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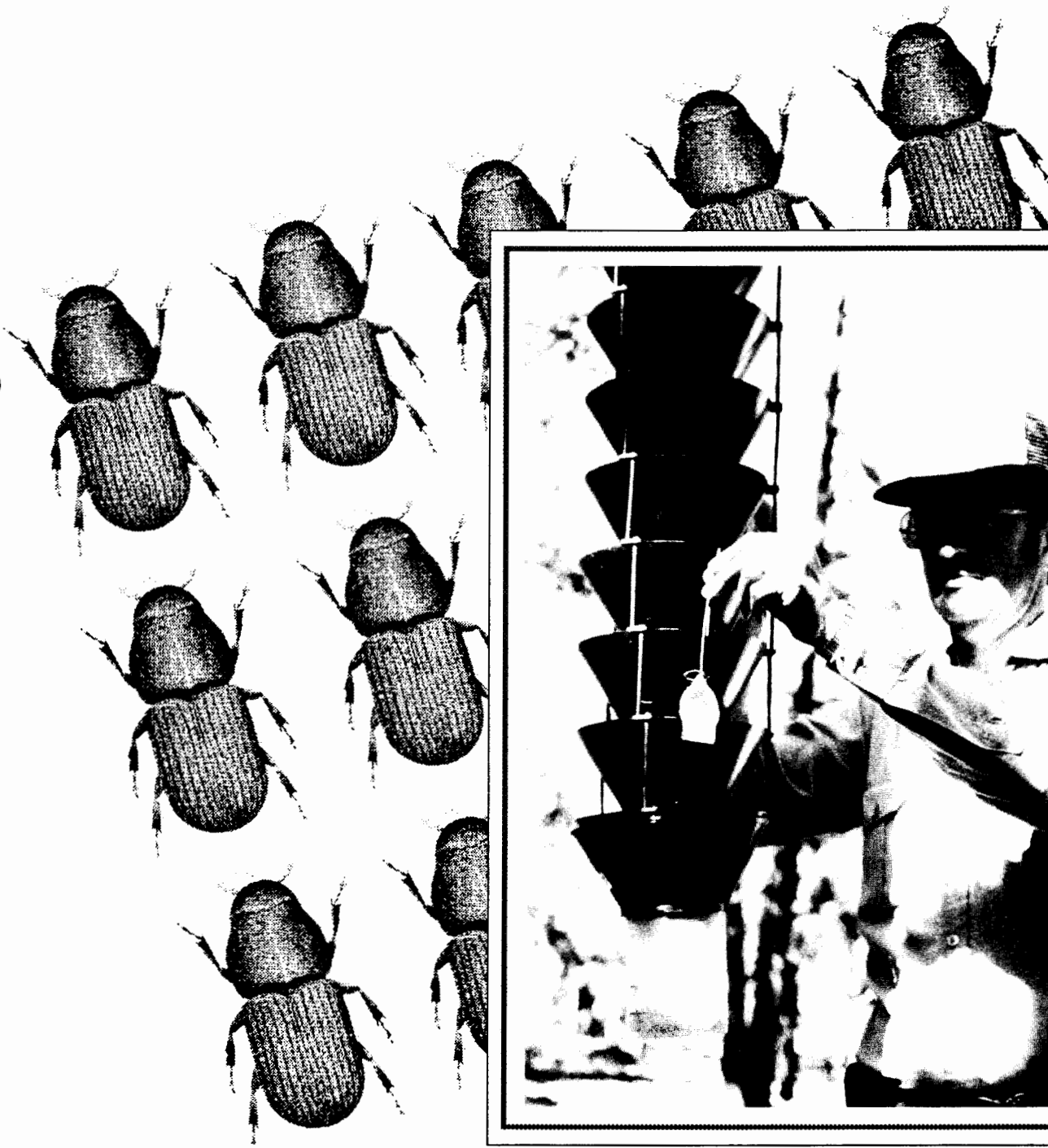
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# Antiaggregative Effect of Verbenone on Response of the Mountain Pine Beetle to Baited Traps

