *Ips pini* and the length of time until entry were not influenced by the presence of aggregation pheromones.

3. Entry into amended media was influenced by the presence of other beetles on the surface of, or constructing galleries in, the substrate. The effects of alpha-pinene and limonene on host entry behaviour were mediated by the density of beetles on the surface of the assay arena, and by the density of beetles constructing galleries within the medium.

4. The percentage of beetles entering medium amended with higher concentrations of monoterpenes increased with increased density of beetles on the surface of the assay arena, until a threshold density of three or four beetles per assay arena, after which entrance rate declined.

5. The presence of other beetles constructing galleries elicited more rapid entry by the test beetles.

6. Gallery lengths were generally higher in the presence of aggregation pheromones.

7. Gallery lengths increased with increased density of beetles within the assay arena.

8. These results suggest a link between the density of bark beetles and responses of individuals. This linkage may partially explain behavioural changes observed during population eruptions.

Key words. Bark beetle, density, host selection behaviour, *Ips pini*, monoterpene, *Pinus resinosa*, population dynamics.

Introduction

Host selection by bark beetles (Coleoptera: Scolytidae) is a complex process that includes initial location, landing in response to visual and chemical stimuli, and subsequent

acceptance or rejection based largely on host chemical cues. The likelihood of a beetle either entering the host or resuming flight, and the interval between its contacting and entering the substrate, are mediated by the types and concentrations of monoterpenes present in the phloem (Klepzig *et al.*, 1996; Wallin & Raffa, 2000). After entry, beetles orient towards regions of relatively low monoterpene concentrations (Klepzig *et al.*, 1996; Wallin & Raffa, 2000), which enable them to avoid resin glands during gallery construction (Ferrell, 1983). Beetles copulate beneath the

Correspondence: Kimberly F. Wallin, USDA Forest Service, 860N 1200E, Logan, UT 84321, U.S.A. E-mail: kwallin_99@yahoo.com

bark, and females construct ovipositional galleries. The number of eggs deposited is related to gallery length (Sahota *et al.*, 1987). As with entry and orientation, different monoterpene concentrations can either elicit or inhibit gallery construction (Klepzig *et al.*, 1996; Wallin & Raffa, 2000).

Bark beetles colonise trees in aggregations that are mediated by pheromones. High densities of beetles include elements of both competition, in that they reduce the amount of substrate available per beetle, and co-operation, in that simultaneous entry by a large number of beetles can collectively exhaust host defences (Wood, 1982; Raffa & Berryman, 1983; Robins & Reid, 1997; Raffa, 2001).

The population dynamics of some bark beetles are characterised by lengthy non-eruptive periods followed by eruptions to high beetle densities and extensive mortality to host trees (Berryman, 1982; Logan *et al.*, 1998; Logan & Bentz, 1999). During periods of low beetle density, populations are limited to stressed trees, and are apparently unable to exploit the large pool of healthy individuals (Bentz *et al.*, 1993, 1996). During periods of high beetle density, however, relatively healthy trees are killed and mortality to the local host population is often over 90% (Rudinsky, 1962; Amman, 1984; Bentz *et al.*, 1993). The underlying factors that favour shifts from stable to eruptive population behaviour are only partly understood but appear to reflect the variable role of host resistance in limiting population growth (Berryman, 1982).

The ability of trees to expel or kill up to several dozen beetles per square metre, versus the ability of high beetle densities to deplete almost all effects of pre-attack defence capacity on brood development, suggests that communication among beetles may modify host acceptance behaviour; however the potential interaction between host chemicals that mediate individual beetles' decisions, and cues associated with the presence of other beetles, are not understood. It is not known, for example, whether aggregation pheromones that elicit landing behaviour also affect beetle entry behaviour. Although references to *mass attack* might suggest that pheromones cause beetles to enter trees that they would otherwise reject, there is no direct evidence for this assumption. An alternative is that aggregation pheromones attract large numbers of potential colonisers, each of which makes its own decision based on external cues and internal physiology. For example, Hynum and Berryman (1980) reported that the proportion of landing female *Dendroctonus ponderosae* Hopkins that initiates attacks did not increase during aggregation but instead remained at pre-aggregation levels. This suggests that a higher number of entries might simply reflect a higher number of arrivals to the potential host. In reviewing the evolutionary origin and specific behaviours elicited by bark beetle pheromones, Wood (1982) cautioned against assuming undocumented functions. In addition to aggregation pheromones, other cues associated with beetle density could influence host entry or, alternatively, each beetle may make an independent decision once it has landed on the plant surface.

