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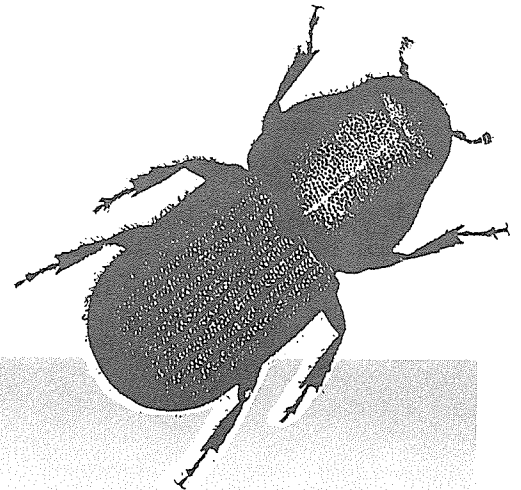
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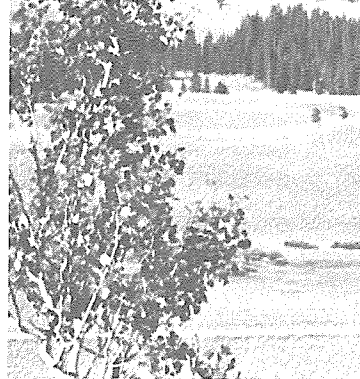


Optimum Dosage of Verbenone to Reduce Infestation of Mountain Pine Beetle in Lodgepole Pine Stands of Central Idaho

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RESEARCH SUMMARY

Five dosages of verbenone, a bark beetle anti-aggregative pheromone, were deployed in lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) stands in Idaho to determine optimum level for

prevention of infestation by mountain pine beetle (*Dendroctonus ponderosae* Hopk.). In 1988, the least amount of beetle infestation occurred where verbenone bubble capsules (released at the rate of 5 mg per capsule per 24 hours at 25 °C) were deployed in a grid pattern at a rate of 49 per hectare. The lowest percentage of trees infested was 0.21 percent in the 49 capsules per hectare treatment, compared to 5.39 percent in check stands. In 1989, the least infestation was obtained with 100 capsules per hectare. Infested trees were 0.72 percent, compared to 4.93 percent in check stands. Beetle infestation did not increase in the immediate area surrounding treatment plots.

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INTRODUCTION

Verbenone (4,6,6-trimethylbicyclo[3.1.1]-hept-3-en-2-one) is considered the principal antiaggregative pheromone component of the mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins, and apparently serves to space attacking beetles, thus preventing overcrowding of larvae. The presence of verbenone in the MPB pheromone complex was first reported by Pitman and others (1969).

Verbenone is derived from three sources: female beetles, autoxidation of alpha-pinene, and microorganisms (primarily yeasts) growing in established galleries (Hunt and Borden 1989). Field tests of MPB response in British Columbia, Canada, revealed that verbenone, when released in funnel traps at 1 or 5 mg per 24 hours in the presence of the attractive synthetic MPB lure (*trans*-Verbenol, *exo*-Brevicom, and myrcene), reduced the response of males by approximately 75 percent. Female response was similar, although the reduction was not statistically significant (Borden and others 1987). In Utah, the addition of verbenone (released at the rate of 5 mg per 24 hours at 25 °C) to funnel traps baited with MPB lure resulted in a 97 percent reduction in catch of MPB, compared with catch in traps baited with MPB lure alone (Schmitz 1989; Schmitz and McGregor 1990).

Synthetic verbenone deployed in clear plastic bubble capsules (obtained from Phero Tech Inc., Delta, BC, Canada) at 100 capsules per hectare was tested in both the Western United States and western Canada to reduce MPB infestation (Amman and others 1989; Lindgren and others 1989). Both tests demonstrated that verbenone reduced MPB infestations in stands baited or unbaited with the MPB lure. Two questions arose from these tests: (1) What is the optimum dosage of verbenone

needed to reduce MPB infestations? (2) Would MPB be attracted or dispersed into areas adjacent to verbenone-treated stands, resulting in increased numbers of infested trees in adjacent stands?

METHODS

A study area was selected in the Sawtooth Valley of the Sawtooth National Recreation Area, ID, at an average elevation of 2,256 m above sea level. Lodgepole pines of 15.2 cm and larger diameter at breast height (d.b.h.) averaged 20 cm d.b.h. and 144 years old. Species composition within the stands averaged 75 percent lodgepole pine, with the remainder being mostly Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) and a few quaking aspen (*Populus tremuloides* Michx.).