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## RESEARCH SUMMARY

Response of the mountain pine beetle (MPB) (*Dendroctonus ponderosae* Hopkins) to Lindgren funnel traps baited with a commercial lure (*trans*-verbenol, *exo*-brevicomin, and myrcene), alone and in combination with verbenone,

an aggregation-inhibiting pheromone, was measured in a lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) stand in Utah. Treatments tested were (1) single verbenone capsule alone, (2) single three-component MPB lure, (3) single three-component MPB lure and verbenone bubble cap, and (4) unbaited trap. Treatments were randomly assigned to four traps in each of eight test blocks. A total of 1,130 MPB were trapped during the flight period, with the greatest percentage (95.8) responding to the MPB bait alone. ANOVA revealed the numbers of MPB caught among treatments for the eight blocks combined were significantly different ( $P < 0.001$ ). Tukey's Studentized Range Test revealed the treatments separated into two significantly different sets: (1) those three treatments for which fewest (4.2 percent) MPB were caught (verbenone, 0.6 percent; lure with verbenone, 1.7 percent; unbaited trap, 1.9 percent), and (2) that catching the greatest percentage (95.8 percent), the MPB lure alone. The lure alone, verbenone alone, and unbaited trap caught more females than males; respective sex ratios (males: females) were 0.57, 0.40, 0.50, while verbenone combined with the lure caught more males than females, sex ratio 2:1. Fisher's Exact Test revealed that treatment and response by sex were not independent, and when verbenone is added to the lure, more males than females will be caught.

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## INTRODUCTION

The chemical ecology of the mountain pine beetle (MPB) (*Dendroctonus ponderosae* Hopkins) like that of most other bark beetles, has proven to be especially difficult and costly to solve. The difficulty is due in large measure to the many compounds present in each of these semiochemical systems, the difficulty of isolating and identifying these chemical messengers, and the extensive field testing required to determine the behavioral response each elicits alone and in combination with the other components throughout a species' range. Components of these semiochemical systems and their effect on beetle behavior are yet to be determined (Lindgren and Borden 1989). As a result, most synthetic systems currently being tested do not elicit the same magnitude of beetle response as the natural systems (Lindgren and Borden 1989).

The advent of improved technology for isolating and identifying candidate compounds promises to speed total understanding of these semiochemical communication systems (Gries and others 1988). Until then, compounds that have been isolated and identified need to be field tested to determine the extent of variation in beetle response throughout its geographic range and under different test conditions—including a range in release rates, stand conditions, stand microclimate, and interactions with other pheromone and host tree components. The knowledge is needed to develop new strategies or to refine existing strategies (McGregor and others 1989) for manipulating localized populations. The intent is to integrate these strategies with existing silvicultural prescriptions (McGregor and others 1987) to prevent populations from reaching intolerable levels that require costly control. The purpose of the test reported here was to assess the antiaggregative effect of the (-) - enantiomer of verbenone when added to the standard commercial MPB lure, consisting of *exo*-brevicomin, *trans*-verbenol, and myrcene, on response of MPB populations in Utah. More specifically, we sought to determine if the anticipated reduction in MPB trapped would warrant further testing of verbenone for use in preventing MPB infestations in high-value stands, such as those in riparian zones, campgrounds, and critical big game habitat.

## PRIOR RESEARCH

Discovery that bark beetles use a complex semiochemical communication system to attract and concentrate populations in sufficient numbers to overcome host resistance has led to efforts to exploit the system for bark beetle control (Anderson 1948; Chapman 1966; Wood 1962). Early efforts deployed attractive elements in a manner designed to lure flying beetles to lethal traps or to trees scheduled for harvesting (Pitman and Vité 1969). Unfortunately, beetle populations attracted to baited stands often "spilled over" into adjacent stands which the land manager intended to protect (Borden and others 1983; Rudinsky and others 1972). At the same time, efforts to isolate and identify the remaining major components of bark beetle pheromones revealed the presence of an antiaggregative element in the pheromones of several species, that, when deployed alone or in combination with the attractive elements, significantly reduced the number of beetles caught (Rudinsky and others 1972). A granular controlled-release formulation of MCH (3-methyl-2-cyclohexen-1-one), the antiaggregative element in the Douglas-fir beetle (DFB) (*D. pseudotsugae* Hopkins) pheromone, reduced infestations in windthrown trees (McGregor and others 1984).