There appears to be an advantage to avoiding healthy trees when beetle populations are low, and conversely to exploiting vigorous trees when population densities are high. First, an individual's likelihood of being joined by enough conspecifics to overwhelm host resistance is probably related to population density. Thus, beetles that enter vigorous trees when populations are low may fail to attract enough conspecifics to exhaust host defences. Conversely, if there are enough beetles in the local population to overcome host defences, healthy trees can provide higher nutritional quality and lower intraspecific competition to developing brood (Amman, 1972; Raffa & Berryman, 1983; Reid & Robb, 1999). Secondly, increasing population densities could exhaust the availability of less vigorous trees after several generations, which could supplement a progression from the stressed tree habitat to the general host population.

The pine engraver *Ips pini* (Say) occurs across the northern United States and southern Canada, and colonises most species of *Pinus*. *Ips pini* show a distinct preference for stressed trees under both field and laboratory conditions (Klepzig *et al.*, 1991, 1996), and avoid substrate with high concentrations of monoterpenes (Wallin & Raffa, 2000). During periods of high population densities that follow a sudden abundance of stressed hosts, *I. pini* can exert substantial mortality to relatively less stressed trees (Geiszler *et al.*, 1984). Males select potential hosts, and in Wisconsin (U.S.A.) produce a pheromone plume consisting of racemic ipsdienol and lanierone (Seybold *et al.*, 1992, 1995; Seybold, 1993).

The objective of the work reported here was to determine whether chemical and physical cues associated with the presence of other beetles influence the host acceptance behaviour of individual male *I. pini*. Specifically, the influences of aggregation pheromones, the number of beetles on the surface of the substrate, and the number of beetles constructing galleries within the substrate were tested on the likelihood of entry into and extent of gallery construction in phloem-based media amended with host monoterpenes.

Methods

Test beetles were from a laboratory culture that was initiated with Wisconsin *I. pini* in 1994 and replenished with wild individuals in the spring and autumn each year (Wallin & Raffa, 2000). For rearing, 10 males were introduced into newly cut 0.3-m long sections of *Pinus resinosa* (Ait), and three females were added to each entry site 72 h later. Logs were placed singly into metal rearing cans. Approximately 3–4 weeks later, adult progeny emerged into clear jars attached to the side of the rearing can. They were collected three times a day and sorted according to gender. Emerging beetles were used either to maintain the colony or for behavioural assays. To minimise the effects of beetle age and starvation, males that emerged within 8-h intervals were used for behavioural assays. The sample size

was 30 males per treatment for each bioassay. Bioassays were conducted in 1998.

Are the effects of host monoterpenes on entrance and gallery construction behaviour by Ips pini altered by the presence of aggregation pheromones?

A modified assay arena of Wallin and Raffa (2000) was used. Each arena consisted of a 9-cm plastic Petri dish containing a mixture of agar, *P. resinosa* phloem, and water. *Pinus resinosa* phloem was freeze-dried in a lyophiliser for 48–72 h, ground through a mill (0.5-mm screen), and autoclaved to sterilise and remove monoterpenes (Wallin & Raffa, 2000). Batch-agar[®] (Disco, Detroit, Michigan) was mixed into boiling distilled water, and ground phloem was added to the agar–water mixture (Klepzig *et al.*, 1996; Wallin & Raffa, 2000). Approximately 2.0 mm of the medium was poured into each Petri dish and dried at 22 °C in a fume hood. After 24 h, the medium was removed and placed in the centre of the 11-cm lid of the Petri dish, which left a 1-cm open space around the circumference.

Racemic alpha-pinene and limonene were used as test monoterpenes (Aldrich, Milwaukee, Wisconsin). Previous studies demonstrated that the chirality of alpha-pinene does not affect *I. pini* entrance behaviour (Wallin & Raffa, 2000). Monoterpenes were administered in units of mg g⁻¹ medium, to simulate the subcortical environment of the host tree (Klepzig *et al.*, 1996; Wallin & Raffa, 2000). Host phloem monoterpene concentrations vary from undetectable or very low levels in dead or severely stressed trees, to several-fold higher in vigorous trees, and can experience an order of magnitude increase during induced reactions against beetle attack (Klepzig *et al.*, 1995; Raffa & Smalley, 1995). Based on these observations, concentrations of 0.0, 0.5, 1.4, 3.4, and 30.0 mg g⁻¹, dissolved in pentane, were applied to the dried surface of the media. These monoterpenes penetrated evenly throughout the medium, as determined by GLC of various medium sections. Monoterpenes remain stable under these conditions for 48 h (Wallin & Raffa, 2000). After the monoterpene treatment or pentane control was applied, a 9-cm circle of transparency film was placed over the amended medium to minimise volatilisation and provide a walking surface for the beetles. The pheromone treatments, consisting of 0.2 mg each of racemic ipsdienol, lanierone, ipsdienol, and lanierone, or distilled water controls, were applied to the surface of filter paper (Whatman, 55 mm, grade 1). Pheromones were obtained from Phero Tech, Inc., Delta, British Columbia. The test beetle was then placed on the centre of the transparency film disc, and the lid was replaced. Beetles could remain on the transparency film, walk around the edge of the medium inside the 1-cm open space, or enter the medium from the side.

