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The response of *Ips plastographus maritimus* to volatiles in the laboratory

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THE RESPONSE OF *Ips plastographus maritimus*
TO VOLATILES IN THE LABORATORY

A Thesis
Presented to
The Faculty of the Department of Biological Sciences
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Carolyn Evalena Warren
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ABSTRACT

THE RESPONSE OF *Ips plastographus maritimus* TO VOLATILES IN THE LABORATORY

by Carolyn E. Warren

Attraction to bark/phloem/xylem disks of *Pinus radiata* D. Don was demonstrated, with *I. p. maritimus* Lanier (Coleoptera: Scolytidae) females exhibiting a higher attraction than males. Attraction of males and females to male infested disks and to captured male volatiles increased with increasing dosage. Attraction of males and females to female infested disks and to captured female volatiles was demonstrated. Increasing numbers of females in male galleries reduced the attractiveness of infested disks to either sex. Increasing numbers of females, tunneling separately from males in the same disk, reduced attraction of males. Increasing concentrations of male and female volatiles released in a one-to-one ratio decreased attraction for both sexes combined. Attraction of both sexes to volatiles derived from males and females tunneling together, increased with increasing dose. Hence, both sexes produce an aggregation pheromone, and interaction between the sexes often reduces male and female attraction.

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TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
Introduction	1
Methods	3
Collection of Beetles and Host Material	3
Preparation of Behavioral Stimuli	3
a. Bark Disks.	3
b. Captured Volatiles	4
Behavioral Assay	5
a. Bark Disks.	5
b. Captured Volatiles	6
Statistical Analyses	7
Results	10
a. Infested and Uninfested Host Disks	10
b. Captured-Volatiles.	11
Discussion.....	17
Literature Cited.....	21

LIST OF FIGURES AND TABLES

Figure 1. Olfactometer.....	9
Table 1: Proportions of <i>I. p. maritimus</i> attracted to beetle-infested <i>P. radiata</i> disks.....	14
Table 2: Proportions of <i>I. p. maritimus</i> attracted to individual captured-volatiles.....	15
Table 3: Proportions of <i>I. p. maritimus</i> attracted to combinations of captured-volatiles.....	16

INTRODUCTION

Most bark beetles (Coleoptera: Scolytidae) colonizing host trees produce pheromones to attract conspecifics of both sexes, resulting in mass attack of the host (Borden, 1985; Wood, 1982). There are exceptions to this pattern of attack; for example, *Dendroctonus micans* Kug. adults are not known to produce aggregation pheromones, and do not mass attack host trees (Gregoire, 1988). *Tomicus piniperda* (L.) adults also are not known to produce aggregation pheromones, but they mass attack trees (Byers *et al.*, 1985). *Ips* spp. males initiate galleries on the host by tunneling through the bark and feeding in fresh phloem (Anderson, 1948). In all species studied males produce a pheromone that mediates the resultant aggregation (Wood, 1982; Borden, 1985; Seybold, 1992; Raffa *et al.*, 1993). For example, the aggregation pheromone released by male *Ips paraconfusus* Lanier was identified as a mixture of three monoterpene alcohols, i.e., ipsenol, ipsdienol, and *cis*-verbenol (Wood *et al.*, 1966; Silverstein *et al.*, 1966), none of which elicited a response when delivered individually (Wood *et al.*, 1967, 1968). These studies demonstrated a multi-component attractant pheromone and synergistic effects among pheromone components (Silverstein, 1977). In these studies, an olfactometer was used to assay the upwind walking behavior of *I. paraconfusus* (Wood and Bushing, 1963). More recently this upwind response was shown to be stimulated both by the presence of multiple pheromone components (chemotaxis) and by the airstream (anemotaxis) (Akers, 1989; Akers and Wood, 1989; Akers *et al.*, 1993).

There are twenty-seven species of *Ips* recognized in North America (Wood, S. L., 1982; Lanier, 1987; Lanier *et al.*, 1991), yet only a few species have been subjects of studies of behavioral chemistry. *Ips plastographus* has been described based on two subspecies of geographic origin: coastal [*maritimus* Lanier] and sub-alpine [*plastographus* (LeConte)] (Wood, S. L., 1982; Lanier, 1970). Lanier (1970) demonstrated reduced fertility of backcross and F₂ generations between the coastal and sub-alpine populations, indicating a genetic difference in the populations. Both subspecies of *I. plastographus* have yielded ~93%-(+)-ipsdienol from a mixture of captured volatiles produced from male infested logs (Seybold, 1992). The attractiveness of this compound to conspecifics has not been demonstrated. *Ips plastographus* *maritimus* frequently colonizes *Pinus radiata* D. Don (Monterey pine), *P. muricata* D. Don (bishop pine), and *P. contorta* var. *contorta* Dougl. (shore pine) (Bright and Stark, 1973, Wood, S. L., 1982). *Ips plastographus* *plastographus* infests *P. contorta* var. *murrayana* Grev. and Balf. (Sierra lodgepole pine), and *P. contorta* var. *latifolia* Engelm. (Rocky Mountain lodgepole pine) (Wood, S. L., 1982).