Early isolation efforts revealed verbenone was a component of semiochemical systems of several species of *Dendroctonus*, and ensuing field tests showed it had antiaggregative properties (Renwick 1967). Renwick and Vité (1970) determined that the addition of verbenone to the attractive lure for the southern pine beetle (SPB) (*Dendroctonus frontalis* Zimmerman) reduced the number of males and females responding to traps, while Richerson and Payne (1979) found that a mixture of verbenone plus *exo*-brevicomin and *endo*-brevicomin reduced landing of SPB on unattacked trees, as well as reducing the density of emerging beetles on infested trees (Watterson and others 1982). But laboratory bioassays of SPB pheromones produced by beetles collected from three regions within its range have shown a significantly higher percentage of positive responses to pheromones produced by SPB from their respective regions, suggesting regional variation in their content (Berisford and Payne 1989). Similar geographic variation in pheromone composition and beetle response has been reported for the striped ambrosia beetle (*Trypodendron lineatum* Oliver) (Borden and others 1982) and the pine engraver beetle (*Ips pini* [Say]) (Lanier and others 1972; Miller and others 1989). Bedard and coworkers

(1980) found the addition of verbenone to the attractive lure reduced the catch of the western pine beetle (WPB) (*Dendroctonus brevicomis* Hopkins). Field tests utilizing verbenone 60:40 mix of (+) and (-) enantiomers released at four rates revealed that elution rates of 4.74 mg/24 h or greater reduced WPB catch at traps baited with its attractive lure by 92 percent or more (Tilden and Bedard 1988). Verbenone has also been shown to reduce response of *Dendroctonus adjunctus* (Blandford) to attractive bolts (Livingston and others 1983) and inhibit response of *Ips paraconfusus* (Lanier) (Byers and Wood 1981) and *Ips typographus* (L.) (Bakke 1981; Schlyter and others 1989). The first evidence that verbenone might function as an antiaggregant for the MPB resulted from laboratory and field bioassays that revealed (-) - verbenone inhibited response to selected semiochemicals (Ryker and Yandell 1983), although Pitman and collaborators (1969) were first to isolate trace amounts from the MPB. This isolation was later confirmed by Rudinsky and coworkers (1974).

In addition to its presence in the beetle, verbenone is produced by the auto-oxidation of *trans*-verbenol and *cis*-verbenol (Lindgren and Borden 1989), and there is also evidence for production of verbenone by oxidation of these compounds by yeasts commonly associated with the MPB (Hunt and Borden 1989). Lindgren and Borden (1989) have described the sequence of pheromone production for the MPB in lodgepole pine and the role of verbenone in generating an antiaggregative signal. This signal limits the density of beetle attack to a range that prevents overcrowding of the host tree.

Field tests in British Columbia to determine the antiaggregative activity of verbenone at two release rates (1.0 or 5.0 mg/24 h) revealed that verbenone significantly reduced male response to the commercial synthetic lure (*trans*-verbenol, *exo*-brevicomine, and myrcene) at both release rates, but not female response, although the reduction in female response approached significance (Borden and others 1987). Two large-scale field tests in Idaho (Amman and others 1989) and British Columbia (Lindgren and others 1989) that evaluated the effectiveness of verbenone for preventing attacks in susceptible lodgepole pine stands confirmed that verbenone has promise for manipulating local MPB populations.

## STUDY DESCRIPTION

### Test Location

The test was performed in a 110-year-old lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) stand, elevation 9,450 ft, approximately 8 miles south of Mountain View, WY, in the Wasatch National Forest. Stands within the test blocks were pure lodgepole pine, with exception of a few quaking aspen (*Populus tremuloides* Michx.). Average diameter breast height (d.b.h.) of lodgepole pines 5 inches and larger was 9.1 inches. The surrounding forest and stands within the eight test blocks had been infested by MPB populations that had been building in numbers in previous years. The test blocks were located on a flat bench without intervening physiographic features that might alter air movement within or between blocks.

## Test Design

The antiaggregative effect of verbenone was assessed in eight blocks 100 ft square. The blocks were arranged in two rows of four, with the rows oriented in an east-west direction and a separation of 100 ft between blocks (fig. 1). The 16-unit Lindgren multiple funnel traps (Lindgren 1983) were positioned at 100-ft intervals, at the corners of each block (fig. 2). Traps were suspended by nylon cord from tree limbs so that they could be lowered to collect the beetles that were caught. A standard trap height was obtained by positioning the plastic containment device at the base of the funnels, 6 ft above ground. Traps were suspended from nonhost trees, old dead lodgepole pines, or lodgepole pines with diameters less than 5 inches (breast height) to minimize the possibility that responding MPB might infest trees on which traps were hung and thereby alter the composition of the assigned treatment.