Verbenone capsules consisted of a clear plastic bubble and a clear membrane, with a cardboard cover to shade the capsule. Each capsule contained 0.5 g of verbenone, which was released at the rate of 5 mg per capsule per 24 hours at 25 °C. The capsules were stapled to the north sides of trees, 2 m above ground. Verbenone had a chemical purity of 98.6 percent and an optical purity of (–)–72 percent.

In 1988, a complete block design, replicated seven times, was used to test five densities of verbenone capsules (0, 25, 49, 100, and 169 per hectare, spaced in a grid pattern within the treated plots. Plots were 1 ha in size and were located 100 m apart.

Treatments within the first replicate were randomly assigned. This order was followed throughout the remaining replicates to avoid placing the same treatment in adjacent plots in areas where more than one replicate was established. Three replicates were established at Alturas Lake Creek, two at Vat Creek, and one each at Beaver Creek and Pole Creek.

In 1989, the dosage-response portion of the study (objective 1) was repeated, with the following changes:

1. Treatments were replicated eight times instead of seven.
2. A newly designed capsule was used, consisting of an opaque bubble with clear plastic membrane. Each capsule contained 0.8 g of verbenone.
3. Treatments were randomized within each block, instead of following the same order in all replicates.
4. The Vat Creek area was discontinued from study because of low level of beetle infestation, and three new areas were added—Alturas Lake, State land, and West Beaver Creek—each containing one replicate.

Treatments were applied June 20 and 21, 1988, and June 19 to 21, 1989. Treatment effects were assessed August 16 to 18, 1988, and August 21 to 23, 1989, after all beetles had flown, as determined by beetles emerging into cages stapled on infested trees. A 100 percent survey of each plot was made to measure the d.b.h. of all lodgepole pine 12.7 cm and larger, and to determine tree mortality by MPB for 1987, 1988, and 1989. Treatment effects within plots were evaluated by the percentage of lodgepole pine 12.7 cm and larger d.b.h. in each plot that was mass-attacked by MPB following verbenone treatment. The arcsin transformation was used for percentages of infested trees that were analyzed by

analysis of variance (ANOVA) procedure (SAS 1987). We did not use a multiple-comparisons procedure, which would have been inappropriate (Warren 1986). The item of interest is how beetle response changes as a function of verbenone level. Differences in numbers of green trees available for beetle infestation among treatments were also tested by ANOVA.

To assess whether MPB infestations increased in stands immediately adjacent to the 1988 verbenone plots, two 20-m-wide strips were established around each plot (fig. 1), and all trees infested in 1987 and 1988 were counted and tallied separately for each strip. Numbers of 1987-infested trees show MPB infestation prior to verbenone treatment and ratio of change following treatment. Numbers of 1988-infested trees in each strip were analyzed separately by ANOVA. Numbers, rather than percentages of infested trees, were used in order to save inventorying all surviving trees in the strips for percentage calculations.

RESULTS AND DISCUSSION

Treatment Plots

The percentages of newly infested trees in plots treated with verbenone capsules in 1988 were all significantly lower (means are 25 = 1.80 percent; 49 = 0.21 percent; 100 = 0.51 percent; 169 = 1.71 percent) than the check stand ($P < 0.003$; $\bar{x} = 5.39$ percent) (tables 1 and 2). In 1989, the percentages of newly infested trees were also significantly different among treatments ($P < 0.012$) and among blocks ($P < 0.001$) (table 3). Differences among blocks do not invalidate the test but do indicate that the precision of the experiment was increased by use of the randomized block design, where each treatment appears in each block (Steel and Torrie 1960). The least amount of infestation occurred in the 100 capsules per hectare treatment ($\bar{x} = 0.72$ percent) compared to the check ($\bar{x} = 4.93$ percent) (table 4). In both years, the trend is for reduced infestation with increased verbenone up to 49 capsules per hectare in 1988 and 100 capsules in 1989. Larger numbers of verbenone capsules resulted in increased infestation (fig. 2). Borden and Lindgren (1988) also noted more trees were attacked when high dosages of verbenone were used.

