Verbenone: Dose-Dependent Interruption of Pheromone-Based Attraction of Three Sympatric Species of Pine Bark Beetles (Coleoptera: Scolytidae)

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ABSTRACT Verbenone significantly reduced catches of *Ips latidens* (LeConte), I. *pini* (Say), **and Dendroctonus *ponderosae* Hopkins in multiple-funnel traps, baited with aggregation pheromones, in stands of lodgepole pine in southern British Columbia. Interruption of attraction was dose dependent for all three species. There were no significant differences in attraction between the sexes. Variation in successful use of verbenone in protecting pine stands partially may be explained by the dose-dependent variation in responses.**

KEY WORDS semiochemical, Scolytidae, dose-dependent

VERBENONE (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-e-one) is an antiaggregation pheromone for the mountain pine beetle, ***Dendroctonus ponderosae*** Hopkins, in stands of lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann, and ponderosa pine, ***P. ponderosa*** Lawson. The pheromone is produced by autoxidation and by microorganisms in association with female beetles (Pitman et al. 1969, Rudinsky et al. 1974, Libbey et al. 1985, Hunt et al. 1989, **Hunt & Borden 1990). Verbenone inhibits** the attraction of both sexes to their attractive semiochemicals (Ryker & Yandell 1983, Borden et al. 1987, Schmitz & McGregor 1990).

One current hypothesis of the function of verbenone for ***D. ponderosae,*** and other species of ***Dendroctonus,*** is the regulation of densities of breeding pairs on suitable hosts, thereby minimizing intraspecific competition among brood (Renwick & Vité 1970, Geiszler & Gara 1978, Geiszler et al. 1980, Alcock 1982, Borden 1982, Berryman et al. 1985, Borden et al. 1987). Initially, ***D. ponderosae*** beetles establishing a gallery release aggregation pheromones such as trans-verbenol and exo-brevicomin. Release of verbenone begins after some residency time. This temporal sequence is repeated for all breeding pairs on a host.

However, breeding pairs establish galleries at different times. The release rates of verbenone and aggregation pheromones of breeding pairs are not in synchrony. As a host tree becomes fully colonized, the total release rate of aggregation pheromones from all breeding pairs on the host decreases as the release rate of verbenone increases. Over time, proportionally more beetles deter from the initial host and attack adjacent trees. When a host is fully colonized, the total release rate of verbenone from all breeding pairs on the initial host far exceeds the release rate of aggregation pheromones. Beetle attacks on the initial host cease at that time, and all responding beetles are diverted to adjacent trees.

Beetles are exposed to various doses and ratios of aggregation and antiaggregation pheromones during an attack sequence. If the density regulation hypothesis is correct, then variations in these doses and ratios should be correlated with host quality as determined by densities of beetles on hosts. Selection should favor individuals that utilize this information by exhibiting a dose-dependent response to the antiaggregation pheromone verbenone. We hypothesized, therefore, that verbenone should interrupt the attraction of ***D. ponderosae*** to its aggregation pheromones in a dose-dependent fashion. Dose-dependent interruption of ***D. brevicomis*** LeConte to its pheromones by verbenone seems to occur in California (Paine & Hanlon 1991).

Individuals of sympatric species may also show a dose-dependent response to verbenone. *Ips latidens* (LeConte) and the pine engraver, I. *pini* (Say), are often associated with ***D. ponderosae,*** often breeding in the same hosts (Furniss & Carolin 1980). However, all three species tend to be found in single-species assemblages. Spatial separation of a host occurs, possibly to minimize interspecific

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Table 1. Description of **semiochemical-releasing** devices

Device	Chemical”	Description	Release rate (mg/d)[b]
1	Verbenone (+17/−83)	Open polypropylene centrifuge tube (1.5 ml) containing one disposable pipette (2 μl)[c]	0.03
2	Verbenone (+17/−83)	Open polypropylene centrifuge tube (1.5 ml) containing one 2-cm-long glass capillary tube (1.5 mm i.d.)[c]	0.09
3	Verbenone (+17/−83)	Polyethylene/nylon bubble cap	0.6
4	Verbenone (+17/−83)	Open polypropylene centrifuge tube (1.5 ml) containing five 2-cm-long glass capillary tubes (1.5 mm i.d.)[c]	0.62
5	Verbenone (+17/−83)	Black polyethylene bubble cap	13.58
6	verbenone (+17/−83)	White polyethylene bubble cap	3.08
7	Verbenols (+17/−83)[d]	Polyethylene bubble cap	2.9
8	em-Brevicomin (+50/−50)	Laminar lure	0.01
9	Ipsenol (+50/−50)	Polyvinyl bubble cap	0.2
10	Ipsdienol (+50/−50)	Polyvinyl bubble cap	0.2
11	β-Myrcene	Polyethylene screw-cap bottle (15 ml)	281.0