The upwind walking response of *I. p. maritimus* was measured against various sources of volatiles in an open arena olfactometer. Volatiles originated either directly from uninfested host tissue, or host tissue infested with male or female *I. p. maritimus*, or from volatiles trapped from air passing over infested or uninfested host tissue.

METHODS

Collection of Beetles and Host Material

Branch and stem sections of recently fallen *P. radiata* that had been colonized by *I. p. maritimus* were collected in 1994 from Santa Cruz and San Mateo Counties in California, and placed in rearing cages (Browne, 1972). Following emergence, males and females were separated by the presence of the *pars stridens*, a stridulatory structure on the vertex of the female's head (Lanier and Cameron, 1969), and stored at 4-5^o C in Petri dishes containing moist filter paper. Sources of uninfested *P. radiata* logs for production of volatiles and for disk assays were collected in 1994 and 1995 from Santa Cruz and Contra Costa Counties, California.

Preparation of Behavioral Stimuli

a. Beetle Infested Host Disks

Bark-phloem-xylem disks (hereafter termed 'disks') were prepared by cutting a fresh *P. radiata* log through the bark and phloem and into the xylem with a five centimeter cylindrical drill bit. Disks were excised with a wood chisel and placed in clean Petri dishes. Disks were prepared either immediately before the beetles were introduced, or prepared and refrigerated not more than 24 hours before beetles were introduced. Males or females were introduced into the disks in drilled holes 3 mm in diameter and 5 mm into the disk. Each drilled hole extended through the bark and phloem and onto the surface of the xylem of the disk. Beetles were confined under wire mesh, which was secured on the bark surface by staples or under a gelatin capsule that was

secured by a #2 insect pin. Beetles were allowed to feed in disks for approximately 24 hours before the disks were assayed. Six disk treatments were prepared in which only one sex of *I. p. maritimus* was used. These treatments were: no insects; 3 or 6 males, and 3, 6, or 9 females.

In addition, disks were prepared using both sexes in different sex-ratios. In these cases male galleries were initiated 24 hours before introduction of females into host disks. Females were either added to male galleries or added to their own galleries in freshly drilled holes adjacent to male galleries. If females were to be added to male galleries, males were secured in their galleries by a pinned gelatin capsule, and females were added after removing the capsule. If females were introduced in different galleries from the males, fresh holes were drilled for females immediately before their introduction. In both instances the females were secured in the disks with fine wire mesh. Five disk treatments were prepared in which both sexes were present. All of these contained 6 male beetles. One disk had no females added to it, two had females added to the male galleries in ratios of one female per male and two females per male, and two had females introduced into separate galleries in ratios of one female per male and two females per male.

b. Chemical Extracts of Volatiles Trapped from Beetle Infested Host

Pinus radiata logs, approximately 30 cm in length, and with a phloem thickness of at least 2 mm, were artificially infested with male or female beetles. Phloem with a thickness of at least 2 mm was used to allow enough feeding material for the duration of the collection of volatiles. Three logs of each treatment were prepared and placed in a 19-liter glass carboy. There were

4 artificially colonized log treatments: three logs with no insects, three logs infested with a total of 140 males, three logs infested with a total of 180 females, and three logs infested with a total of 168 males and 168 females. Logs with either males or females tunneling alone were prepared 24 hours before placing them into a carboy, while logs containing male and female beetles tunneling together were placed in the carboy 24 hours after the introduction of the females, i.e., 48 hours after introduction of the males.

To collect host and insect produced volatiles, beetle colonized logs were placed into nineteen-liter glass carboys for approximately 168 hours. Air was drawn through activated charcoal into these chambers and filtered through Porapak Q[®] (50/80 mesh, Supelco Inc., Bellefonte, Pennsylvania) to capture compounds volatilizing from the insect colonized and uncolonized host tissue (Seybold, 1992). The Porapak was extracted with 350 ml of pentane and the volatiles were concentrated by rotary evaporation to produce a 10 ml stock solution of crude volatiles. The stock solution was stored at -35°C.

