# Dose-Dependent Pheromone Responses of Mountain Pine Beetle in Stands of Lodgepole Pine

DANIEL R. MILLER,[1] B. STAFFAN LINDGREN,[2] AND JOHN H. BORDEN[3]

USDA Forest Service, Southern Research Station, 320 Green St., Athens, GA 30602–2044

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**ABSTRACT** We conducted seven behavioral choice tests with Lindgren multiple-funnel traps in stands of mature lodgepole pine in British Columbia, from 1988 to 1994, to determine the dose-dependent responses of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, to its pheromones. A multifunctional dose-dependent response was exhibited by *D. ponderosae* to the pheromones *cis*- and *trans*-verbenol in areas with low population numbers. In an area with a high population level of *D. ponderosae*, the response was directly proportional to release rates. No dose-dependent response was exhibited by *D. ponderosae* to *exo*-brevicomin at low release rates. At rates of release >0.5 mg/d, *exo*-brevicomin interrupted the attraction of *D. ponderosae* in a dose-dependent fashion. The bark beetle predators, *Enoclerus sphegeus* (F.) and *Thanasimus undatulus* (Say), showed dose-dependent responses to only a few pheromones, with trap catches directly proportional to release rates. The multi-functional response of *D. ponderosae* to verbenols is consistent with an optimal attack density hypothesis.

**KEY WORDS** *Dendroctonus ponderosae*, *exo*-brevicomin, verbenols, Cleridae

THE MOUNTAIN PINE BEETLE, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), breeds in the phloem tissue of mature lodgepole pine, *Pinus contorta* variety *latifolia* Engelmann (Furniss and Carolin 1980). To overcome host defenses and colonize live trees, individual beetles must aggregate and attack the same host at the same time. If the density of attacks exceeds 40/$m^2$ of bark surface, the beetles are successful and can establish breeding galleries (Raffa and Berryman 1983); otherwise they die. Successful mass attacks by *D. ponderosae* are mediated by the aggregation pheromones *exo*-brevicomin, and *cis*- and *trans*-verbenol (Borden et al. 1987, Miller and Lafontaine 1991, Pureswaran and Borden 2003). Typically, attacks by bark beetles in such aggregations start with a few beetles and quickly progress to large assemblages (Byers 1989). It is likely that pheromone production first increases as assemblages grow and that beetles show preferences for assemblages with high production of pheromones, thereby facilitating large assemblages. Raffa and Berryman (1983) found that reproductive success of *D. ponderosae* decreased after densities reached high levels. Therefore, we would expect pheromone production and responses by mountain pine beetle to decrease in combination with an increased production of antiaggregation pheromones to further interrupt attraction of beetles (Byers 1989).

Our objective was to determine the effects of dose (release rate) on the attraction of *D. ponderosae* to its pheromones in stands of lodgepole pine. Our expectation is that such data should provide insights into the mass aggregation process and might lead to improved trap lures for *D. ponderosae.* We also determined the dose–response of two common bark beetle predators, *Enoclerus sphegeus* (F.) and *Thanasimus undatulus* (Say) (Coleoptera: Cleridae), to the same mountain pine beetle pheromones. Clerids respond to bark beetle pheromones and may exert selection pressures on pheromone traits (Raffa and Dahlsten 1995, Dahlsten et al. 2003). The combination of *cis*-verbenol and ipsenol is attractive to *E. sphegeus* (Miller et al. 1991), whereas (±)-*exo*-brevicomin is attractive to *T. undatulus* when combined with either myrcene or (−)-α-pinene (Miller and Lindgren 2000).

## Materials and Methods

**Semiochemical Release Devices.** Phero Tech (Delta, Canada) supplied release devices 1–6, 8–10, and 13–14 (Table 1), as well as a 13:87 mixture of *cis*- and *trans*-verbenol (chemical purities > 98%). Release devices 7, 11, and 12 consisted of open polypropylene centrifuge tubes (1.5 ml; Quality Scientific Plastics, Petaluma, CA) containing one or five 2-cm-long glass capillary tubes (ID, 1.5 mm; OD, 1.8 mm), each sealed at one end and filled with the verbenol

[1] Corresponding author, e-mail: dmiller03@fs.fed.us.
[2] College of Science and Management, University of Northern British Columbia, 3333 University Way, Prince George, BC, Canada V2N 4Z9.
[3] Phero Tech Inc., 7572 Progress Way, Delta, BC, Canada V4G 1E9.