The four treatments tested were (1) single verbenone bubble cap alone (fig. 2); (2) single three-component MPB tree bait alone (fig. 3); (3) single verbenone bubble cap and a three-component MPB lure; and (4) unbaited trap. The treatment components were suspended in the funnel openings from wire hangers clipped to the lip of the funnels in the order shown in fig. 3. Verbenone bubble caps were suspended from the hangers in the same manner and position as that for the *trans*-verbenol bubble caps. Release rates and devices for each semiochemical treatment are shown in table 1.

Treatments were randomly assigned to the four traps in each block. Traps were in place and baited 2 weeks prior to the onset of beetle flight. Collections were made weekly thereafter, and the insects caught were placed in 70 percent alcohol and taken to the laboratory for specific determination.

## RESULTS AND DISCUSSION

A total of 1,130 MPB were trapped in the eight blocks, with the greatest percentage (95.8) responding to the MPB bait alone (table 2). Analysis of variance revealed the total numbers of MPB caught among treatments for the eight blocks combined were significantly different ( $P < 0.001$ ). Tukey's Studentized Range Test showed the treatments separated into two sets (table 2). The treatments for which the fewest MPB were caught included the MPB lure with verbenone (1.7 percent), verbenone alone (0.6 percent), and the unbaited trap (1.9 percent). These three treatments accounted for only 4.2 percent of the MPB trapped. The MPB lure alone, which accounted for 95.8 percent of the MPB trapped, comprised the second set that differed significantly from the first set. The results show that the addition of (-) verbenone significantly reduces the number of MPB responding to the synthetic lure. Comparison of MPB response to the four treatments among the eight blocks revealed that the variation in the percentage of MPB caught by treatment was minimal (fig. 4). The single exception was recorded in block eight, where the percentage caught in the unbaited trap was slightly higher than in the remaining seven blocks, despite the fact fewer beetles were trapped in this block than any other block. This trap was positioned in the extreme southwest corner of the test