Behavioral Assay

a. Bark Disks

An open arena olfactometer (Figure 1) was used to assay the attraction of walking *I. p. maritimus* to various olfactory stimuli. The experimental method used was adapted from Wood and Bushing (1963) as modified by Seybold (1992). Airflow into the arena at 0.76 +/- 0.2 meters per second from a hair dryer was maintained with a rheostat. A receptacle just downwind of the air stream was designated as the target and contained an infested or uninfested disk of *P. radiata* or a chemical extract of volatiles. The olfactometer chamber

was prepared before each assay by cleaning the arena with 70% ethanol and covering the arena with disposable newsprint sheets. The ethanol-treated arena was allowed to evaporate under maximum air speed for a minimum of twenty minutes before use. Disposable sheets allowed the test beetles to maintain a secure footing. A pencil-mark was placed on the paper 2 cm immediately preceding the target. Test beetles were grouped into batches of 30 individuals. Each individual was placed facing the target 30 cm downwind of the target. A beetle passing the 2 cm mark within the 2 min. time limit was considered to have responded to the source. Whenever possible, 30 individual males and females were exposed to all treatments, except where the supply of beetles was limited. All beetles were examined to ensure they were alive and with legs and antennae.

b. Captured Volatiles

To assay each beetle against a dose of captured volatiles, extracts were loaded into 5 ml micropipettes, placed on a foil-covered holder, which was placed under the airstream in the olfactometer. Newly loaded micropipettes were used for each beetle. The diluted volatiles were stored at 4°C. The stock solutions of extracts of volatiles were designated as having a concentration of 10^0 , and dilutions were prepared from the extracts to produce concentrations of 10^{-1} to 10^{-6} . During the assays volatiles were placed on dry ice to reduce evaporation. Captured beetle volatiles were used alone and in combination to test for attractiveness to male and female *I. p. maritimus*. Thirty male and 30 female beetles were tested in each of the experiments using volatiles, except where beetles or dilutions became limited.

Increasing concentrations of captured male, female, and male and female volatiles were tested to determine their attractiveness to individual male and female *I. p. maritimus*. Similarly, males and females were exposed to captured volatiles from each sex released simultaneously at increasing concentrations to simulate males and females tunneling separately in a ratio of 1:1. Additional assays were carried out to determine the attraction of male and female *I. p. maritimus* to a known attractive dose of male captured volatiles (10^{-2}), and adding increasing doses of female captured volatiles, or increasing dosages of captured volatiles from males and females tunneling together.

Statistical Analyses:

Hierarchical log-linear analyses (Zar, 1984) were conducted to determine the relationship among three variables: Response, Dose and Sex. Response was determined from the number of beetles reaching the target within the 2 min. time limit. Dose was the number of beetles in the host disk or the concentration of the volatiles. Sex was the gender of the responding beetle. Analyses were carried out using SPSS (SPSS Inc., Chicago, Illinois), which produces a best fit final model for each data set.

Where the factor of Dose was a component of the best fit model, orthogonal pairwise comparisons were carried out between doses by calculating the G-statistic for each comparison (Sokal and Rohlf, 1981). If Sex was not dependent on Dose, and the two-way interaction between Dose and Response was significant, the sexes were grouped for pairwise comparisons. If the final model included a three-way interaction, pairwise comparisons were

computed by Sex for Response versus Dose. Comparisons were made between least and most dilute doses. In each case, the comparisons were designed to contrast response to higher doses with response to lower doses.

For the data from the volatiles derived from beetle infested logs, Spearman rank correlation's were calculated to compare the ranking of the Dose, and the ranking of the Response, to determine if there was a significant dose-response relationship present. The significance of the R_s values calculated were assessed using tables of R_s with a low N (Siegel, 1956). If the correlation coefficients were significant, pairwise comparisons were not calculated. When the factor of Dose was a component of the best fit model, orthogonal pairwise comparisons were carried out as described previously.

Figure 1. Olfactometer (Wood and Bushing, 1963). Arena, air source and direction, target, and release point are indicated (adapted from Seybold, 1992).

RESULTS

a. Infested and Uninfested Host Disks

The attraction of male and female *I. p. maritimus* was significantly greater when a host disk was present, than if it was not (Dose* Response, $p<0.0001$). Of those beetles exposed to uninfested host, 40.8% ($n=250$) approached the target, while 25.0% ($n=220$) of the beetles approached the target when the host was not present. Females were more likely to approach the receptacle than males (38.0%, $n=250$ vs. 28.2%, $n=220$) (Sex * Response, $p=0.024$).

The attraction of male and female *I. p. maritimus* to hosts disks infested with males was dependent on the number of males tunneling in the host (Dose * Response, $p<0.001$) and increased with increasing numbers of males in the host (Table 1a). Response to the host was greater when the host contained 3 or 6 males than it was to host containing no beetles (66.7% vs. 30.0%, $G=21.83$, 1 d.f., $p<0.01$). More beetles were attracted to host containing 6 males than host containing 3 males (78.3% vs. 55.0%, $G=7.36$, 1 d.f., $p<0.05$). Response did not depend on the sex of the responding beetle.