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

| Devices | Chemical[a] | Description | Release rate (mg/d at 24°C) |
|---|---|---|---|
| 1 | (±)-*exo*-Brevicomin | Flex lure | 0.002 |
| 2 | (±)-*exo*-Brevicomin | Flex lure | 0.003 |
| 3 | (±)-*exo*-Brevicomin | Laminar lure | 0.01 |
| 4 | (±)-*exo*-Brevicomin | Flex lure | 0.03 |
| 5 | (±)-*exo*-Brevicomin | Laminar lure | 0.04 |
| 6 | (±)-*exo*-Brevicomin | Laminar lure | 0.10 |
| 7 | (±)-*exo*-Brevicomin | One glass capillary in centrifuge tube (1.5 ml) | 0.11 |
| 8 | (±)-*exo*-Brevicomin | Flex lure | 0.31 |
| 9 | (±)-*exo*-Brevicomin | Closed polyethylene centrifuge tube (250 μl) | 2.06 |
| 10 | Myrcene | Closed polyethylene screw-cap bottle (15 ml) | 280 |
| 11 | Verbenols[b] | One glass capillary in centrifuge tube (1.5 ml) | 0.12 |
| 12 | Verbenols[b] | Five glass capillaries in centrifuge tube (1.5 ml) | 0.23 |
| 13 | Verbenols[b] | Closed polyethylene centrifuge tube (250 μl) | 0.21 |
| 14 | Verbenols[b] | Polyethylene bubblecap | 2.58 |

[a] All chemical purities >95%.
[b] A 13:87 mixture of *cis*- and *trans*-verbenol (+17/−83).

mixture. Release rates for devices 1–6 and 8 (Table 1) were determined by collection of volatiles on Porapak-Q and quantitative analysis by gas chromatography. Release rates for all remaining devices were determined by weight loss.

**Experiments.** Seven experiments were conducted from 1988 to 1994 (Table 2) in an attempt to determine the effect of release rate (dose) on attraction of *D. ponderosae* to *cis*- and *trans*-verbenol (experiments 1–4) and *exo*-brevicomin (experiments 5–7). We employed a behavioral choice type of design in all our experiments. All treatments within a block were grouped within the same area, with traps spaced 10–15 m apart. Because all treatments had the same component pheromones, we would expect that the pheromone plumes from each treatment would blend together, spreading over a single area. Choices by beetles would be based on changes in pheromone concentrations in the air as they approach various traps rather than attraction from separate catchment areas of different sizes.

All experiments were set in mature stands of lodgepole pine, with experiments 1–3 and 5–7 conducted near Princeton, Canada, and experiment 4 conducted near Penticton, Canada. Population levels of *D. ponderosae* were low at locations used in experiments 1–3, 5, and 6, with infestation rates of standing trees by *D. ponderosae* at <5% in all these stands. The infestation rate by *D. ponderosae* was higher (>15%) in stands used in experiments 4 and 7. The trapping periods for experiments 1–7 were as follows: 14–24 August 1998; 20 August to 26 September 1991; 15 August to 22 September 1990; 17 August to 26 September 1991; 24 August to 4 September 1988; 6–20 August 1989; and 16–30 August 1994, respectively.

In experiments 1–3, 5, and 6, blocks of four or six eight-unit, multiple-funnel traps (Phero Tech) were set in grids of 2 by 2 or 2 by 3, respectively. In experiments 4 and 7, five traps were set in a pentagon grid formation. Replicate grids were placed at least 100 m apart, and traps were spaced 10–15 m apart within each replicate. Each trap was suspended between trees by rope such that the bottom of each trap was 0.2–0.5 m above ground level. No trap was within 2 m of any tree. A small piece (3 by 3 cm) of dichlorvos-impregnated wax bar (No Pest Strip; Loveland Industries, Greeley, CO) was added to each cup to kill beetles and prevent damage by predators.

In experiments 1–3, 5, and 6, treatments were randomly assigned to traps within each block as follows: a control treatment lacking the pheromone and three or five treatments consisting of the target pheromone released at three or five different rates, respectively (Table 2). In experiment 1, the five different release rates of verbenols, in increasing order, were obtained by the following device (Table 1) combinations: (1)

**Table 2. Summary of pheromone dose response experiments**

| Experiment | Pheromone | Replicates | Release rate class (mg/d at 24°C) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Control | 1 | 2 | 3 | 4 | 5 |
| 1 | Verbenol mix[a] | 4 | 0 | 0.12 | 0.23 | 1.15 | 5.16 | 25.8 |
| 2 | Verbenol mix[a] | 10 | 0 | 0.23 | 2.58 | 12.91 | — | — |
| 3 | Verbenol mix[a] | 8 | 0 | 0.21 | 0.84 | 2.58 | 7.74 | 25.8 |
| 4 | Verbenol mix[a] | 7 | — | 0.21 | 0.63 | 1.68 | 2.58 | 5.16 |
| 5 | *exo*-Brevicomin[b] | 5 | 0 | 0.11 | 0.31 | 1.24 | 2.06 | 12.36 |
| 6 | *exo*-Brevicomin[b] | 10 | 0 | 0.01 | 0.04 | 0.10 | — | — |
| 7 | *exo*-Brevicomin[b] | 8 | — | 0.002 | 0.005 | 0.009 | 0.03 | 0.31 |

[a] All baited with *exo*-brevicomin and myrcene (0.1 and 280 mg/d, respectively, at 24°C).
[b] All baited with verbenol mix and myrcene (2.58 and 280 mg/d, respectively, at 24°C).