Newly emerged beetles were assigned randomly to treatments consisting of the above monoterpene and pheromone combinations. Because *I. pini* are phototrophic, assays were conducted in a room illuminated with red light only. Beetles were observed for 15 min and entry was recorded. Among

beetles that entered the medium, times until entrance and gallery lengths constructed over the next 48 h were quantified. The arenas were arranged randomly in stacks of five, and stacks were assigned randomly to positions in environmental chambers (24 °C, dark, 80% RH). The gallery constructed by each beetle was traced onto the lid of the dish, recorded, and quantified using a map measurer (PECO, Jackson, Mississippi).

Are the effects of host monoterpenes on entrance and gallery construction behaviour altered by the presence of other beetles?

Density of beetles on the surface of the assay arena. The above assay was repeated using varying numbers of male beetles on the transparency film, instead of pheromone treatments. The number of test beetles was one, two, or four, which equates to 0.63, 1.27, and 2.55 males per 100-cm² host surface. Each beetle was scored as entering or rejecting the media within 15 min. Mean time until entrance was determined for each assay arena. Arenas were arranged randomly in environmental chambers as described above. Gallery lengths were recorded and quantified as above.

Density of beetles constructing galleries within the assay arena. The above assay was repeated, except that the number of beetles constructing galleries in the medium, rather than on its surface, was varied. The effects of this parameter on the entrance behaviour of a single test beetle on the surface were evaluated. Five evenly spaced 2-mm holes were drilled around the base of each 9-cm Petri dish. Phloem-based medium was poured into the Petri dish base, dried, and amended with monoterpenes or solvent control as described above. Zero, one, three, or five beetles were inserted gently through the holes into the medium. These densities equate to 0.63, 1.91, and 3.18 males per 100 cm². As an added control, one, three, or five dead beetles were inserted into the holes of separate assay arenas. These beetles were killed by placing them in individual vials and freezing them at 0 °C for 24 h. Each hole was sealed with Parafilm. Assay arenas were kept in darkness at 24 °C as described above. The assay arenas were removed from the environmental chambers after 24 h, and the gallery lengths were measured as described above. The test medium was removed from the 9-cm base and placed in the 11-cm lid of the Petri dish, with care not to displace the live or dead beetles. A separate male was designated as the test beetle and placed on the centre of the transparency film. The test beetles were observed in a room illuminated with red light for 15 min, and time until entry was recorded. After the entrance assay, the arenas were returned to environmental chambers, and gallery lengths were measured as described above.

Individual whole male *I. pini* were extracted in hexane for 24 h at 24 °C. Five µl of either beetle extract or hexane control was applied to the edge of the medium, and its location was marked on the bottom of the Petri dish. Test beetles were placed on the centre of each medium and

entrance behaviour, time until entrance, and gallery lengths constructed were recorded as described above.

Statistical analyses

The number of entered beetles and their gallery lengths were compared among different monoterpene types, monoterpene concentrations, and pheromones using a three-way ANOVA (SAS, 1999). Data were square-root transformed prior to analysis. Values shown are those prior to transformation. Where treatments were significant in the overall model, Fisher's Protected Least Square Difference was used to make multiple comparisons among treatments (SAS, 1999).

In assays testing the effects of other beetles, individuals within an assay arena were not independent replicates, so their responses were averaged. The probability of entry relative to the number of beetles on the surface of the substrate was analysed by ANOVA, using monoterpene type, monoterpene concentration, density, and their interactions as fixed main effects. The time until entry was analysed using the non-parametric Kruskal–Wallis rank sum test, which is more robust for data that cannot be transformed to a normal distribution. The effects of the number of beetles constructing galleries on entry by the test beetle were analysed using the Mann–Whitney rank-sum test (SAS, 1999), where each density was paired with the control of no beetles constructing galleries. This provided a means for evaluating potential differences between each number of beetles present vs. a *pioneer* beetle arriving at an unentered host. Significance was determined at $P < 0.05$. The effects of existing gallery length on entry by the test beetle were fitted as a response to monoterpene type, monoterpene concentration, and their interactions. The assay chamber was treated as the experimental unit, so the total gallery length by each group provided one value. Based on the resulting distribution of data, Spearman's measure of correlation was used to test the relationship of test beetle entry time to the length of existing galleries, because rank is more robust to outliers and can identify both linear and non-linear relationships.

Results

Are the effects of host monoterpenes on entrance and gallery construction behaviour by Ips pini altered by the presence of aggregation pheromones?