Male and female *I. p. maritimus* were attracted to host disks containing females (Dose * Response, $p=0.003$) (Table 1a). Attraction was greater when the host contained 3, 6 or 9 female beetles than to host containing no beetles (55.0% vs. 30.0%, $G=11.42$, 1 d.f., $p<0.01$). There was no significant difference in attraction between host containing 3, 6, or 9 females. Response did not depend on the sex of the responding beetle.

Increasing numbers of females tunneling in male galleries decreased attraction of males and females (Table 1b) when compared to males tunneling

alone. Response was dependent on Dose (Dose * Response, $p < 0.004$), and Response decreased when beetles were exposed to host disks containing 1 or 2 females compared to males tunneling alone (47.5% vs. 70.0%, $G = 8.29$, 1 d.f., $p < 0.01$). There was no significant difference in attraction when beetles were exposed to either 1 or 2 females in a male gallery. Overall response of females was higher than males (63.3% vs. 46.7%) (Response * Sex, $p = 0.024$).

When the number of females tunneling separately from males increased, male response decreased significantly while female response did not (Table 1b). Response was dependent on both Dose and Sex (Dose * Response * Sex, $p < 0.001$). Male response was lower, when 2 females were tunneling side-by-side with males than when 1 female was tunneling side-by-side with a male (13.3% vs. 63.3%, $G = 16.45$, 1 d.f., $p < 0.01$), while female response remained unaffected.

b. Captured Volatiles

Exposure to volatiles captured from uninfested logs of *P. radatia*, did not significantly affect beetle attraction when compared to pentane alone. None of the interactions involving volatiles were significant.

The response of male and female *I. p. maritimus* to volatiles of *P. radiata* logs infested with males, increased as the dose of volatiles increased (Table 2). Response was dependent on Dose (Dose * Response, $p < 0.001$), and the ranking of Response was positively correlated with the ranking of the Dose ($R_s = 1.00$, $N = 6$, $p < 0.01$). Overall female attraction was greater than males (48.9% vs. 37.2%; Response * Sex, $p = 0.025$).

The attraction of male and female *I. p. maritimus* to volatiles from logs infested with females was dependent on the dose of the volatiles (Table 2)

(Dose * Response, $p < 0.001$). Although the ranks of Dose and Response did not correlate, orthogonal comparisons indicated that the greatest attraction occurs before the highest dose. Overall response was higher to volatiles captured from female beetles tunneling in a host than to pentane alone (47.1% vs. 25.0%, $G = 9.69$, 1 d.f., $p < 0.05$). Attraction to the two highest doses of female volatiles (10^{-2} , 10^0) was lower than to the two lowest doses (10^{-4} , 10^{-3}) (35.8% vs. 62.5%, $G = 14.52$, 1 d.f., $p < 0.01$). There was no significant difference in attraction between the two lowest doses of volatiles. The highest dose (10^0) was less attractive than the next highest dose (10^{-2}) (15.0% vs. 56.7%, $G = 24.43$, 1 d.f., $p < 0.01$). Overall, females were more likely to respond than males (48.1% vs. 36.1%; Response * Sex, $p = 0.045$).

Male and female *I. p. maritimus* were attracted to volatiles from logs in which males and females were tunneling in the same gallery, and attraction increased with increasing dose (Table 2). Response was dependent on Dose (Dose * Response, $p < 0.001$) and there was a positive correlation between the ranks of Dose and Response ($R_s = 1.00$, $N = 5$, $p < 0.05$). Response did not depend on the sex of the responding beetle.

Response of males and females, to male-derived volatiles and female-derived volatiles released simultaneously, decreased with increasing concentration of the volatiles (Table 3). Response was dependent on Dose (Dose * Response, $p < 0.001$). There was no significant correlation between the ranks of Dose and Response. Attraction was higher to all combinations of beetle-derived volatiles than to pentane alone (60.1% vs. 33.3%, $G = 14.37$, 1 d.f., $p < 0.01$). The three lowest doses were more attractive than the two highest doses of volatiles (65.7% vs. 52.1%, $G = 5.37$, 1 d.f., $p < 0.05$), and the highest of the

three lower concentrations (10^{-2}) was less attractive than the other two lowest doses (53.3% vs. 72.3%, $G=6.05$, 1 d.f., $p<0.05$). There were no differences in response between the two highest or the two lowest concentrations. Sex of the responding beetle was not a factor affecting response.

When a constant attractive dose of male volatiles was released with increasing doses of female volatiles, Response was dependent on the Dose of female volatiles (Dose * Response, $p=0.025$) (Table 3). There was no significant correlation between the ranks of Dose and Response. There was no difference in response when female volatiles were present at any level compared to the male volatiles alone. Attraction was lower at the lowest dose (10^{-4}), than at the next lowest dose (10^{-3}) (55.0% vs. 78.3%, $G=7.36$, 1 d.f., $p<0.05$). No other Doses were significantly different. Regardless of Dose, females were more likely to walk towards the target than males (75.0% vs. 59.4%; Response * Sex, $p=0.002$).

