

INTERSPECIFIC EFFECTS OF PHEROMONES
ON THE ATTRACTION OF THE BARK BEETLES,
Dendroctonus brevicomis AND *Ips paraconfusus*¹
IN THE LABORATORY

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(Received November 13, 1979; revised February 25, 1980)

Abstract—*Dendroctonus brevicomis* was attracted to a mixture of the *Ips paraconfusus* pheromones, ipsenol, *cis*-verbenol, and ipsdienol at 10⁻⁹ g each/ μ l but was not attracted to these pheromones at higher and lower release rates. *I. paraconfusus* was not attracted to the *D. brevicomis* pheromones *exo*-brevicomin, frontalin, and myrcene at any release rate tested. Increased release rates of a mixture of the three pheromones of *I. paraconfusus* inhibited the attraction of *D. brevicomis* to its synthetic pheromones. A mixture of ipsenol + ipsdienol or *cis*-verbenol alone failed to cause inhibition indicating that at least two of the *I. paraconfusus* pheromones are required to inhibit the response of *D. brevicomis*. The pheromones of *D. brevicomis* did not inhibit the attraction of *I. paraconfusus* to its pheromones; however, verbenone was a potent inhibitor.

Key Words—*Dendroctonus brevicomis*, *Ips paraconfusus*, Coleoptera, Scolytidae, *Pinus ponderosa*, bark beetle, *exo*-brevicomin, frontalin, myrcene, verbenone, ipsenol, ipsdienol, *cis*-verbenol, attractants, inhibition, semiochemicals, pheromones.

INTRODUCTION

In California *D. brevicomis* LeConte and *I. paraconfusus* Lanier colonize the phloem-cambium tissues of ponderosa pine, *Pinus ponderosa* Laws.,

¹Coleoptera: Scolytidae. This paper is based in part upon a dissertation submitted by J.A.B. to the University of California, Berkeley, in partial fulfillment of the requirements for the Ph.D. degree in entomology, June 1978. These studies were supported by the Rockefeller Foundation; USDA Forest Service; Regional research project W-110, SEA/USDA; and the National Science Foundation and Environmental Protection Agency through a grant (NSF GB-34719/BMS 75-04223) to the University of California. The findings, opinions, and recommendations are not necessarily those of the University of California or the funding agencies.

during the same period of time. Byers and Wood (1980) found that the attraction of both species to their naturally produced pheromones was inhibited by the presence of the other species feeding in logs of the host tree. The function of this mutual inhibition is believed to reduce interspecific competition for food and space. They showed that the response of *D. brevicomis* to its synthetic pheromones, *exo*-brevicommin (E) and frontalin (F) and the host terpene, myrcene (M) (Silverstein et al., 1968; Kinzer et al., 1969) was inhibited by the presence of logs infested with male *I. paraconfusus*. Byers and Wood (1980) also found that E + F + M had no effect on the attraction of *I. paraconfusus* to its natural pheromone. However, they established that synthetic verbenone inhibited the response of *I. paraconfusus* to its pheromone. Furthermore, they showed that verbenone was present in male *D. brevicomis* feeding in logs cut from pheromone-baited trees that had inhibited the response of *I. paraconfusus* to its pheromone. However, they did not test the pheromones of *I. paraconfusus*, ipsenol (I), *cis*-verbenol (II), and ipsdienol (III) (Silverstein and Rodin, 1965; Silverstein et al., 1966; Wood et al., 1966) as possible inhibitors of the response of *D. brevicomis* to its pheromone or as attractants for *D. brevicomis*, nor did they test for inhibition of *I. paraconfusus* attraction to I + II + III by verbenone. The objectives of this study were to investigate the responses of these beetles to semiochemicals, both intra- and interspecific, under laboratory conditions.

METHODS AND MATERIALS

Both *I. paraconfusus* and *D. brevicomis* were collected from the Sierra National Forest near Bass Lake, Madera County, California, at an approximate elevation of 1000 m. The *D. brevicomis* were obtained by removing bark from naturally attacked ponderosa pines, while *I. paraconfusus* were obtained from infested logging debris. The rearing, preparation, and determination of sex were as described in Byers and Wood (1980).