The proportion of beetles entering amended media varied with concentrations of alpha-pinene or limonene (Table 1). Entrance did not vary between these monoterpenes, and there was no monoterpene \times concentration interaction. Entry rates into non-amended or amended media were not affected by the presence of ipsdienol, lanierone, or ipsdienol plus lanierone, or all pheromone treatments pooled (Table 1). Pheromones did not interact with monoterpene

Table 1. Effects of monoterpenes, concentration of monoterpenes, and pheromones on entrance behaviour and gallery lengths in media. Sources of variation for proportion entering and gallery lengths in phloem-based media.

Source of variation	d.f.	Proportion entered		Gallery length	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Monoterpene (Mono)	1	0.65	0.57	0.73	0.42
Concentration (Conc)	4	16.38	0.001	14.34	0.001
Pheromone (Pher)	3	1.87	0.132	10.37	0.001
Mono \times Conc	4	0.34	0.79	0.44	0.64
Mono \times Pher	3	1.95	0.16	8.6	0.002
Conc \times Pher	12	1.53	0.14	1.78	0.05
Mono \times Conc \times Pher	12	1.63	0.06	1.4	0.19

type, concentration, or both to affect beetle entrance into phloem-based medium.

Increased concentrations of monoterpenes increased the interval before beetles entered test media, however this effect did not vary between monoterpenes. The duration before entry into non-amended or amended media was not affected by the presence of ipsdienol, lanierone, ipsdienol plus lanierone, or all pheromone treatments pooled.

Increasing concentrations of monoterpenes resulted in decreasing gallery lengths (Table 1). This effect occurred with both alpha-pinene (Fig. 1) and limonene (Fig. 2), and did not vary between these compounds (Table 1). Gallery lengths generally ranged from 1.75 cm in non-amended media to 0.4 cm at 30.0 mg alpha-pinene g⁻¹ media.

Gallery length was influenced by the presence of *I. pini* aggregation pheromones (Table 1). This occurred in both non-amended ($F = 3.4$, d.f. = 3, $P < 0.01$) and monoterpene amended ($F = 2.34$, d.f. = 1, $P < 0.05$) media, but in different directions (Figs 1 and 2). In non-amended media, the presence of pheromones always decreased the gallery lengths constructed by *I. pini*. In media amended with monoterpenes, the presence of pheromones almost always resulted in longer galleries than the absence of pheromones.

Although beetle responses to alpha-pinene and limonene were equivalent, there were strong interactions between monoterpene type and concentration with the presence of pheromones. In media amended with 0.5 mg alpha-pinene g⁻¹, *I. pini* constructed galleries over twice as long in the presence than in the absence of ipsdienol (Fig. 1a), and 20% longer in the presence than in the absence of lanierone. At 3.4 mg alpha-pinene g⁻¹, neither ipsdienol nor lanierone had an effect but their combination increased gallery length four-fold (Fig. 1c). These galleries were substantially longer than those constructed in any other combination of host and beetle semiochemicals (Figs 1 and 2). At 30.0 mg alpha-pinene g⁻¹, galleries were always very short, regardless of whether or not pheromones were present (Fig. 1). The presence of pheromones also elicited more extensive gallery construction in the presence of limonene. At 0.5, 1.4, and 3.4 mg limonene g⁻¹, *I. pini* constructed longer galleries in the presence of ipsdienol, lanierone, and their combination