1 device 11; (2) 1 device 12: (3) 4 device 12; (4) 2 device 14; and (5) 10 device 14. The two lowest rates used in experiment 2 were obtained by using one device 12 and one device 14, respectively, whereas the highest rate was obtained with five device 14. The following device combinations were used for verbenols in experiment 3: (1) 1 device 13; (2) 3 device 13; (3) 1 device 14; (4) 3 device 14; and (5) 10 device 14. In experiment 4, the five release rates were obtained with the following: (1) one device 13; (2) three device 13; (3) eight device 13; (4) one device 14; and (5) two device 14.

In experiment 5, the five different release rates of *exo*-brevicomin, in increasing order, were obtained by the following device combinations: (1) one device 7; (2) one device 8; (3) four device 8; (4) one device 9; and (5) six device 9. In experiment 6, the three rates were obtained by using one device 3, one device 5, and one device 6. The following device combinations were used in experiment 7: (1) one device 1; (2) one device 1 and one device 2; (3) three device 2; (4) one device 4; and (5) one device 8.

In experiments 1–3, the control traps were baited with myrcene and *exo*-brevicomin, whereas control traps in experiments 5 and 6 were baited with myrcene and *cis*- and *trans*-verbenol. Myrcene is a host compound that synergizes the attraction of *D. ponderosae* to the pheromones *trans*-verbenol and *exo*-brevicomin (Billings et al. 1976, Borden et al. 1983, Miller and Lindgren 2000). In all experiments, the compounds used in the control trap were present in all traps within each block. There were no control traps in experiments 4 and 7. Myrcene was present in all traps in experiments 4 and 7 with *exo*-brevicomin, released from device 3, in all traps in experiment 4, and the verbenol mixture, released from device 9, was present in all traps in experiment 7.

Sexes of *D. ponderosae* in trap catches in experiment 3 were determined by dissection and examination of genitalia. Voucher specimens were deposited at the Entomology Museum, Simon Fraser University.

**Statistical Analyses.** Data were analyzed using the SYSTAT statistical package version 10.2 and the SYSTAT TableCurve 2D curve fitting package ver. 5.01 (SYSTAT Software, Richmond, CA). Trap catch data (total number of beetles caught by a single trap) were transformed by $\ln(Y)$, or $\ln(Y + 1)$ if at least one trap had no beetles, to remove heteroscedasticity (Pepper et al. 1997). Sex ratio data (expressed as proportion of males in trap catches) were not transformed. Data in all experiments were subjected to two-way analysis of variance (ANOVA), using replicate and treatment as model factors, and by Tukey's honestly significant difference (HSD) multiple comparison test. Regression analyses were conducted against the release rate of pheromones, transformed by $\ln(X)$, for all data excepting catches associated with controls, because zero release rate is not definable by the transformation. For each regression, residuals were examined to ensure the appropriateness of either a linear or curvilinear model.

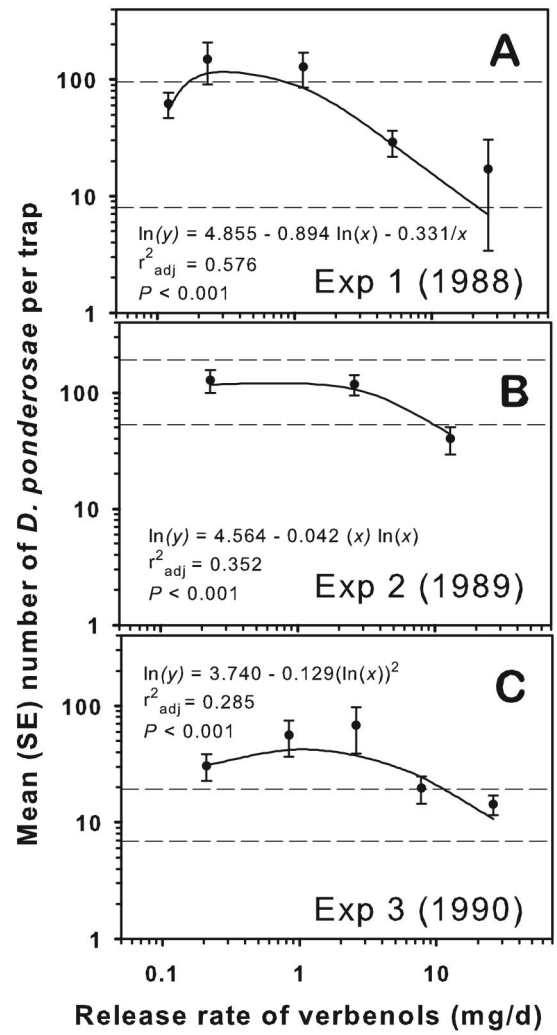


**Fig. 1.** The effects of a 13:87 mixture of *cis*- and *trans*-verbenol, released at various rates, on catches (±SE) of *D. ponderosae* in multiple-funnel traps baited with myrcene and *exo*-brevicomin in 1988 (A), 1989 (B), and 1990 (C) (experiments 1–3, respectively). Dashed horizontal lines represent confidence limits (95%) for mean catches in control traps with no release of verbenols.