When a constant attractive dose of male volatiles was released with increasing concentrations of volatiles derived from males and females, attraction of *I. p. maritimus* decreased (Table 3). Response was dependent on Dose (Dose * Response, $p=0.037$), and the ranks of Response and Dose were negatively correlated ($R_s = -0.90$, $N=5$, $p<0.05$). The overall level of attraction of females was higher than for males (64.7% vs. 50.0%; Response * Sex, $p=0.012$).

Table 1: Proportions of male and female *Ips plastographus* *maritimus* attracted to *Pinus radiata* disks containing male and/or female beetles.a/

a) <u>Single Sex-disks</u> – Tunneling in a disk						
Interactions from log linear model (p<0.05)		Sex of responding beetle	#beetles / disk			
			0	3	6	9
Males	Dose * Response	Male	0.30	0.70	0.77	-
		Female	0.30	0.40	0.80	-
Females	Dose * Response	Male	0.23	0.47	0.63	0.57
		Female	0.37	0.47	0.57	0.60
b) <u>Multiple Sex-disks</u>						
Gallery type	Interactions from log linear model (p<0.05)	Sex of responding beetle	# of females per male			
			0	1	2	
Same	Dose * Response Response * Sex	Male	0.57	0.53	0.30	
		Female	0.83	0.57	0.50	
Different	Dose * Response * Sex	Male	0.58	0.63	0.13	
		Female	0.83	0.60	0.80	

a/ In all cases, $n = 30$.

Table 2: Proportions of male and female *Ips plastographus maritimus* attracted to individual captured-volatiles from *Pinus radiata* logs containing either male or female beetles, or male and female beetles in the same tunnel.^{a/}

Sex of beetles in log from which volatile was captured	Interactions from log linear model (p<0.05)	Sex of responding beetle	Dosage of volatile						
			0	10 ⁻⁶	10 ⁻⁴	10 ⁻³	10 ⁻²	10 ⁻¹	10 ⁰
Male	Dose * Response Response * Sex	Male	0.27	-	0.23	0.23	0.53	0.43	0.53
		Female	0.23	-	0.33	0.50	0.43	0.73	0.70
Female	Dose * Response Response * Sex	Male	0.27	-	0.46 ¹	0.60 ²	0.43	-	0.17
		Female	0.23	-	0.70	0.80 ²	0.70	-	0.13
Male & Female	Dose * Response	Male	0.17	0.23	0.47	-	0.53	-	0.80
		Female	0.20	0.20	0.20	-	0.57	-	0.57

a/ n = 30, except ¹n=28, ²n=15.

Table 3: Proportions of male and female *Ips plastographus maritimus* attracted to combinations of captured-volatiles from *Pinus radiata* logs containing male or female beetles. a/

Combination(s) of volatiles	Interactions from log linear model (p<0.05)	Sex of responding beetle	Dosage of volatile						
			0	10 ⁻⁵	10 ⁻⁴	10 ⁻³	10 ⁻²	10 ⁻¹	10 ⁰
Male volatile + Female volatile (1:1).	Dose * Response	Male Female	0.33 0.33	- -	0.60 0.76 ¹	0.73 0.82 ²	0.60 0.47	0.40 0.52 ⁵	0.43 0.73
Fixed-attractive male dose, w/ increasing dose from tunneling females.	Dose * Response Response * Sex	Male Female	0.53 0.73	- -	0.43 0.67	0.73 0.83	0.73 0.83	0.57 0.77	0.57 0.64 ³
Fixed-attractive male dose, w/ increasing dose from males and females tunneling together.	Dose * Response Response * Sex	Male Female	0.63 0.73	0.53 0.57	- -	0.63 0.67	0.37 0.67	- -	0.33 0.56 ⁴

a/ n = 30 except ¹n=25, ²n=27, ³n=22, ⁴n=16.

DISCUSSION

Although *I. p. maritimus* were attracted to cut disks of *P. radiata*, they were not attracted to volatiles captured from uninfested logs of *P. radiata*. This lack of attraction may have been because the doses chosen for assay were too concentrated, or the compounds eliciting attraction may not have been captured by the Porapak Q[®]. In some Scolytidae attraction to the host is a subtle effect requiring extensive sampling (Moeck, *et al.*, 1981). *Ips latidens* LeConte and *Hylastes gracilis* LeConte have been shown to respond in the field to host volatiles from lodgepole pine in British Columbia (Miller *et al.*, 1986; Miller and Borden, 1990). *Ips grandicollis* (Eichh.) has been shown to respond to physiologically weakened *Pinus taeda* L. (Werner, 1971, 1972). The attraction of *I. p. maritimus* to *P. radiata* increases the likelihood that beetles will find a suitable host shortly after dispersal. Less exposure time to environmental hazards will increase the likelihood of survival and reproduction (Wood, 1982).