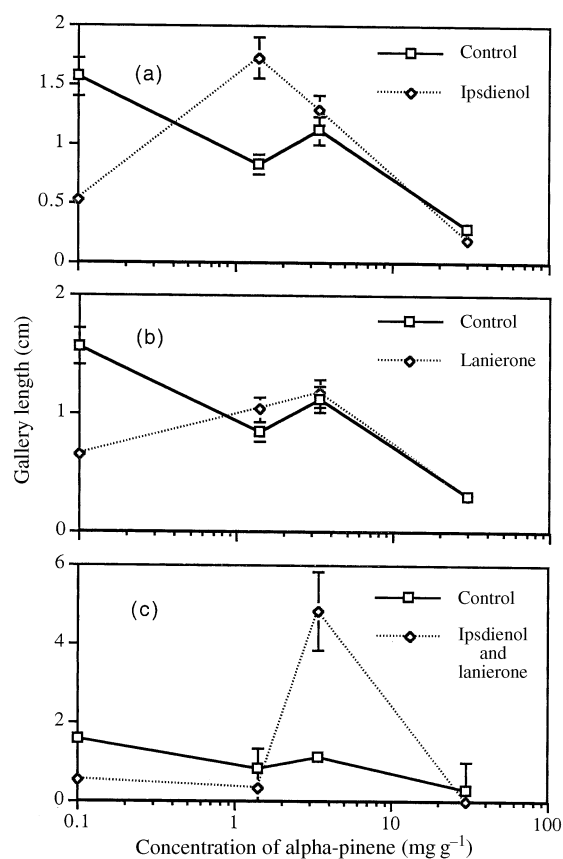


Fig. 1. Effects of synthetic pheromones on lengths of galleries constructed by *Ips pini* in alpha-pinene amended media. (a) Ipsdienol present and control ($F=3.05$, d.f. = 4, $P < 0.05$), (b) lanierone present and control ($F=2.1$, d.f. = 4, $P < 0.05$), (c) ipsdienol and lanierone present and control ($F=4.5$, d.f. = 4, $P < 0.05$). Standard error bars are present but not always visible.

than the controls (Fig. 2). *Ips pini* constructed longer galleries in media amended with the highest concentration of limonene in the presence of both pheromones than with either one alone or in the absence of pheromones.

Are the effects of host monoterpenes on entrance behaviour and gallery construction behaviour altered by the presence of other beetles?

Density of beetles on the surface of the assay arena. The number of beetles on the surface of the assay arena affected the percentage of beetles that entered the phloem-based media (overall model: $F=6.4$, d.f. = 29,870, $P < 0.05$; Table 2). This occurred in both non-amended and amended media (Table 2). In non-amended media, increasing from one to two beetles increased entry by 3.1 times, however increasing the density from two to four beetles decreased beetle entry into non-amended media by 22%.

The presence of other beetles on the surface of the assay arena generally increased entry into media amended with

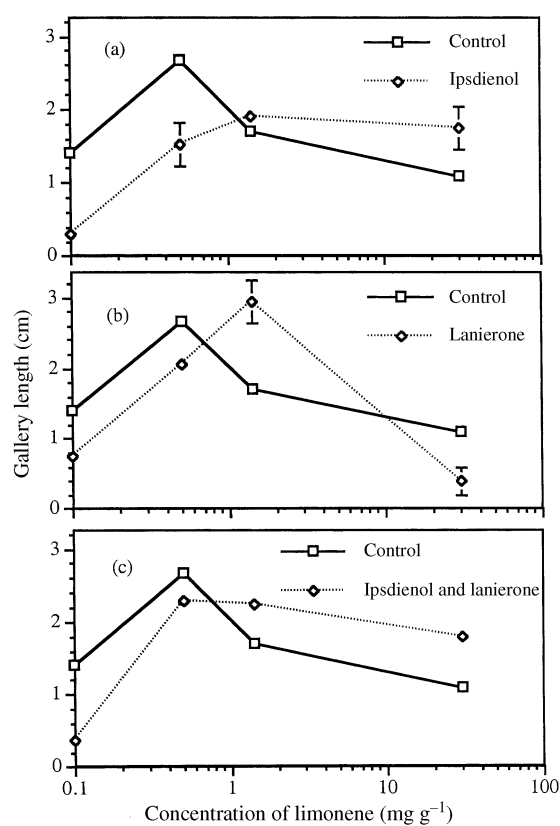


Fig. 2. Effects of synthetic pheromones on lengths of galleries constructed by *Ips pini* in limonene-amended media. (a) Ipsdienol present and control ($F=3.0$, d.f. = 4, $P < 0.05$), (b) lanierone present and control ($F=5.3$, d.f. = 4, $P < 0.05$), (c) ipsdienol and lanierone present and control ($F=6.03$, d.f. = 4, $P < 0.05$). Standard error bars are present but not always visible.

either monoterpene ($F=2.01$, d.f. = 2, $P < 0.05$; Table 2). Overall entry into media amended with all concentrations of alpha-pinene was 36% with one beetle, 46% with two beetles, and 63% with four beetles (Table 2). Increasing from one to two beetles on the surface of the assay arena increased entry rates in media amended with 0.5 mg and 3.4 mg alpha-pinene g⁻¹, and 1.4 mg and 3.4 mg limonene g⁻¹ (Table 2). Entry rates were always low in media amended with the highest concentration of alpha-pinene or limonene. The presence of four beetles elicited significantly higher entrance rates than entrance with one beetle in media amended with 0.5 or 3.4 mg of alpha-pinene and 1.4, 3.4, or 30.0 mg limonene g⁻¹ (Table 2). Increasing from two to four beetles increased entry into media amended with alpha-pinene but did not have a strong influence on entry into media amended with limonene. Beetle density on the surface of the assay arena did not affect the mean time to entry ($r_s = 0.145$, $P = \text{NS}$), which averaged 10.5 ± 0.4 min.