The responses of *D. brevicomis* and *I. paraconfusus* to semiochemicals were tested in the laboratory olfactometer developed by Browne et al (1974) for *I. pini* Say. However, several modifications were used. The polyurethane foam was removed from the plexiglass manifold to maintain the air speed at 0.9 m/sec at the semiochemical source and 0.6 m/sec where the beetles were released (21 ± 2 cm "downwind"). A positive response was recorded when a beetle arrived within 1 cm of the attractive source in the time required for various mixtures of the semiochemicals in diethyl ether to elute from a 5- μ l capillary tube (126 ± 10 sec). The release rate of semiochemicals in the bioassay (g/min) from the 5- μ l capillary pipette was estimated to be 2.2 times

the concentration of the starting solution ($\text{g}/\mu\text{l}$), assuming that the compounds were released in proportion to the volume reduction of the solvent. However, the actual release rates of compounds from the pipette probably was not linear due to chemical interactions and different rates of distillation (vapor pressures) of the semiochemicals and the solvent. At least 30 beetles of each sex were tested for each release rate of the compounds. Differences in the percent responding between various release rates were determined by a chi-square test.

Both sexes of *D. brevicomis* and *I. paraconfusus* was tested for interspecific attraction to the appropriate synthetic pheromones: I + II + III (each >98%) (Figure 1) or E (>95%) + F (>95%) + M (>99%) (Figure 2). *I. paraconfusus* were also tested for their response to verbenone (>99.8%, GLC purified) (Figure 2). I, III, E, and F were all racemic, the enantiomeric composition of II was not known, and verbenone was $[\alpha]_{25}^D = +90^\circ$. All compounds were obtained from Chemical Samples Co., Cleveland, Ohio.

The three pheromones of each species must be released simultaneously for maximum attraction in the laboratory assay (Wood et al., 1967, 1968, 1976; Silverstein et al., 1968; Wood, 1970; Byers et al., 1979). Each sex of *D. brevicomis* was tested for attraction to E + F + M to determine a release rate that elicited an approximate 50% response (Figure 3). *D. brevicomis* then were tested at this concentration (10^{-9} g E + F + M/ μl) for inhibition of their response by increasing the release rate ($\text{g}/\mu\text{l}$) of either I + II + III (Figure 4), I + III, or II (Table 1). Similarly, each sex of *I. paraconfusus* was tested for

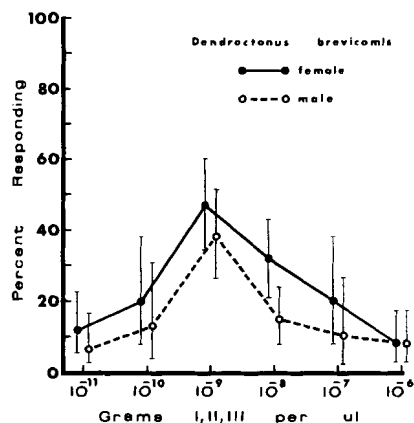


FIG. 1. Response of male and female *Dendroctonus brevicomis* to ipsenol (I), *cis*-verbenol (II), and ipsdienol (III) at 10^{-11} to 10^{-6} g each/ μl (October 5-7, 1976). Brackets represent 95% binomial confidence limits.

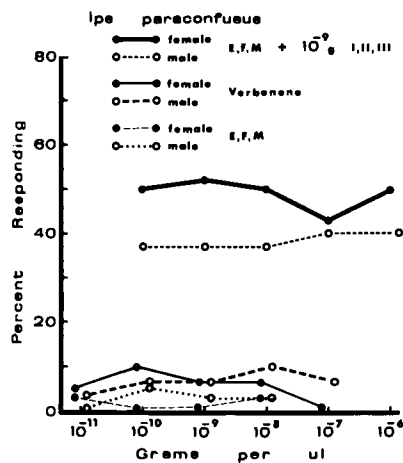


FIG. 2. Response of male and female *Ips paraconfusus* to *exo*-brevicomins (E), frontalin (F), and myrcene (M) at 10^{-11} to 10^{-8} g each/ μ l; ipsenol (I), *cis*-verbenol (II), and ipsdienol (III) at 10^{-9} g each/ μ l in mixtures with E, F, M at 10^{-10} to 10^{-6} g each/ μ l; and verbenone alone at 10^{-11} to 10^{-7} g/ μ l (July 28-29, 1976).

inhibition of their response to 10^{-9} g I + II + III/ μ l by increasing the release rate of either E + F + M (Figure 2) or verbenone (Figure 5).