Tunneling male *I. p. maritimus* elicits mass attack on the host. The presence of a male aggregation-pheromone is supported by earlier investigations of *Ips* spp., (see reviews by Wood, 1982; Borden, 1985; and Raffa *et al.*, 1993). In nature, male *Ips* spp. initiate galleries on a new host and are later joined by one to four females. A mass attack by bark beetles concentrates the same species on material suitable for feeding and reproduction and is dependent on pheromone production by the sex that initiates attacks on the host (Wood, 1962, 1982; Wood and Vité, 1961). This study demonstrates the

first dose-response relationship to be demonstrated for male volatiles produced by males of an *Ips* spp.

Female *I. p. maritimus* also produce an attractant pheromone, but at higher concentrations female-volatiles appear to decrease in attraction. This decrease of attraction suggests that, at high levels, pheromone components produced by females may prevent over-exploitation of the host. *Ips latidens* females also produce a pheromone, but it is not an attractant as shown for *I. p. maritimus*. However, female *I. latidens* pheromone reduces attraction to the male-produced pheromone (Seybold, 1992). In nature, females tunnel only in the presence of the male in the gallery system. This joining of males and females may influence the production of pheromone compounds in both sexes. An attractant pheromone produced by a *Ips* spp. females has not been reported previously.

Although mating is thought to decrease attraction (Borden, 1967), such an effect was not observed for *I. p. maritimus* when males were tunneling with females in a ratio of one-to-one. However, the addition of two females to male galleries will disrupt attraction of both sexes. Attraction was also reduced when increasing doses of volatiles captured from male and females tunneling together was released with an attractive dose of male volatiles. Borden (1967) showed that attraction of *I. paraconfusus* females to excised guts in the laboratory decreased as the number of females joining males increased. This indicates that the female anti-attractant is produced following mating. These results do not support Byers (1981), who showed that logs infested with male and female *I. paraconfusus* in a one-to three ratio reduced attraction of males, but not females, to male-infested logs in the field.

Alternatively, stridulation by females (Barr, 1969) may stimulate male-produced anti-attractant volatiles, as has been shown for *Dendroctonus pseudotsugae* Hopkins (Rudinsky, 1969). Male *D. brevicomis* LeConte also produce attractant (frontalin) and anti-attractant (verbenone) compounds which regulate the response of the attacking population to a female attractant-pheromone (*exo-brevicomin*) (Byers *et al.*, 1984). In *Dendroctonus* spp. females initiate galleries on the host and only one male joins the female gallery. Reduced attraction may prevent over-exploitation of the host, therefore increasing the probability of brood survival.

When male and female *I. p. maritimus* were introduced into separate galleries only male response was interrupted. In nature, these volatiles would be produced after joining because female-initiated galleries are rarely observed. Female attraction to male-produced volatiles is reduced only after joining has occurred. This result indicates that there is an interaction between the sexes that produces a unique volatile(s) that reduces female attraction.

Female *I. p. maritimus* response, to all pheromone sources, was greater than male response. *Tomicus piniperda* (Byers, 1989), *I. pini* (Birch *et al.*, 1980), *I. paraconfusus* (Wood, 1962; Seybold, 1992) *I. confusus* LeConte (Birch, *et al.*, 1977), and *I. latidens* (Wood, *et al.*, 1962; Seybold, 1992), have also shown this sex-specific response. Polygamous joining increases the chances for male reproductive success. The lowered attraction of males to male galleries and galleries joined by females increases the chances for mass attack to occur on nearby trees and thus prevents over-utilization of host material.

This study demonstrates that *I. p. maritimus* produces a male and female aggregation pheromone component. Also, two females present in the

male gallery decreases attraction of both males and females. Identification of the male and female attractant pheromone components would provide a useful technique for trapping *I. p. maritimus* in order to determine the phoresy (capacity of insects to carry fungus) of *Fusarium subglutinans* f. sp. *pini* (pitch canker), a pathogen first discovered in California in 1986 (McCain *et al.*, 1987). This bark beetle is known to carry this pathogen (Storer and Dallara, 1992), but its role as a vector of the fungus is unknown. Identification of the female-produced anti-attractant(s) that interrupts male and female attraction to male-produced pheromones may also provide a useful method for preventing attacks by *I. p. maritimus* and perhaps other *Ips* spp. Further work is needed to verify host response by *I. p. maritimus*. Although there was a significant attraction to host disks, the response to captured volatiles could not be demonstrated. Additionally, further work is needed to determine if the response of *I. p. maritimus* to host volatiles can be interrupted. Such a tactic would prevent production of aggregation pheromones and thereby be more effective in preventing host colonization than interrupting pheromone production after the host has been attacked.