Gallery lengths were influenced by the original density of beetles on the surface of the assay arena (overall model: $F=4.5$, d.f. = 29,870, $P < 0.05$; Fig. 3). This occurred in both non-amended and amended media. Galleries in

Table 2. Effect of varying densities of beetles on the surface of the assay arena on host entrance behaviour of *Ips pini*. Percentages of beetles entering phloem-based media are given. Different letters indicate significant differences of host entrance. Upper case letters indicate significant concentration effect on host entrance (columns). Lower case letters indicate significant density effects on host entrance (rows).

Monoterpene	Concentration	Number of beetles on surface of assay arena		
		1	2	4
Alpha-pinene	0	20 Aa	62 Ab	48 Ac
	0.5	30 Ba	53 Bb	97 Bc
	1.4	50 Ca	48 Ba	56 Aa
	3.4	50 Da	85 Cd	95 Cc
	30	15 Ea	0 Db	3 Dc
Limonene	0	15 Aa	61 Ab	50 Ac
	0.5	60 Ba	57 Ab	55 Bc
	1.4	50 Ca	59 Ab	60 Bb
	3.4	25 Da	95 Bb	97 Cb
	30	5 Ea	14 Cb	9 Dc

non-amended media were longer in arenas with three or four beetles than in arenas with one or two beetles. Increasing from one to two beetles yielded longer galleries across the three intermediate concentrations of alpha-pinene and limonene (Fig. 3). A similar relationship occurred when beetle density was increased from two to three beetles. Increasing from three to four beetles decreased gallery lengths constructed in media amended with 0.5 mg alpha-pinene but increased gallery lengths at 3.4 mg alpha-pinene and 1.4 and 3.4 mg limonene.

Density of beetles constructing galleries within the assay arena. The density of beetles constructing galleries influenced the percentage of test beetles entering non-amended and amended media significantly (overall model: $F=3.0$, d.f. = 38,1160, $P<0.05$; Table 3). The likelihood of a test beetle entering non-amended media was not affected by one other beetle constructing a gallery, reached 100% at two beetles, and declined to about 50% (twice the control entrance) at five beetles constructing galleries (Table 3).

The presence of at least one other beetle constructing a gallery increased entry by test beetles significantly in 92% of the treatments containing alpha-pinene and 56% of the treatments containing limonene (Table 3). At 30.0 mg alpha-pinene, entrance by the test beetle increased in the presence of one other beetle but decreased in the presence of additional beetles. The percentage of test beetles entering the media amended with 30.0 mg limonene increased consistently with the number of beetles constructing galleries.

Overall, the average entry rate into media amended with alpha-pinene was 16% with no beetles, 90% with one beetle, 76% with two beetles, 65% with three beetles, 64% with four beetles, and 32% with five beetles constructing galleries. The average entry rate into media amended with limonene was 58% with no beetles, 55% with one beetle, 59% with two beetles, 49% with three beetles, 56% with four

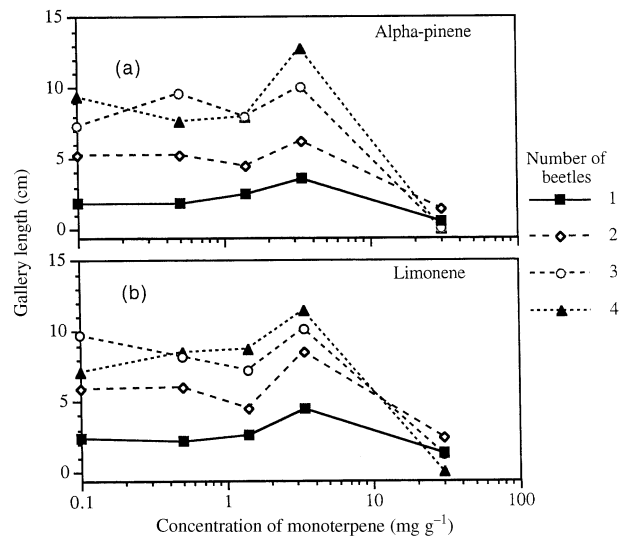


Fig. 3. Effect of various densities of beetles constructing galleries in monoterpene-amended media on mean gallery length per assay arena. Standard error bars are present but not always visible.