Since verbenone is structurally related to II and thus might compete for acceptor sites of II on the antennae of *I. paraconfusus*, we wanted to know how much II was required to significantly enhance the beetles' attraction to

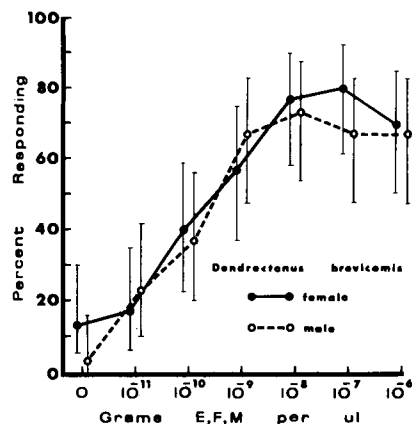


FIG. 3. Response of male and female *Dendroctonus brevicomis* to *exo*-brevicomins (E), frontalin (F), and myrcene (M) at 0 and 10^{-11} to 10^{-6} g each/ μ l (October 8, 1976). Brackets represent 95% binomial confidence limits.

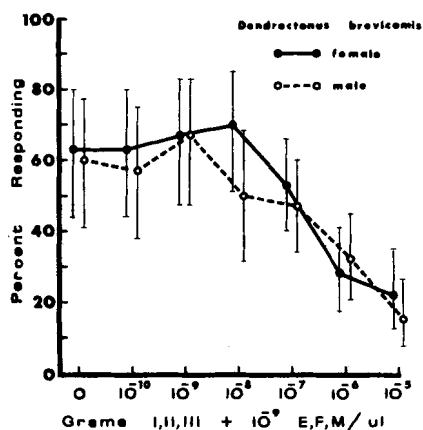


FIG. 4. Response of male and female *Dendroctonus brevicomis* to *exo*-brevicomin (E), frontalin (F), and myrcene (M) at 10^{-9} g each/ μ l in mixtures with ipsenol (I), *cis*-verbenol (II), and ipsdienol (III) at 0 and 10^{-10} to 10^{-5} g each/ μ l (October 11-12, 1976). Brackets represent 95% binomial confidence limits.

TABLE I. RESPONSE OF MALE AND FEMALE *Dendroctonus brevicomis* TO *EXO*-BREVICOMIN (E), FRONTALIN (F), AND MYRCENE (M) IN VARIOUS MIXTURES WITH IPSENOI (I), *CIS*-VERBENOL (II), AND IPSDIENOL (III) (OCTOBER 16, 1976).

Compounds tested	Dose (g/ μ l)	Sex	Number tested	Percent responding	Confidence interval (95%)
E, F, M	10^{-9}	♀	90	63	52-73
		♂	120	68	59-76
E, F, M + I, III	10^{-9}	♀	30	87	69-97
		♂	30	83	64-94
E, F, M + II	10^{-9}	♀	60	53	40-68
		♂	60	60	46-72
E, F, M + I, II, III	10^{-9}	♀	90	30	20-41 ^a
		♂	90	31	21-42 ^a

^aSignificantly different from above treatments ($P < 0.05$).

I + III. This was determined by testing female response to mixtures of a 10-fold concentration series of II from 10^{-13} to 10^{-9} g/ μ l with 5×10^{-9} g each I + III/ μ l.

RESULTS

Both sexes of *D. brevicomis* responded in significantly greater proportions to I + II + III at 10^{-9} g each/ μ l than to these pheromones released at either higher or lower rates (Figure 1). The percent of males responding at 10^{-9} g/ μ l was different than the percent responding at 10^{-11} , 10^{-7} , or 10^{-6} g I + II + III/ μ l ($P < 0.05$). The percent of females responding at 10^{-9} g/ μ l was different than at either 10^{-11} , 10^{-10} , or 10^{-6} g I + II + III/ μ l ($P < 0.05$). In contrast, *I. paraconfusus* was not attracted to E + F + M or verbenone at any concentration tested ($P > 0.1$) (Figure 2).

Both sexes of *D. brevicomis* responded similarly to each release rate of E + F + M. The percent of males and females responding to 10^{-10} and 10^{-9} g E + F + M/ μ l, respectively, and all higher rates were significantly greater than the percent responding to solvent controls ($P < 0.05$). There were no significant differences in percent responding between the sexes at any concentration of E + F + M ($P > 0.1$) (Figure 3). The response of both sexes of *D. brevicomis* to E + F + M was inhibited by I + II + III released at 10^{-6} and 10^{-5} g each/ μ l compared to 0, 10^{-10} , and 10^{-9} g each I + II + III/ μ l ($P < 0.05$) (Figure 4). Neither I + III nor II inhibited the response of *D. brevicomis* to E + F + M (Table 1) which indicates a mixture of at least two (II + I or II + III) and possibly all three pheromones of *I. paraconfusus* are necessary.