REFERENCES

- Akers, R. P. 1989. Counterturns initiated by decrease in rate of increase of concentration: Possible mechanism of chemiotaxis by walking female *Ips paraconfusus* bark beetles. J. Chem. Ecol. 15: 183-208.
- Akers, R. P., and Wood, D. L., 1989. Olfactory orientation responses by walking female *Ips paraconfusus*. II. In an anemotaxis assay. J. Chem. Ecol. 15: 1147-1159.
- Akers, R. P., Preisler, H. M., and Wood, D. L. 1993. Interactions between components of the aggregation pheromone during chemotaxis by the bark beetle *Ips paraconfusus*. J. Chem. Ecol. 19: 863-879.
- Anderson, R. F. 1948. Host selection by the pine engraver. J. Econ. Entomol. 41: 596-602.
- Barr, B. A. 1969. Sound production in the Scolytidae (Coleoptera) with emphasis on the genus *Ips*. Can Entomol. 101: 636-672.
- Birch, M. C., Light, D. M., Wood, D. L., Browne, L. E., Silverstein, R. M., Bergot, B. J., Ohloff, G., West, J. R., and Young, J. C. 1980. Pheromonal response and allomonal interruption of *Ips pini* in California by the two enantiomers of ipsdienol. J. Chem. Ecol. 6: 703-717.
- Birch, M. C., Tilden, P. E., Wood, D. L., Browne, L. E., Young, J. C., and Silverstein, M. C. 1977. Biological activity of compounds isolated from air condensates and from the bark beetle, *Ips confusus*. J. Insect Physiol., 23: 1373-1376.
- Bright, D. E., Jr. and Stark, R. W. 1973. The bark and ambrosia beetles of California. Coleoptera: Scolytidae and Platypodidae. Bull. Calif. Insect Surv., Univ. Calif. Press, vol. 16: 169 pp.
- Borden, J. H. 1967. Factors influencing the response of *Ips confusus* to male attractant. Can. Entomol. 99: 1164-1193.

- Borden, J. H. 1985. Aggregation pheromones. In G. A. Kerkut and L. I. Gilbert (eds.) *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*. pp. 257-258. Pergamon Press, Oxford.
- Browne, L. E. 1972. An emergence cage and refrigerated collector for wood boring insects and their associates. *J. Econ. Entomol.* 65: 1499-1501.
- Byers, J. A. 1981. Effect of mating on terminating aggregation during host colonization in the bark beetle, *Ips paraconfusus*. *J. Chem. Ecol.* 7: 1135-1147.
- Byers, J. A., 1989. Behavioral mechanisms involved in reducing competition in bark beetles. *Holarctic Ecol.* 12: 466-476.
- Byers, J. A., Lanne, B. S., Löfquist, J., Schlyter, F., and Bergström, G. 1985. Olfactory recognition of host-tree susceptibility by pine shoot beetles. *Naturwissenschaften.* 72: 324-326.
- Byers, J. A., Wood, D. L., Craig, J., and Hendry, L. B. 1984. Attractive and inhibitory pheromones produced in the bark beetle *Dendroctonus brevicornis*, during host colonization. *J. Chem. Ecol.* 10: 861-877.
- Grégoire, J. C. 1988. The greater European spruce bark beetle. In A. A. Berryman (ed.) *Dynamics of Forest Insect Populations. Patterns, Causes, Implications*. pp. 455-78. Plenum, New York.
- Lanier, G. N. 1970. Biosystematics of the genus *Ips* (Coleoptera: Scolytidae) in North America. Hopping's group III. *Can. Entomol.* 102:1404-1423.
- Lanier, G. N. 1987. The validity of *Ips cribricollis* (Eich.)(Coleoptera: Scolytidae) as distinct from the *Ips grandicollis* and the occurrence of both species in Central America. *Can. Entomol.* 119: 179-187.
- Lanier, G. N., S. A. Teale, and Pajares, J. A. 1991. Biosystematics of the genus *Ips* (Coleoptera: Scolytidae) in North America: Review of the *Ips calligraphus* group. *Can Entomol.* 123: 1103-1124.