Laminar lures supplied by Hercon Environmental Company (Emigsville, PA).

[a] All chemical purities >98%.

[b] At 24–28°C.

[c] Each capillary tube and pipette was sealed at one end and filled with verbenone.

[d] 13:87 mixture of *cis*- and *trans*-verbenol.

competition among brood (Rankin & Borden 1991).

Verbenone interrupts the attraction of I. *pini* to its pheromone, ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) (Borden et al. 1992). We hypothesized that this phenomenon for *I. pini* should be dependent on the release rate of verbenone. We further hypothesized that verbenone should also interrupt the attraction of *I. latidens* to its pheromone, ipsenol (2-methyl-6-methylene-7-octen-4-ol) (Miller & Borden 1990, Miller et al. 1991), and that interruption should be exhibited in a dose-dependent fashion.

Materials and Methods

Semiochemical-Releasing Devices. All devices used to release semiochemicals were supplied by Phero Tech (Delta, BC) (Table 1). Release rates for devices 8, 9, and 10 were determined by collection of volatiles on Porapak-Q and analysis by capillary gas chromatography. Release rates for all remaining devices were determined by weight loss analyses. Myrcene is a host kairomone for ***D. ponderosae*** (Billings et al. 1976).

Experiments. We conducted three experiments, one for each hypothesis. Experiments 1, 2, and 3 were targeted for ***D. ponderosae, I. pini,*** and ***I. latidens,*** respectively. In each experiment, blocks of six multiple-funnel traps (Phero Tech) (Lindgren 1983) were set at least 100 m apart in stands of lodgepole pine near Princeton, BC. Traps were spaced 10-15 m apart in grids of 2 by 3 within each block. Twelve-unit traps were used in experiment 2; eight-unit traps were used in experiments 1 and 3. Each trap was at least 2 m from any tree and suspended such that the bottom of each trap was 0.2–0.5 m above ground level.

Experiment 1 consisted of four blocks of traps, set from 1 September to 18 October 1988. Experiment 2 consisted of eight blocks, set from 3 to 29 August 1990. Experiment 3 consisted of five blocks, set from 17 June to 20 July 1988. Populations of ***I. latidens*** and I. *pini* were endemic; those of ***D. ponderosae*** were post-epidemic.

In each experiment, treatments were assigned randomly to traps within each block as follows: attractive semiochemical(s) alone or with devices resulting in one of five verbenone release rates. The control treatment in each experiment was a trap baited only with attractive semiochemicals. In experiment 1, these semiochemicals were the aggregation pheromones exo-brevicomin and cis- and *trans*-verbenol, and the host kairomone myrcene. In experiments 2 and 3, the semiochemicals were the pheromones ipsdienol and ipsenol, respectively.

In experiments 1 and 3, the release rates of verbenone were ≈0.03, 0.09, 0.62, 13.58, and 54.02 mg/d at 24-28°C. The four lowest rates were obtained by the use of devices 1, 2, 4, and 5, respectively (Table 1). The highest rate was obtained through the use of four device 5. In experiment 2, the release rates of verbenone were ≈0.01, 0.19, 1.82, 3.08, and 12.32 mg/d at 24–28°C. The two lowest rates were obtained with devices 1 and 2. The second highest rate was obtained with device 7. The third lowest rate was obtained with three device 3 and the highest rate was obtained with four device 7.

Sexes of captured ***I. latidens*** and ***D. ponderosae*** were determined by dissection and internal examination of genitalia. Sexes of captured *I. pini* were determined using declivital characters (Lanier & Cameron 1969).