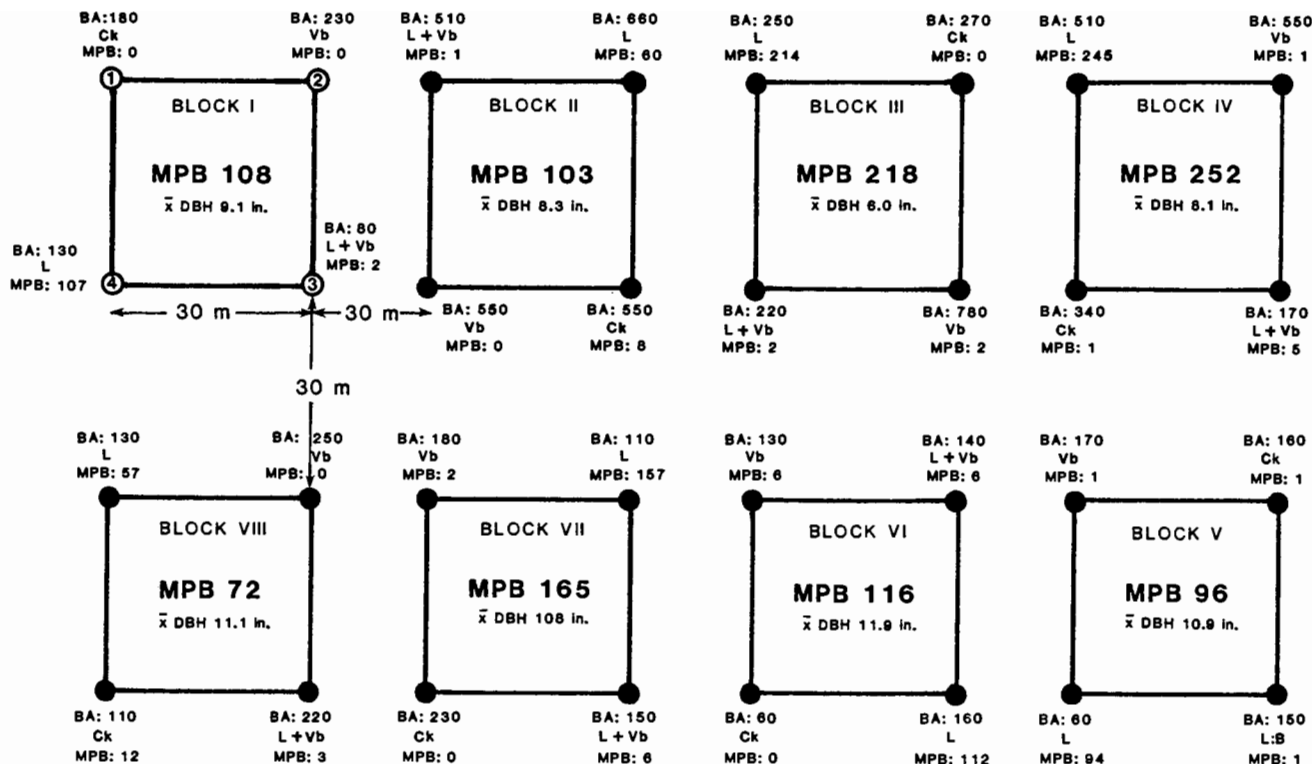


Figure 1—Test block alignment, including mean lodgepole pine diameter (breast height) and MPB trapped per block, as well as basal area (BA), treatment assignments (L = lure, Vb = verbenone, Ck = unbaited trap), and MPB caught (MPB), by trap position within each block.

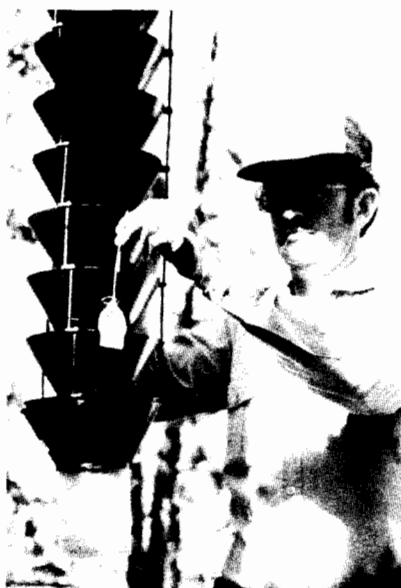


Figure 2—Lindgren funnel trap showing plastic container at funnel base that retains beetles caught, and wire hanger with bubble cap that is suspended within funnel opening by clipping hanger over the edge of the funnel.

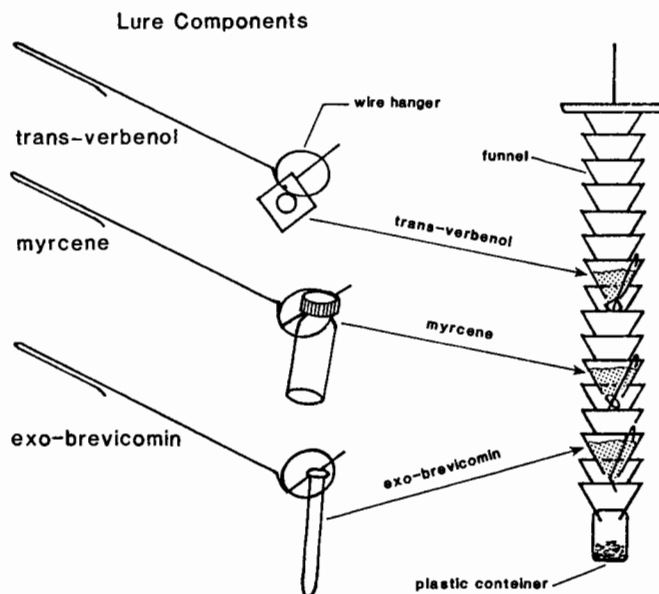


Figure 3—Positions of release devices used to dispense the three-component mountain pine beetle lure and verbenone on the 16-unit funnel traps. *Trans*-verbenol and verbenone were released from polyethylene bubble caps, myrcene from a closed 20-mL polyethylene bottle, and *exo*-brevicomin from a glass capillary tube within an open polyethylene centrifuge tube.

**Table 1—Semiocemical release devices and rates**

Compound <sup>1</sup>	Release device	Release rate <sup>2</sup>
Verbenone	Polyethylene bubble cap	5.0
MPB lure (three components):		
<i>trans</i> -verbenol	Polyethylene bubble cap	2.0
<i>exo</i> -brevicommin	Glass capillary tube (1.0 mm i.d.) in open 400- $\mu$ L polyethylene centrifuge tube	.2
myrcene	Closed 20-mL polyethylene bottle	17.6

<sup>1</sup>Chemical names, supplier and purity of semiocemicals tested:

1. Verbenone: 4,6,6-trimethylbicyclo [3.1.1] hept-3-en-one; Phero Tech Inc., Vancouver, BC; 98.2% pure.
2. *trans*-verbenol: *trans*-4,6,4-trimethylbicyclo [3.1.1] hept-3-en-2-ol; Phero Tech Inc., Vancouver, BC; 93.6% pure.
3. *exo*-brevicommin: *exo*-7-ethyl-5-methyl-6,8-dioxabicyclo [3.2.1] 1, octane; Phero Tech Inc., Vancouver, BC; 99.7% pure.
4. Myrcene: 2-methyl-6-methylene-octa-2,7 diene; Phero Tech Inc., Vancouver, BC; 98% pure.