## Results

Regression analyses showed a strong curvilinear relationship between trap catches of *D. ponderosae* and release rate of verbenols in 1988–1990 (Fig. 1), consistent with a multifunctional pheromone. Typically, multifunctional pheromones are attractive to conspecifics at low rates of release but become interruptive or repellent at high release rates (Borden 1996). We found that catches of *D. ponderosae* initially increased with increasing rates of release of verbenols but decreased as release rates reached higher levels. For data collected in 1988–1990 (experiments 1–3), pheromone dose accounted for 29–58% of the variation in trap catches. The total numbers of beetles captured in

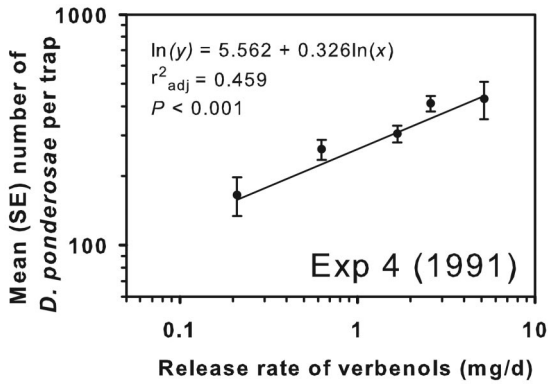


**Fig. 2.** The effects of a 13:87 mixture of *cis*- and *trans*-verbenol, released at various rates, on catches (±SE) of *D. ponderosae* in multiple-funnel traps baited with myrcene and *exo*-brevicomin in 1991 (experiment 4).

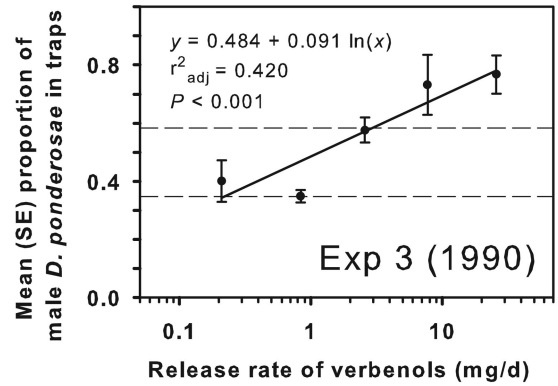


**Fig. 3.** The effects of a 13:87 mixture of *cis*- and *trans*-verbenol, released at various rates, on the proportion of males (±SE) in trap catches of *D. ponderosae* in multiple-funnel traps baited with myrcene and *exo*-brevicomin in 1990 (experiment 3). Dashed horizontal lines represent confidence limits (95%) for proportion of males in control traps with no release of verbenols.

experiments 1–3 were 1,749, 4,073, and 1,610, respectively, with maximum catches of 276, 359, and 265 beetles/trap, respectively.

In 1988 (experiment 1), there was a significant treatment effect on catches of *D. ponderosae* ($F = 9.830$; df = 5,15; $P < 0.001$), with catches in traps baited with verbenol released at the highest rate significantly lower than those baited with verbenol released at the three lowest rates (Tukey's HSD test, $P < 0.05$; Fig. 1A). Similarly, there were significant treatment effects in 1989 (experiment 2) and 1990 (experiment 3) on catches of beetles ($F = 10.160$; df = 3,27; $P < 0.001$ and $F = 7.174$; df = 5,32; $P < 0.001$, respectively). Catches of beetles in experiment 2 were lower in traps baited with verbenols released at the highest rate than in all other traps (Tukey's HSD test, $P < 0.05$; Fig. 1B). In experiment 3, the lowest catches were in control traps and those baited with verbenol released at the highest rate (Tukey's HSD test, $P < 0.05$; Fig. 1C).

Catches of *D. ponderosae* were affected by treatments in experiment 4 as well ($F = 10.464$; df = 4,24; $P < 0.001$). However, in contrast to 1988–1990, catches of beetles in 1991 (experiment 4) were directly proportional to the release rate of verbenols without showing any evidence of interruption (Fig. 2). Pheromone dose accounted for almost one-half of the variation in trap catches. The stands used in 1991 differed from those used in previous years by having infestation rates >15% of standing lodgepole pine by *D. ponderosae.* The total number of *D. ponderosae* captured in experiment 4 was 11,408, with catches ranging from 51 to 746 beetles/trap.

The dose-dependent effect of verbenols on catches of *D. ponderosae* to traps baited with myrcene and *exo*-brevicomin in 1990 was not the same for both sexes ($F = 6.223$; df = 5,32; $P < 0.001$). The proportion of males in catches of beetles in experiment 3 (1990) increased as the release rate of verbenols increased, with pheromone dose accounting for 42% of the variation in proportion of males in trap catches (Fig. 3). Data on sex ratios were not collected in 1988, 1989, and 1991.