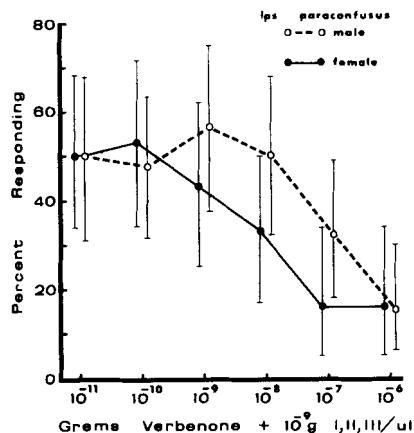


FIG. 5. Response of male and female *Ips paraconfusus* to ipsenol (I), *cis*-verbenol (II), and ipsdienol (III) at 10^{-9} g each/ μ l in mixtures with verbenone at 10^{-11} to 10^{-6} g/ μ l (July 27-28, 1976). Brackets represent 95% binomial confidence limits.

The response of either sex of *I. paraconfusus* to 10^{-9} g I + II + III/ μ l was unaffected by E + F + M at concentrations of 10^{-10} g to 10^{-6} g/ μ l (Figure 2). However, verbenone at 10^{-7} g to 10^{-6} g/ μ l was effective in inhibiting the response of both sexes of *I. paraconfusus* to I + II + III (Figure 5). The percent of males responding to I + II + III at 10^{-6} g verbenone/ μ l was significantly less than the percent responding at 10^{-11} or 10^{-10} g/ μ l ($P < 0.05$). The percent of females responding to I + II + III at 10^{-7} or 10^{-6} g verbenone/ μ l was significantly less than the percent responding at 10^{-11} or 10^{-10} g/ μ l ($P < 0.05$) (Figure 5).

The response of female *I. paraconfusus* to 5×10^{-9} g each I + III/ μ l was increased from 25% (95% binomial confidence limits, BCL, 16% and 38%) to 57% (BCL 38% and 75%) (different at $P < 0.05$) by adding as little as 10^{-10} g II/ μ l to the I + III mixture.

DISCUSSION

Dethier (1947) and Dethier et al. (1952) described a phenomenon in which a gustatory response to an optimum dosage of a stimulant could be lowered by either increasing or decreasing the dosage. A similar relationship has been shown for *D. frontalis* Zimm. and *D. pseudotsugae* Hopk., where response to a certain release rate of a pheromone was lowered by both increasing and decreasing the release rate of the intraspecific compounds, verbenone or 3-methyl-2-cyclohexen-1-one (MCH), for each species, respectively (Rudinsky 1973a,b). We report the first instance of response curve for interspecifically active compounds, i.e., I + II + III at 10^{-9} g each/ μ l was attractive to *D. brevicomis* but either lower or higher release rates were not (Figure 1). Further experiments with various mixtures of I, II, and III are needed to elucidate which compounds are involved. In contrast, *I. paraconfusus* was not attracted to either E + F + M or to verbenone (Figure 2). There have been no reports of *I. paraconfusus* trapped in the field at sources of naturally produced or synthetic pheromones of *D. brevicomis*. The attraction of *D. brevicomis* to I + II + III in our laboratory studies, but apparently not to natural or synthetic pheromones in the field (Wood et al. 1968, 1976), may be explained by a high release rate of the pheromones thus inhibiting the beetle before encountering the trap or by differences in the walking and flight response to I + II + III.

Struble and Hall (1955) and Miller and Keen (1960) have summarized several reports that *I. paraconfusus* may precede *D. brevicomis* in the successful colonization of a ponderosa pine. The attraction of *D. brevicomis* to trees that were top-killed by *I. paraconfusus* also appeared to be greater than to trees that had their tops removed (Miller and Keen, 1960). *D. brevicomis* may exploit weakened and more susceptible hosts by responding to the pheromone produced by *I. paraconfusus* which is believed to be a less aggressive tree-killer. However, field experiments utilizing various release

rates of synthetic and naturally produced pheromones are required before this sequence of host selection can be attributed to interspecific attraction.