<sup>2</sup>Release rate = mg/24 h at 25 °C.

area, and because prevailing winds were from the south-southwest, it is unlikely that the catch was affected by treatments present in adjacent traps (fig. 3).

The reduction (95.8 percent) in MPB response to the current commercial attractive lure in the presence of verbenone in this test was greater than that recorded by others. Ryker and Yandell (1983), working in eastern Oregon, reported an 86 percent reduction in MPB response after a 9-day-long test, when verbenone (91.4 percent (-) at 1,000 ppm) was added to an MPB lure (*trans*-verbenol, 20 mg/h; 1:1 mixture of myrcene and alpha pinene at 600 mg; plus an additional vial of alpha pinene at 1 to 2 mg/h). In British Columbia, Borden and collaborators (1987) recorded a reduction of approximately 65 percent in the mean number of beetles trapped over a 3-day period when (-) verbenone (5.0 mg/24 h) was added to the MPB

lure (myrcene 8 mg/24 h, *trans*-verbenol 1.0 mg/24 h, *exo*-brevicommin 0.1 mg/h).

Differences in test methodology among the three tests, including use of different lure components, release rates of test components, and test durations, preclude any inference about inherent differences in response among the Oregon, British Columbia, and Utah MPB populations.

Of the 1,130 MPB trapped, 410 were males and 720 were females (sex ratio 0.56) (table 2). The lure alone, verbenone alone, and unbaited trap caught more females than males; respective sex ratios (males:females) were 0.57, 0.40, and 0.50 (table 2). In contrast, the lure with verbenone caught more males than females, resulting in a sex ratio of 2.1. A contingency table analysis using Fisher's Exact Test (2-Tail) was used to test for independence between treatment and response by sex (table 3). The analysis revealed

**Table 2—Response of the mountain pine beetle to funnel traps baited with synthetic MPB lure and verbenone, alone and in combination, Wasatch National Forest, UT, 1986**

Block	Average d.b.h.	MPB lure alone			MPB lure with verbenone			Verbenone alone			Unbaited trap			Total		
		LPP	Females	Males	Total	Females	Males	Total	Females	Males	Total	Females	Males	Total	Females	Males
	<i>Inches</i>	<i>Number</i>														
1	9.1	77	30	107										77	31	108
2	8.3	58	38	96		1	1				4	2	6	62	41	103
3	8.0	130	84	214	1	1	2	2		2				133	85	218
4	8.1	171	74	245	1	4	5	1	1		1	1	1	173	79	252
5	10.9	66	28	94		1	1	1		1				67	29	96
6	11.9	68	44	112		1	1		1	1	2		2	70	46	116
7	10.8	89	68	157	3	3	6	1	1	2				93	72	165
8	11.1	36	21	57	1	2	3				8	4	12	45	27	72
Total		695	397	1,082	6	13	19	5	2	7	14	8	22	720	240	1,130
Percent		64	36	95.8	32	68	1.7	71	29	0.6	75	25	1.9			
Sex ratio (males:females)		0.57			2.1			0.40			0.50					
Mean		<sup>1</sup> 11.433a			1.759b			1.333b			1.726b					

<sup>1</sup>Means with the same letter are not significantly different ( $P < 0.001$ ).