There was little effect of *exo*-brevicomin release rate on catches of *D. ponderosae* in traps baited with myrcene and verbenols (Fig. 4). The total numbers of beetles captured in experiments 5–7 were 4,200, 3,547, and 9,230, respectively, with maximum trap catches of 378, 241, and 572, respectively. There was a treatment effect in 1988 (experiment 5; $F = 3.990$; df = 5,20; $P = 0.011$), with a significant negative relationship between catches of beetles and release rate of *exo*-brevicomin (Fig. 4A). However, pheromone dose accounted for only 17% of the variation in trap catches. There was no significant treatment effect in the low-range experiments conducted in 1989 (experiment 6) and 1994 (experiment 7; $F = 0.897$; df = 3,27; $P = 0.456$ and $F = 0.835$; df = 4,28; $P = 0.515$, respectively). Sex ratio data were not collected in experiments 5–7. The mean catch of beetles in traps baited with myrcene, *exo*-brevicomin, and verbenols in experiments 6 and 7 were 82.9 ± 8.1 and 230.8 ± 22.4/trap (SE), respectively.

Predators were not very abundant, with total catches not exceeding 265 beetles for any single experiment. The total number of *E. sphegeus* caught in experiments 1 and 3 was only four. In experiment 2, there was a significant verbenol treatment effect on *E. sphegeus* ($F = 7.416$; df = 3,24; $P = 0.001$), with a total of 34 beetles caught. Catches of beetles were directly proportional to the release rate of verbenols, although pheromone dose accounted for only 38% of the variation in trap catches (Fig. 5A). A similar but weaker relationship was found for *E. sphegeus* in experiment 4, with a total of 48 beetles captured (Fig. 5B). Pheromone dose explained very little of the variation in trap catches (6%). The total number of *T. undatulus* captured in experiments 1–3 was only 14. In experiment 4, there were 128 *T. undatulus* captured, with no significant verbenol treatment effect ($F = 1.807$; df = 4,28; $P = 0.155$). The mean trap catch of *T. undatulus* in experiment 4 was 3.2 ± 0.5.

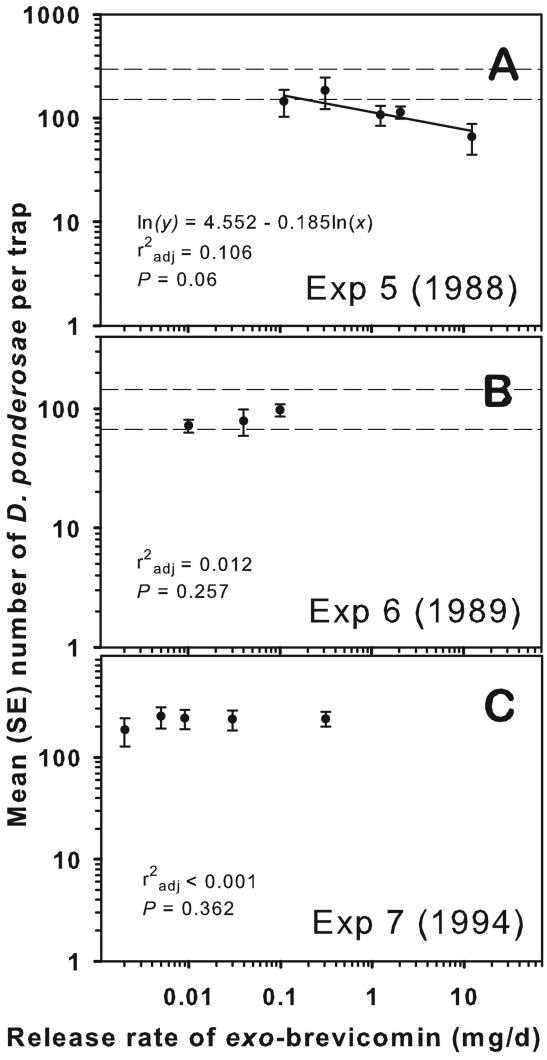


**Fig. 4.** The effects of *exo*-brevicomin, released at various rates, on catches (±SE) of *D. ponderosae* in multiple-funnel traps baited with myrcene and a 13:87 mixture of *cis*- and *trans*-verbenol in 1988 (A), 1989 (B), and 1994 (C) (experiments 5–7, respectively). Dashed horizontal lines represent confidence limits (95%) for catches in control traps with no release of *exo*-brevicomin.

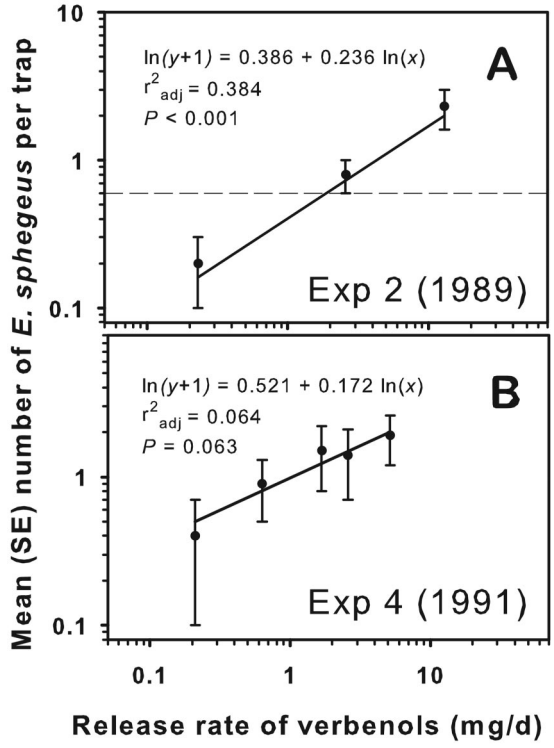


**Fig. 5.** The effects of a 13:87 mixture of *cis*- and *trans*-verbenol, released at various rates, on catches (±SE) of *E. sphegeus* in multiple-funnel traps baited with myrcene and *exo*-brevicomin in 1989 (experiment 2) (A) and 1991 (experiment 4) (B). Dashed horizontal line represents upper bound of confidence limits (95%) for catches in control traps with no release of verbenols. There were no control traps in experiment 4.