- Lanier, G. N., and Cameron, E. A. 1969. Secondary sexual characters in the North American species of the genus *Ips* (Coleoptera: Scolytidae). *Can. Entomol.* 101: 862-870.
- McCain, A. H., Koehler, C. S., and Tjosvold, S. A. 1987. Pitch canker threatens California pines. *Calif. Agric.* 41 (11): 22-23.
- Miller, D. R., and Borden, J. H., 1990. The use of monoterpenes as kairmones by *Ips latidens* (LeConte) by *Ips pini* (Say) (Coleoptera: Scolytidae). *J. Chem. Ecol.* 17: 1517-1527.
- Miller, D. R., Madden, J. L., and Borden, J. H. 1986. Primary attraction of *Ips latidens* (LeConte) and *Hylastes gracillis* (Coleoptera: Scolytidae) to high-girdled lodgepole pine, *Pinus contorta* var. *latifolia* Engelman. *Can. Entomol.* 118: 85-88.
- Moeck, H. A., Wood, D. L., and Lindahl, K. Q. 1981. Host selection behavior of bark beetles attacking *Pinus ponderosa* with special emphasis on the western pine beetle *Dendroctonus brevicornis*. *J. Chem. Ecol.* 7: 49-83
- Raffa, K. F., Phillips, T. W., and Salom, S. M. 1993. in Strategies and mechanisms of host colonization by bark beetles, T. D. Schowalter and G. M. Filip, (eds.), *Beetle-pathogen Interactions in Conifer Forests*. pp. 103-128. Academic Press, London.
- Rudinsky, J. A. 1969. Masking of the aggregation pheromone in *Dendroctonus pseudotsugae* Hopkins. *Science.* 166: 884-885.
- Seybold, S. J. 1992. The role of chirality in the olfactory-directed aggregation behavior of pine engraver beetles in the genus *Ips* (Coleoptera: Scolytidae). Ph. D. Thesis. Univ. of California, at Berkeley. 355 pp.
- Siegel, S. 1956. *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York. 312 pp.

- Silverstein, R. M. 1977, Complexity, diversity and specificity of behavior-modifying chemicals: Examples mainly from Coleoptera and Hymenoptera, in H. H. Shorey, and J. J. McKelvey Jr. (eds.). *Chemical Control of Insect Behavior: Theory and Application*, pp. 231-251, J. Wiley and Sons, New York.
- Silverstein, R. M., Rodin, J. O., and Wood, D. L. 1966. Sex attractants in frass produced by male *Ips paraconfusus* in ponderosa pine. *Science*. 154: 509-510.
- Sokal, R. R., and Rohlf, F. J. 1981. *Biometry*. 2nd. Edition. W. H. Freeman and Co., New York. 859 pp.
- Storer, A. J. and Dallara, P. L. 1992. Pitch canker disease in California. Calif. Dept. Forestry and Fire Protection, Tree Note Number 15. 2 pp.
- Werner, R. A. 1971. Aggregation behavior of the beetle *Ips grandicollis* in response to host-produced attractants. *J. Insect Physiol.* 18: 423-437.
- Werner, R. A. 1972. Response of the beetle, *Ips grandicollis*, to combinations of host and insect produced attractants. *J. Insect Physiol.* 18: 1403-1412.
- Wood, D. L. 1962. The response created by males of a bark beetle *Ips confusus* attacking ponderosa pine (Coleoptera: Scolytidae). *Pan-Pacific Entomol.* 38: 141-145.
- Wood, D. L. 1982. The role of pheromones, kairmones, and allomones in the host selection and colonization behavior of bark beetles. *Ann. Rev. Entomol.* 27: 441-446.
- Wood, D. L., and Bushing, R. W. 1963. The olfactory response of *Ips confusus* (LeConte) to the secondary attraction in the laboratory. *Can Entomol.* 95: 1066-1078.
- Wood, D. L., and Vité, J. P. 1961. Studies on the host selection behavior of *Ips confusus* (LeConte) (Coleoptera: Scolytidae) attacking *Pinus ponderosa*. *Contribs. Boyce Thompson Inst.* 21: 79-96.

Wood, D. L., Browne, L. E., Bedard, W. D., Tilden, P. E., Silverstein, R. M., and Rodin, J. O. 1968. Response of *Ips confusus* to synthetic sex pheromones in nature. *Science* 159: 1373-1374.

Wood, D. L., Browne, L. E., Silverstein, R. M. and Rodin, J. O. 1966. Sex pheromones of bark beetles. I. Mass production, bio-assay, source, and isolation of sex pheromones of *Ips confusus* (LeC). *J. Insect Physiol.* 12: 523-536.

Wood, D. L., Stark, R. W., Silverstein, R. M., and Rodin, J. O. 1967. Unique synergistic effects produced by the principal sex attractant compounds of *Ips confusus* (LeConte). *Nature*. 215: 206.

Wood, S. L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), A Taxonomic Monograph. Great Basin Nat. No. 6, 1359 pp.

Zar, J. H. 1984. *Biostatistical Analysis*. 2nd Edition. Prentice Hall, New Jersey. 718 pp.