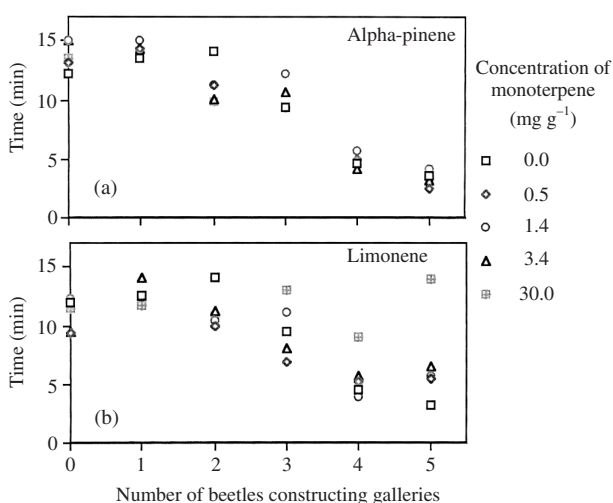
beetles, and 64% with five beetles constructing galleries. Increasing from one to three beetles constructing galleries resulted in 100% entrance by test beetles in half of the alpha-pinene treatments (Table 3), however increasing from three to five beetles decreased entrances at all but the highest concentrations of alpha-pinene. Increasing from one to three beetles resulted in increased entry by test beetles in three of the four limonene treatments. Increasing from three to five beetles resulted in increased entry at three concentrations of limonene and a decrease at one concentration. All of the test beetles entered media amended with 30.0 mg limonene when five beetles were constructing galleries, compared with only 2% in the absence of beetles constructing galleries.

The density of beetles constructing galleries influenced the time until the test beetle entered the phloem-based media (Fig. 4). Differences in mean entry time of test beetles were greater over the gradient of beetle densities ($r_s=0.86$, $P<0.05$) than across all monoterpene concentrations (Fig. 4). Time to entrance decreased with increased beetle density 53% of the time, remained the same 3% of the time, and increased 44% of the time. In two-thirds of the treatments, there was a significant, negative correlation between the time to entrance of the test beetle and total gallery lengths constructed by the beetles.

The addition of dead beetles did not influence entrance rate ($F=1.0$, d.f. = 4, $P=NS$), time until entrance ($F=1.05$, d.f. = 4, $P=NS$), or gallery length ($F=1.0$, d.f. = 4, $P=NS$) of the test beetle across all monoterpene treatments. In addition, the application of whole beetle extracts onto the surface of the media did not influence entrance rates ($F=0.9$, d.f. = 4, $P=NS$), time until entrance ($F=1.8$, d.f. = 4, $P=NS$), or gallery length ($F=1.2$, d.f. = 4, $P=NS$) of test beetles across all monoterpene treatments.

Table 3. Effect of varying densities of beetles constructing galleries on host entrance behaviour of *Ips pini*. Percentages of beetles entering phloem-based media are given. Different letters indicate significant differences of host entrance. Upper case letters indicate significant concentration effect on host entrance (columns). Lower case letters indicate a significant density effects on host entrance (rows).

Monoterpene	Concentration	Number of beetles constructing galleries					
		0	1	2	3	4	5
Alpha-pinene	0	26 Aa	35 Aa	100 Ab	46 Ac	80 Ad	52 Ae
	0.5	41 Ba	100 Bb	62 Bc	63 Bc	67 Bc	38 ABa
	1.4	10 Ca	91 Bb	100 Ac	100 Cc	100 Cc	23 Bd
	3.4	10 Ca	86 BCb	83 Cb	100 Cc	90 ACd	67 Ce
		4 Da	83 Cb	60 Bc	0 Dd	0 Dd	0 Dd
Limonene	0	25 Aa	25 Aa	100 Ab	44 Ac	75 Ad	50 Ac
	0.5	65 Ba	50 Bb	67 Ba	61 Ba	50 Bb	73 Bc
	1.4	37 Aa	36 Ba	100 Ab	68 Bc	50 Bd	41 Aa
	3.4	45 Ba	43 Ba	40 Ba	31 Ab	75 Ac	42 Aa
		2 Ca	16 Ab	29 Cc	38 Ac	50 Bd	100 Ce

**Fig. 4.** Effect of various densities of beetles constructing galleries in monoterpene-amended media on time until entrance by test beetle. (a) alpha-pinene concentration of 0.0: $y = -2.4x^2 + 2.9x + 13.5$, $r^2 = 1.0$; alpha-pinene concentration of 0.5 mg g⁻¹: $y = -0.33x^2 - 0.642x + 15.23$, $r^2 = 0.91$; alpha-pinene concentration of 1.4 mg g⁻¹: $y = -0.6x^2 + 0.838x + 12.9$, $r^2 = 0.9$; alpha-pinene concentration of 3.4 mg g⁻¹: $y = -0.19x^2 - 1.6x + 15.3$, $r^2 = 1.0$; alpha-pinene concentration of 30.0 mg g⁻¹: $y = -0.42x^2 - 0.23x + 13.8$, $r^2 = 0.97$. (b) Limonene concentration of 0.0: $y = -0.63x^2 + 1.1x + 12.55$, $r^2 = 0.98$; limonene concentration of 0.5 mg g⁻¹: $y = -0.23x^2 - 0.51x + 12.73$, $r^2 = 0.92$; limonene concentration of 1.4 mg g⁻¹: $y = -0.19x^2 + 0.54x + 11.9$, $r^2 = 0.9$; limonene concentration of 3.4 mg g⁻¹: $y = -0.2x^2 - 0.8x + 11.2$, $r^2 = 0.91$; limonene concentration of 30.0 mg g⁻¹: $y = -0.31x^2 - 0.31x + 11.8$, $r^2 = 0.96$.