The total numbers of *T. undatulus* and *E. sphegeus* captured in experiment 5 were 68 and 21. There was a significant effect of *exo*-brevicomin treatment on *T. undatulus* ($F = 4.841$; df = 5,20; $P = 0.005$) but not on *E. sphegeus* ($F = 2.291$; df = 5,20; $P = 0.084$). Catches of *T. undatulus* were directly proportional to the release rate of *exo*-brevicomin, with pheromone dose explaining 25% of the variation in trap catches (Fig. 6A). The mean catch of *E. sphegeus* in traps baited with myrcene, *exo*-brevicomin, and verbenols in experiment 5 was 0.7 ± 0.2 beetles/trap.

In experiment 6, we captured 23 *T. undatulus* and 17 *E. sphegeus*, with no significant treatment effect for either species ($F = 0.746$; df 3,18; $P = 0.539$ and $F = 1.092$; df = 3,24; $P = 0.371$, respectively). There was no significant relationship between catches of *T. undatulus* and release rate of *exo*-brevicomin in the low-range experiment (Fig. 6B). The mean catch of *E. sphegeus* in traps baited with myrcene, *exo*-brevicomin, and verbenols in experiment 6 was 0.6 ± 0.1 beetles. There were no predators captured in experiment 7.

## Discussion

The benefit of mass aggregation behavior in bark beetles has generally been ascribed to overcoming tree defenses to establish breeding galleries, often involving pathogenic fungi (Raffa et al. 1993). For *D. ponderosae* in lodgepole pine stands in northeastern Oregon, trees survived and beetles died when attack densities were <40 galleries/$m^2$ of bark surface (Raffa and Berryman 1983). Reproductive success of individual beetles decreased when attack densities exceeded 80 galleries/$m^2$, suggesting an optimal density range of 40–80 attacks/$m^2$.

Our results in 1988–1990 on the response of *D. ponderosae* to the verbenols are consistent with the

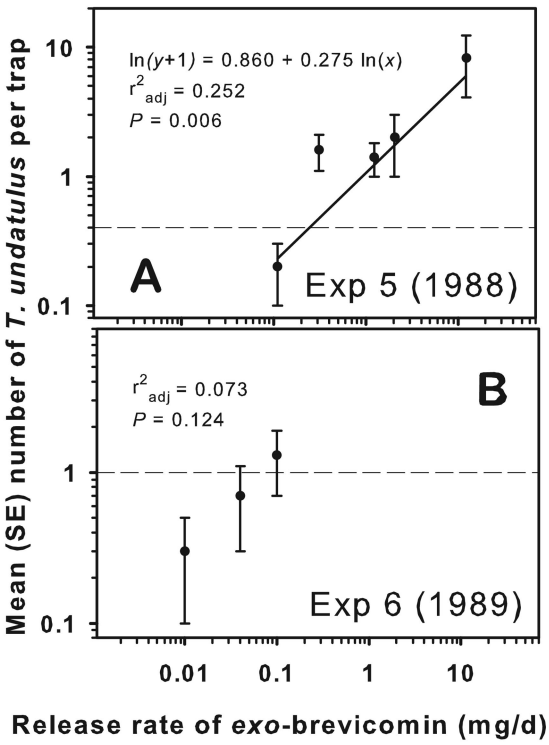


**Fig. 6.** The effects of *exo*-brevicomin, released at various rates, on catches (±SE) of *T. undatulus* in multiple-funnel traps baited with myrcene and a 13:87 mixture of *cis*- and *trans*-verbenol in 1988 (A) and 1989 (B) (experiments 5 and 6, respectively). Dashed horizontal line represents upper bound of confidence limits (95%) for catches in control traps with no release of *exo*-brevicomin.

optimal attack density hypothesis suggested by Raffa and Berryman (1983) for attacks on a single tree, suggesting that pheromone dose could be a mechanism facilitating selection for optimal densities. Female *D. ponderosae* are the first to arrive and initiate attacks, producing verbenols that attract both male and female beetles (Borden et al. 1987, Miller and Lafontaine 1991). We found that beetle attraction first increases and then decreases with increasing release rate of verbenols (Fig. 1A–C). Using the critical range of 40–80 galleries/$m^2$ for attacks by *D. ponderosae* on lodgepole pine (Raffa and Berryman 1983), one would expect a range of 16–32 individual galleries over the surface area of a cylinder defined by an eight-unit funnel trap (0.4 $m^2$) with release rates of 6–13 mg/d for *trans*-verbenol over the same area, assuming a production rate of 0.4 mg/d/individual female (Hunt et al. 1989). Our results showing maximal attraction of beetles to traps releasing verbenols at 0.3–3 mg/d (Fig. 1A–C) are not inconsistent with this very rough approximation, particularly when one considers that females cease producing verbenols once they are joined by male consorts (Pureswaran and Borden 2003). Our data in 1990 show that male beetles are less sensitive than females to release rate of verbenols (Fig. 3), suggesting different optimality curves for the sexes.