The proportion of *D. brevicomis* responding to a constant dose of E + F + M was reduced as the release rate of I + II + III was increased (Figure 4). The inhibition occurred only at the two higher release rates tested, which suggests that *D. brevicomis* would be inhibited in the field only at close range to substrates containing a mixture of these species. The inhibition of attraction of *D. brevicomis* to naturally infested substrates and synthetic pheromones in the field by logs infested with *I. paraconfusus* males (Byers and Wood, 1980) may be due, at least in part, to the release of I + II + III from these logs. In this regard, the laboratory assay has provided the first evidence that a mixture of at least two compounds is necessary to cause this interspecific inhibition.

The response of *I. paraconfusus* to male-produced pheromone was not inhibited by E + F + M in the laboratory. This supports the field observation where E + F + M did not inhibit the response of *I. paraconfusus* to male-infested logs (Byers and Wood, 1980). However, in the present study, verbenone at relatively high release rates inhibited the response of *I. paraconfusus* to I + II + III (Figure 5), which complements the field results where verbenone inhibited the attraction of *I. paraconfusus* to naturally produced pheromone (Byers and Wood, 1980). *I. paraconfusus* probably would be attracted in flight to a tree under colonization by both species but inhibition of *I. paraconfusus* would increase as the beetles approached the *D. brevicomis*-infested areas where the concentration of verbenone is highest.

Certain sensory cells on the antennae of *I. paraconfusus* and *I. pini* are known to be sensitive to several monoterpenes (verbenone, II, and *trans*-verbenol) that are structurally related (Mustaparta, 1979). It is possible that less evolutionary change in *D. brevicomis* and *I. paraconfusus* would be necessary to acquire sensory systems responsive to interspecific compounds, if each species utilized compounds structurally similar to their own pheromones. Thus, the acceptor sites for myrcene (2-methyl-6-methylene-2,7-octadiene) on the antennae of *D. brevicomis* may have evolved into new sites capable of accepting the structurally similar I (2-methyl-6-methylene-7-octene-4-ol) and/or III (2-methyl-6-methylene-2,7-octadiene-4-ol). Similarly, the *D. brevicomis* acceptor site for the intraspecific inhibitor verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) (Bedard et al., 1980) may have evolved into new sites capable of accepting structurally similar II (*cis*-verbenol = 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol). *I. paraconfusus* may have evolved acceptor sites for verbenone from acceptor sites for II.

On the other hand, verbenone may compete with II for the acceptor sites of II in *I. paraconfusus* so that the beetle effectively can respond only to I + III which has been shown to be essentially unattractive in the field (Wood et al., 1967, 1968). However, in competitive interactions verbenone would probably have to have a much stronger affinity for the acceptor site than II to

cause the observed inhibition of the behavioral response since as little as 10^{-10} g II/ μ l significantly increased the response of female *I. paraconfusus* to 5×10^{-9} g/ μ l each of I + III. Further, the enhancement of attraction by adding II to I + III does not support the hypothesis of Kikuchi and Ogura (1976), based on molecular binding-site models, that II interacts with acceptor sites in a similar conformational way as III or by the conversion of III into II.

Verbenone primarily from male *D. brevicomis* (Renwick, 1967; Byers and Wood, 1980), I + II + III from male, and II from female (Renwick et al., 1976) *I. paraconfusus* appear to cause, at least in part, the observed interspecific inhibition of the responses of these species to their pheromones. We do not know how closely the release rates of I, II, III, and verbenone reported in our study represent the release rates from infested pine substrates in nature. However, Browne et al. (1979) have quantified the release rates of E, F, and M per beetle per day in a tree as 4.1×10^{-6} g E, 8.6×10^{-7} g F, and 4.1×10^{-4} g M. Our release rates were estimated to range from 3×10^{-8} to 3×10^{-3} g/day (Figure 3). Inhibition of the response of *D. brevicomis* to naturally produced pheromone by I + II + III has not been tested in the laboratory or field, nor has the test of inhibition of response to E + F + M by I + II + III been conducted in the field. Further work would be necessary to elucidate the role of each semiochemical at the enantiomeric level (Wood et al., 1976; Borden et al., 1976). Verbenone and I + II + III may prove useful in inhibiting aggregation and host colonization by *I. paraconfusus* and *D. brevicomis* and thus function to reduce tree mortality caused by these bark beetles.

Acknowledgments—We would like to thank L.E. Browne, W.J. Loher, P.A. Rauch, and E. Zavarin of the University of California at Berkeley; M.C. Birch and D.M. Light of the University of California at Davis; and W.D. Bedard of the Forest Service, USDA, for their helpful suggestions and review of the manuscript. We also thank K.Q. Lindahl, Jr., University of California at Berkeley, for review of statistical methods.

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