Discussion

These results indicate a linkage between host selection behaviour by individual bark beetles and beetle density on the substrate surface. The density-related cues that affect a beetle's response to host allelochemicals vary with the stage of the host selection sequence.

Contrary to the prediction, the effects of host allelochemicals on beetle entrance behaviour were not modified by the presence of aggregation pheromones. This contrasts with the well-documented locomotory function of aggregation pheromones, which have been shown to elicit beetle movement towards a point source under similar laboratory conditions (Borden, 1982; Camacho *et al.*, 1994). Thus, these results do not support the assumption that aggregation pheromones cause a beetle to enter a tree that it would otherwise reject. The presence of pheromones likewise did not affect the interval before entry of those beetles that entered the substrate.

In contrast to initial entry, aggregation pheromones have strong effects on gallery construction. Increased gallery lengths in the presence of pheromones may reflect competition among individuals for breeding substrate (Sahota & Peet, 1988), i.e. longer galleries may increase space and substrate for developing larvae, giving them a competitive advantage (Haack *et al.*, 1987; Sahota *et al.*, 1987).

In contrast to aggregation pheromones, the density of beetles either on the surface of the assay arena (Table 2) or constructing galleries in the medium (Table 3) influenced beetle entrance behaviour strongly. Both the likelihood and speed of entry are influenced by the presence of other live beetles. This relationship was non-linear, which suggests an upper limit to the number of beetles on the bark surface that elicits entry into trees of a specific quality. For example, low concentrations of monoterpenes may signal a host of low defensive capacity, and elicitation of more beetles to overcome defences is terminated sooner in such hosts. Because hosts with low defences often have low nutritional quality and moisture, behaviour that limits competition within a nutrient-poor resource may be favoured. By contrast, media amended with concentrations of monoterpenes that are similar to those occurring in more resistant hosts (Raffa & Smalley, 1995; Klepzig *et al.*, 1996) continued to elicit increased beetle entry when more beetles were present in the current study.

Increased entry in response to the presence of other beetles constructing galleries also (Table 3) supports the idea of a threshold effect on beetle entrance. The density of beetles constructing galleries at which the test beetles exhibited maximum entrance varied with the concentration of each monoterpene. The particular beetle densities that influenced the test beetles in these assays may relate to natural conditions. For example, Robins and Reid (1997) reported that *I. pini* colonisation densities ranged from 4.5 to 9.0 females per 100 cm², which occurred between the three- and four-beetle treatment.

The mechanisms by which beetles on the bark surface detect the presence of other beetles are unknown. The most likely cues are short-distance chemical signals other than aggregation pheromones, and vibration associated with gallery construction. The absence of effects elicited by either dead beetles or extracts of beetles suggests that chemical cues are probably not solely responsible.

The interactions among monoterpene type, monoterpene concentration, and beetle density in affecting the time before entering the host may reflect elements of scramble competition (Reeve *et al.*, 1998). If a beetle correctly perceives a tree as suitable, due to either low defences or the presence of enough beetles to overcome its defences, it may be advantageous to enter rapidly. At least three ecological factors may favour such behaviour. First, entering a host quickly decreases the efficacy of the tree's mobilisation of induced defences (Raffa & Smalley, 1995). Second, entering a host quickly may confer an advantage against competing conspecifics in securing adequate space and resources for their progeny. Third, beetles entering hosts quickly may minimise the potential risk posed by predators (Raffa, 2001), which are attracted to beetle pheromones.

These results support the view that the overall process of host selection by bark beetles involves a sequence of behavioural events that are influenced by multiple factors. Specifically, beetle density on or in the plant can interact with host tree monoterpenes to influence the entry behaviour of subsequent beetles. Bark beetles can reach relatively high numbers on tree surfaces prior to any host entry, due to *switching* from neighbouring trees undergoing the late stages of mass attack (Gara & Coster, 1968; Geiszler *et al.*, 1980). Likewise, beetles may arrive at trees in response to aggregation pheromones of early arrivers, at a time when successful exhaustion of host defences is not assured (Raffa & Berryman, 1983). The observation that aggregation may increase the likelihood of a beetle accepting a broader physiological range of potential hosts than it would accept by itself, suggests one linkage between individual herbivore behaviour and population dynamics that can generate positive feedback and thereby contribute to eruptive patterns.

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