After attraction to suitable hosts, male *D. ponderosae* produce *exo*-brevicomin, which attracts more beetles, especially females (Rudinsky et al. 1974, Borden et al. 1983, 1987, Conn et al. 1983, Shore et al. 1992). The production of *exo*-brevicomin by males drops sharply, however, after they join females in their galleries (Pureswaran and Borden 2003). We were unable to show an increase in attraction of beetles to traps with *exo*-brevicomin, even at low release rates (Fig. 4B and C).

Some of the discrepancy between our results and those of other researchers may relate to distance between traps. In our experiments, all treatments were grouped within the same area, with traps spaced 10–15 m apart in grid patterns. In contrast, Rudinsky et al. (1974) set sticky screen traps at a spacing of >100 m, whereas Conn et al. (1983) and Borden et al. (1987) set multiple-funnel traps at distances >25 m, all in linear arrangements. It was our intention that the catchment area for traps would overlap, providing opportunities for beetles to select among traps with lures releasing at different rates. In designs used by Rudinsky et al. (1974), Conn et al. (1983), and Borden et al. (1987), the spacing between traps may have been sufficient to produce separate catchment areas, resulting in beetles choosing an area with *exo*-brevicomin as opposed to one without *exo*-brevicomin. In analyzing results from previous studies on *Ips paraconfusus* Lanier and *I. typographus* L., Byers (1999) determined that the radii of the catchment area around a baited trap ranged from 3.27 to 15.9 m for *I. typographus* and from 0.25 to 34.5 m for *I. paraconfusus.*

Some of the variation may also be because of the lack of tree silhouette and associated volatiles. Attacks by *D. ponderosae* are generally conducted in the context of whole trees, usually upright, with attacks over the lower 6–12 m of bole, depending on tree diameter (Safranyik et al. 1974, Amman and Cole 1983). In studies by Borden et al. (1983) and Shore et al. (1992), the effectiveness of *exo*-brevicomin was shown by attaching lures directly on mature trees. Activities on one part of the tree might affect activities on another part of the tree, with numerous pheromones and host kairomones playing significant roles in the semiochemical ecology of *D. ponderosae* (Lindgren and Borden 1989, Miller and Lindgren 2000, Miller and Borden 2003; Pureswaran and Borden 2003). Borden et al. (1983) found that *exo*-brevicomin increased the number of mass-attacked lodgepole pines, spaced ≈50 m apart, in combination with 3-carene, 3-carene-10-ol, and *trans*-verbenol.

As gallery density on the bole of a tree increases, the likelihood that beetle attacks switch to adjacent trees increases, possibly mediated by antiaggregation pheromones (Geiszler and Gara 1978). Compounds such as *exo*-brevicomin seem to act as antiaggregation pheromones when they are released at high rates (Rudinsky et al. 1974, Borden et al. 1987). In our studies, interruption of attraction of *D. ponderosae* occurred with devices releasing *exo*-brevicomin at high rates (Fig. 4A). Ryker and Rudinsky (1982) found that *exo*-brevicomin released at 12 mg/d significantly in-

terrupted attraction of *D. ponderosae* to sticky screen traps baited with terpenes and *cis*- and *trans*-verbenol at a trap spacing of 10–30 m.

Some of the interruption seen with verbenols on the attraction of *D. ponderosae* (Fig. 1A–C) may be partly attributed to verbenone. Hunt et al. (1989) showed that verbenol can autoxidise to verbenone, independent of beetles. Verbenone produced from verbenols interrupts attraction of beetles to baited traps (Rudinsky et al. 1974, Ryker and Yandell 1983, Libbey et al. 1985, Hunt and Borden 1990, Miller et al. 1995). As yet, we do not know the fate of verbenols after they are released from lures.

We found that the responses of *D. ponderosae* in experiment 4, conducted in 1991, were significantly different from the responses of *D. ponderosae* in experiments 1–3, conducted in 1988–1990 (Fig. 1A–C). Beetle response in 1991 was directly proportional to the release rate of verbenols, with no evidence of interruption in attraction at high release rates (Fig. 3). The location used in experiment 4 was different from that used for experiments 1–3. However, the distance between the two locations is <50 mi, with extensive and contiguous stands of mature lodgepole pine between them and no physical barrier that might impede gene flow. Stands at both locations were similar in elevation, aspect, and species composition, with similar histories of *D. ponderosae* activity. The only significant difference between the two locations seemed to relate to population levels of *D. ponderosae.* Unlike the other three experiments that were conducted in stands with low population levels of *D. ponderosae* (<5% of standing trees attacked by beetles), experiment 4 was conducted in a stand with epidemic levels of *D. ponderosae* (>15% standing trees attacked).