Statistical Analyses. The data were analyzed using the SYSTAT statistical package (SYSTAT 1990). Trap catches were transformed by $\log_{10}(y + 1)$ to remove heteroscedasticity. Proportions of males in catches were transformed by $\text{arcsine}(y)$ to reduce deviations from normality. Homoscedastic data were subjected to two-way analyses of variance (ANOVA), using block and treatment as model factors, and Tukey's multiple comparison tests set with an experimentwise $P = 0.05$. Catches of beetles, transformed by $\log_{10}(y + 1)$, and proportions of male *I. pini* and ***D. ponderosae,*** transformed by $\arcsin(y)$, in traps baited with verbenone devices, were regressed on the release rate of verbenone, transformed by $\log_{10}(x)$, using a general linear model.

Results

Verbenone significantly interrupted the attraction of ***I. latidens,*** *I. pini,* and ***D. ponderosae*** to pheromone-baited multiple-funnel traps (Fig. 1). Interruption was significantly dose dependent for all three species, with the highest dose having the greatest effect. Catches of ***I. latidens*** in traps with devices releasing verbenone at the highest rate were significantly lower than those in traps without verbenone or with devices releasing verbenone at the two lowest rates (Tukey's test, $P = 0.017$, 0.010, and 0.017, respectively). Catches of ***I. pini*** and ***D. ponderosae*** in traps baited with verbenone devices releasing at the two highest rates were significantly lower than those in all other traps (Tukey's test, $P < 0.05$).

There were no differences in sex ratio of captured beetles among treatments for ***I. latidens*** ($F = 1.38$; df = 2, 4; $P = 0.350$) and *I. pini* ($F = 1.94$; df = 5, 35; $P = 0.113$). The mean (± SEM) proportions of male ***I. latidens*** and *I. pini* caught in traps were 0.17 ± 0.03 ($n = 11$) and 0.25 ± 0.01 ($n = 48$), respectively. Sex ratios of *I. latidens* in catches from traps baited with verbenone devices releasing at the three highest rates were not calculated because of low numbers,

There was a weakly significant difference in sex ratio of captured ***D. ponderosae*** among treatments ($F = 3.24$; df = 3, 8; $P = 0.082$). Control traps had a lower proportion of males than those baited with verbenone released at the third highest rate (Tukey's test, $P = 0.066$). The mean (±SEM) proportion of male ***D. ponderosae*** in control traps was 0.40 ± 0.07; those for traps with verbenone devices releasing at the three lowest rates were 0.48 ± 0.033, 0.47 ± 0.04, and 0.54 ± 0.03, respectively. None of the other comparisons was significant (Tukey's test, $P > 0.25$). Regressions of sex ratio on release rate of verbenone were not significant for either *I. pini* ($r^2 = 0.039$; $P = 0.833$) or ***D. ponderosae*** ($r^2 = 0.203$; $P = 0.165$). Sex ratios for catches of ***D. ponderosae*** and *I. pini* from traps with verbenone devices releasing at the two highest rates were not calculated because of low numbers.