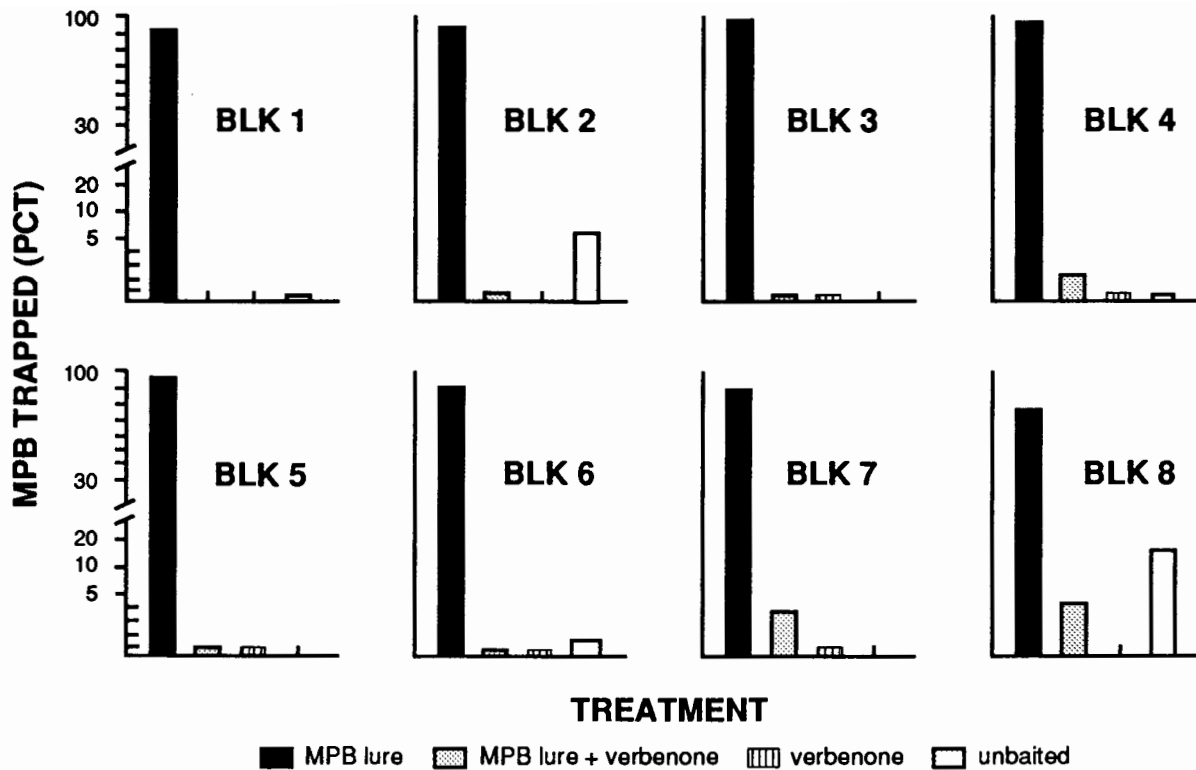


Figure 4—Percentage of mountain pine beetles trapped, by treatment, for the eight test blocks, Wasatch National Forest, UT, 1986.

Table 3—Contingency table comparing mountain pine beetle response, by sex, to the lure alone, lure with verbenone, verbenone alone, and unbaited trap<sup>1</sup>

Treatment	Sex		Total	No.	Percent
	Males	Females			
<b>Lure alone</b>					
Number	387	695	1,082		95.75
Percent	34.25	61.50			
Row percent	35.77	64.23			
Column percent	94.39	96.53			
<b>Lure + verbenone</b>					
Number	13	6	19		1.68
Percent	1.15	0.53			
Row percent	68.42	31.58			
Column percent	3.17	.83			
<b>Verbenone alone</b>					
Number	2	5	7		0.62
Percent	0.18	.44			
Row percent	28.57	71.43			
Column percent	.49	.69			
<b>Unbaited trap</b>					
Number	8	14	22		1.95
Percent	.71	1.24			
Row percent	36.36	63.64			
Column percent	1.95	1.94			
<b>Totals</b>					
Number	410	720	1,130		
Percent	36.28	63.72			100.00

<sup>1</sup>Fisher's Exact Test (2-Tail) value = 0.0336.

that treatment and response by sex are not independent, and when verbenone is added to the lure we can expect more males than females to be trapped.

Given the current understanding of MPB response to verbenone, the reason for the change in sex ratio of MPB responding to treatment (lure with verbenone compared to the lure alone, verbenone alone, and the unbaited trap) is unclear. The number of females responding to the lure with verbenone, and verbenone alone, was almost identical; however, the number of males responding to the lure with verbenone was six times greater. Results from a short-term initial field test in British Columbia revealed a proportional reduction in male and female response when verbenone was added to the lure (Borden and others 1987). Recent electroantennogram (EAG) measures of MPB response to racemic pheromone components revealed recovery rates following exposure to the three-component lure and verbenone were longer for males than females (Whitehead 1986). Recovery rates are thought to be affected by the amount of time a stimulant molecule is bound to a membrane receptor (Roelofs and Comeau 1971). Further, such sex-related differences in response may be the result of a different number or type of antennal receptor. Electroantennogram analyses have also determined the calculated threshold of response to verbenone was higher for males than females, while response to the three-component lure was almost identical (Whitehead and others 1989). The change in sex ratio among treatments could result from a synergistic effect triggered by the addition of verbenone and augmented by the auto-oxidation of the *trans*-verbenol in the lure to verbenone. It has been suggested that the effect of such a reaction may be to stimulate separate sensory cells in the antenna or occupy multiple receptor sites on single sensory cell membranes resulting in a differential response (Whitehead 1986). The effect may differ by sex because of differences in the number or type of receptors common to each sex.