Our results in experiments 1–3 are consistent with the suggestion by Raffa and Berryman (1987) and Birgersson et al. (1988) that selection pressures on beetles may be related to population densities. At low population levels, individual *D. ponderosae* may be selected for strong discriminating behaviors, ensuring that they join groups at the optimal density, because the likelihood of recruiting sufficient beetles to ensure successful attacks may be low. However, at high population levels, selection may not favor individuals with such behaviors. Alternatively, it is possible that beetles are able to assess population numbers and adjust their behavior accordingly (Wallin and Raffa 2004). In areas with abundant numbers of conspecifics, beetles may be selected for their ability to find large spot infestations rather than small spots or individual trees as the former areas might have a higher probability of successful attacks compared with small spot infestations. Individual trees or small spots may become overpopulated more rapidly than large spots. Larger spots would have a greater number of opportunities for new arriving beetles because there would likely be more available sites as well as a greater probability of having sufficient numbers of beetles to mass attack adjacent unattacked trees. The strong dose-dependent increase in attraction of *D. ponderosae* we noted in 1991 is consistent with this argument (Fig. 2).

Attempts to understand the interactions of all possible behavioral choices by bark beetles should be complemented by attempts to understand how such variation is maintained within populations and how changes in behaviors occur over short time frames. Hager and Teale (1996) found a genetic basis to pheromone use by *I. pini.* Host acceptance by *I. pini* based on concentration of $\alpha$-pinene in phloem is strongly heritable (Wallin et al. 2002). Genetic variation related to host types, geographic locality, thickness of phloem, and season has been shown for *D. ponderosae* (Stock and Amman 1980, Sturgeon and Mitton 1986, Langor and Spence 1991, Amman and Stock 1995), and Stock et al. (1992) suggested that population phase of aggressive bark beetles may be related to genetic heterozygosity.

The semiochemical ecology of bark beetles can also be influenced by the behaviors of predators (Raffa 2001). Variation in the use of pheromones by *I. pini* may be related to selection pressures from *T. dubius* (F.) (Herms et al. 1991, Aukema and Raffa 2000). Both predators, *T. undatulus* and *E. sphegeus*, exhibited a dose-dependent response to some of the pheromones produced by *D. ponderosae* (Figs. 5 and 6). In both species, adults and larvae prey on adult and larval *D. ponderosae*, respectively, and sites with higher rates of production are probably associated with higher numbers of adult and larval prey. *Thanasimus undatulus* and *E. lecontei* (Wolcott) respond in a dose-dependent fashion to the bark beetle pheromones, ipsenol and lanierone, respectively, produced by *Orthotomicus latidens* (LeConte) and *I. pini* (Miller et al. 2005). Selection should favor bark beetles that cease pheromone production in a prompt fashion after achieving a critical attack density and an appropriate mate.

However, much is yet to be learned about the semiochemical ecology of predators and the interaction with the semiochemical ecology of bark beetles. It is not clear why *E. sphegeus* responds to verbenols (Fig. 5) but not *exo*-brevicomin, because both are produced by *D. ponderosae.* Similarly, it is not clear why *T. undatulus* responds to *exo*-brevicomin (Fig. 6) but not verbenols. Previously, Miller and Lindgren (2000) found a similar effect of *exo*-brevicomin on *T. undatulus.*

It is possible that the differences in responses may help to minimize interspecific competition between the two species, either for adults or larvae. Pureswaran and Borden (2003) found a distinct temporal sequence in the production of *trans*-verbenol and *exo*-brevicomin by *D. ponderosae. trans*-Verbenol is only produced by unmated females after attacking a tree, whereas *exo*-brevicomin is produced later by unmated males as they attempt to join females. It is possible that the competitive abilities of the two species differ and that beetles such as *E. sphegeus* may do better if their larvae can get established before those of *T. undatulus* even though there is a possibility that the attacks by *D. ponderosae* may not be successful. Waiting until production of *exo*-brevicomin is prominent may indicate a more secure environment for larvae of *T. undatulus*

as production of verbenols may occur before a host is overcome.

Alternatively, the responses of *E. sphegeus* and *T. undatulus* may relate to their preferred prey items when *D. ponderosae* are not abundant. Various secondary bark beetles use pheromones, many of which are yet to de determined. The combination of *cis*-verbenol with ipsenol is attractive *E. sphegeus* (Miller et al. 1991). The opportunities for speculation are endless and will require considerable research (Raffa 2001).

In addition, more research is required to document the variation in the dose–response behavior of *D. ponderosae* to verbenols, particularly as it relates to epidemiology of beetle populations. If our results are found to be consistent with other populations, there is a possibility that the ratio of beetle catches between two to three traps, releasing verbenols at different rates, could be used to monitor populations of *D. ponderosae* and determine whether the population cycle is in incipient, epidemic, or declining phase. Such a possibility would also require knowledge on the phenology of such changes within populations to determine the benefit of such a system in early detection of population changes.

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