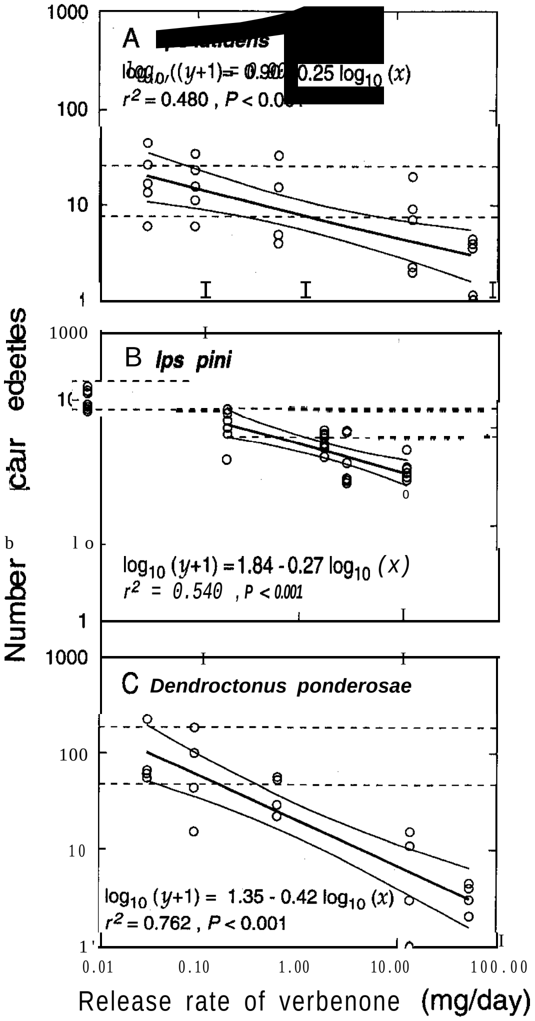

Fig. 1. Effect of verbenone, released at various rates, on the attraction of (A) *I. latidens,* (B) *I. pini,* and (C) *D. ponderosae* to multiple-funnel traps baited with ipsenol (A), ipsdienol (B), or the combination of myrcene, *exo*-brevicomin, and cis- and *trans*-verbenol (C). Slopes of the regression lines are significantly different from zero (t test, $P < 0.001$). Confidence limits (95%) (thin solid lines) are associated with each regression line (thick solid line). Dashed horizontal lines represent confidence limits (95%) for catches in control traps.

Discussion

Over 200 million mature pines were killed by ***D. ponderosae*** in British Columbia between 1972 and 1988 (Van Sickle 1989). Verbenone has been tested as an alternative to harvesting as a tactic to minimize mortality of pines by ***D. ponderosae*** in attempts to protect both timber and nontimber values. The results of these attempts have been inconsistent (Amman 1994). Verbenone released from multiple points in stands of lodgepole pine significantly reduced the levels of infestations in comparison with controls in some trials (Amman et al. 1989, Lindgren et al. 1989, Gibson et al. 1991) but not in others (Gibson et al. 1991; B.S.L., unpublished data). In stands of ponderosa pine, verbenone did not reduce the levels of infestations (Bentz et al. 1989, Lister et al. 1990, Gibson et al. 1991). In two of these trials in ponderosa pine there appeared to be a negative correlation between levels of infestation and the release rate of verbenone. High levels of variation between replicates may have reduced the power of multiple range tests associated with these trials.

In nature, the ratio and release rates of aggregation and antiaggregation pheromones used by *D. ponderosae* vary over time during the attack and establishment phases. This phenomenon may provide information concerning availability of host material and mates to other conspecifics (Borden et al. 1987). Dose-dependent interruption of pheromone attraction of *D. ponderosae* by verbenone demonstrates that beetles can respond to this information (Fig. 1C).

Air space concentrations of aggregation and antiaggregation pheromones vary spatially through a forest as well. Pheromones released from a point source such as a tree diffuse outward into a three-dimensional air space. Pheromone concentrations decrease with increasing distance from the point source. Further variation within stands could occur as a result of variation in crown closure and subsequent variation in penetration by sunlight (Kostyk et al. 1993). Verbenone is photoisomerized by ultraviolet radiation to chrysanthanone, a compound that has no known effect on the response of *D. ponderosae.* Air spaces receiving high levels of sunlight could result in local depletions of verbenone.

D. ponderosae is able to deal with this type of information, as evidenced by attacks on additional trees during epidemics. Dose-dependent responses to verbenone over a broad range of release rates may be the mechanism facilitating navigation through an infested stand. The release of verbenone from multiple points in a stand, as used in attempts to control *D. ponderosae,* should result in similar spatial variation in pheromone concentrations. It is reasonable to expect that beetles should be able to navigate through this type of pheromone landscape as well, ultimately reaching suitable hosts through responses to host kairomones and aggregation pheromones.

The level of variation in responses of *D. ponderosae* tend to suggest that the use of verbenone in mitigating the impacts of *D. ponderosae* may be quite limited. Successes to date tend to have occurred in stands with a low infestation level (40%) (B.S.L., unpublished data). One possible means of improving the efficacy and reliability of verbenone against *D. ponderosae* is the addition of other semiochemicals, such as green leaf volatiles or pheromones of competing species, that help convey the message that trees in the stand are not suitable for attack. The trees may be perceived as resistant, nonhost species, or as trees already occupied by competing species (Paine & Hanlon 1991, Borden et al. 1992, Dickens et al. 1992, Hobson 1995). However, the responses of beetles would have to preclude their ability to navigate through a pheromone landscape, possibly by causing beetles to leave the stand.

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