## CONCLUSIONS

Results from this test suggest that verbenone, when deployed prior to beetle flight, offers considerable promise for interfering with the aggregation behavior of MPB populations and preventing them from reaching unacceptable levels, especially in high-value stands. The fate of beetles deterred from aggregating on suitable host in the presence of verbenone is unknown. Accordingly, at this stage of development its potential for reducing intolerable levels of infestation would likely be enhanced if it were deployed in conjunction with synthetic MPB lures to attract beetles avoiding the area treated with verbenone. The lures could be deployed in lethal traps or less valuable stands that could be treated to destroy any residual infestations. The test results showed that of 1,130 MPB trapped, 1,101 (97.5 percent) responded to traps baited with the attractive lure. If the 1,130 MPB trapped represented the total MPB population in the test area, only 29 (2.5 percent) remained free to infest suitable trees (table 2). A sensible alternative would be to invoke such a strategy when MPB populations are at low or endemic levels, so that the effort required to trap-out or treat residual populations is more manageable. This alternative requires knowledge of the dynamics of

endemic populations so that stands likely to harbor such infestations can be identified and treated in a timely manner. Such strategies will also require continued field testing of verbenone to assess the effectiveness of current elution rates against different MPB population levels throughout the range of LPP and other host species because of potential host, and geographic differences (see Lanier and others 1980). Efforts to gain this level of understanding are part of the Intermountain Research Station's program to devise strategies for preventing MPB populations from building to unacceptable levels.

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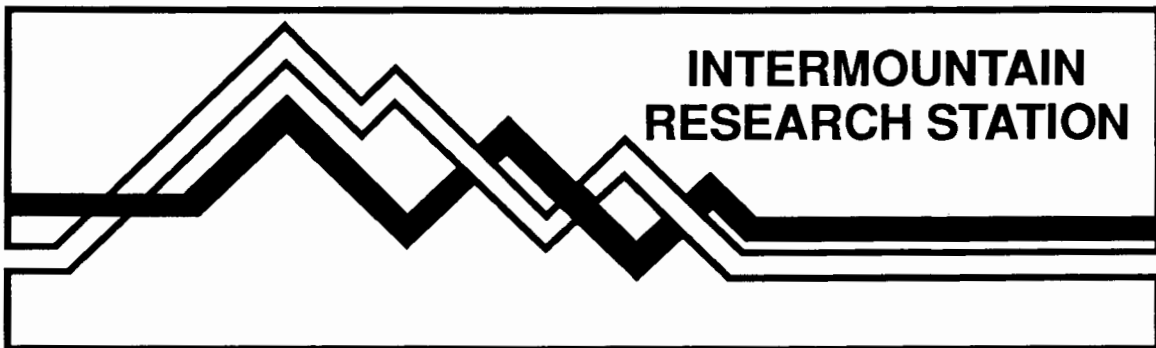
Schmitz, Richard F.; McGregor, Mark D. 1990. Antiaggregative effect of verbenone on response of the mountain pine beetle to baited traps. Res. Pap. INT-423. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station. 7 p.

Mountain pine beetle (MPB) (*Dendroctonus ponderosae*) response to Lindgren funnel traps baited with a commercial lure (*trans*-verbenol, *exo*-brevicomin, and myrcene), alone and in combination with verbenone, an aggregation-inhibiting pheromone, was measured in a lodgepole pine (*Pinus contorta*) stand in Utah. Treatments were randomly assigned to four traps in each of eight test blocks. A total of 1,130 MPB were trapped during the flight period. ANOVA revealed the number of MPB caught among treatments was significantly different ( $P < 0.001$ ). Tukey's studentized range test revealed MPB caught among treatments separated into two significantly different sets: (1) verbenone (0.6 percent), lure with verbenone (1.7 percent), unbaited trap (1.9 percent); and (2) the MPB lure alone (95.8 percent).

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KEYWORDS: *Dendroctonus ponderosae*, Scolytidae, pheromones, *Pinus contorta*

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