Amman and others (1989) and Lindgren and others (1989) showed that 100 capsules per hectare reduced MPB infestation in stands of lodgepole pine, either in the presence or absence of the aggregative MPB lure. Our studies confirm these findings. Although 49 capsules per hectare

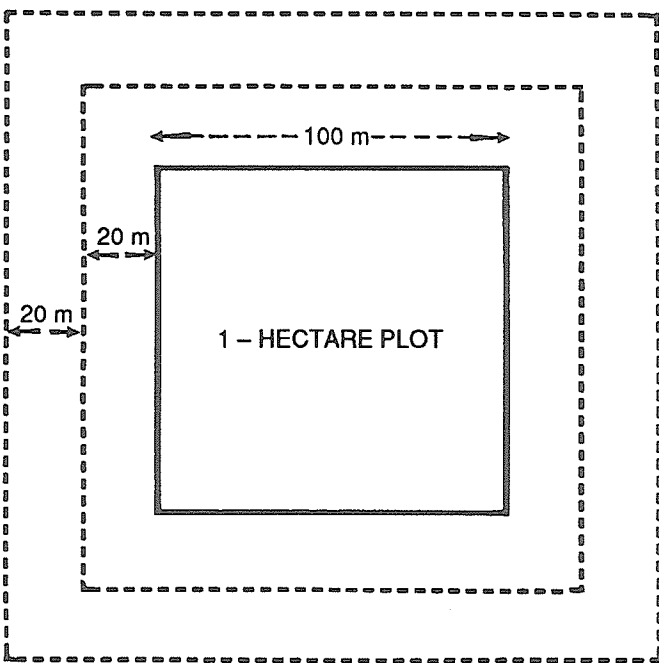


Figure 1—Design of verbenone treatment plot and surrounding strips used in the 1988 tests.

Table 1—Mountain pine beetle infested and uninfested lodgepole pines per hectare in verbenone-treated plots and in surrounding strips, Sawtooth National Recreation Area, ID, 1988

Treatment (No. of capsules)	Treated plot				First 20-m-wide strip			Second 20-m-wide strip		
	Green trees ¹ available for attack		Trees infested		Trees infested		Ratio of infested trees	Trees infested		Ratio of infested trees
	1988	in 1987	In 1988		1987	1988	1988/1987	1987	1988	1988/1987
	\bar{x}	\bar{x}	\bar{x}	Percent ²	\bar{x}	\bar{x}		\bar{x}	\bar{x}	
Check	528	14.9	27.0	5.39	5.7	22.1	3.9	5.1	18.5	3.6
25	582	6.0	10.3	1.80	7.7	16.5	2.1	7.1	19.4	2.7
49	534	6.1	1.1	.21	6.6	12.6	1.9	5.7	18.6	3.3
100	587	14.4	3.0	.51	6.6	14.0	2.1	2.9	12.7	4.4
169	527	9.9	8.9	1.71	8.5	8.2	1.0	5.7	6.6	1.2

¹Trees 12.7 cm and larger d.b.h. available for beetles to infest in 1988.

²Percentage of trees 12.7 cm and larger d.b.h. infested by mountain pine beetles.

Table 2—Randomized block ANOVA statistics for percentages (arcsin transformation) of lodgepole pine infested by mountain pine beetle in plots treated with different dosages of verbenone, Sawtooth National Recreation Area, ID, 1988

Source	df	Mean sum of squares	F ratio	P
Block	6	1.149	1.23	0.324
Treatment	4	5.207	5.59	.003
Error	24	.931		

Table 3—Randomized block ANOVA statistics for percentages (arcsin transformation) of lodgepole pine infested by mountain pine beetle in plots treated with different dosages of verbenone, Sawtooth National Recreation Area, ID, 1989

Source	df	Mean sum of squares	F ratio	P
Block	7	2.093	4.71	0.001
Treatment	4	1.748	3.93	.012
Error	28	.445		

Table 4—Mountain pine beetle infested and uninfested lodgepole pines per hectare in verbenone-treated plots, Sawtooth National Recreation Area, ID, 1989

Treatment (No. of capsules)	Green trees ¹ available for attack, 1989	Trees infested in 1988	Trees infested in 1989	
	\bar{x}	\bar{x}	\bar{x}	Percent ²
Check	614	12.0	30.3	4.93
25	559	11.1	21.8	3.89
49	650	9.9	16.4	2.52
100	606	7.6	4.4	.72
169	507	6.8	8.0	1.58

¹Trees 12.7 cm and larger d.b.h. available for beetles to infest in 1989.

²Percentage of trees 12.7 cm and larger d.b.h. infested by mountain pine beetles.

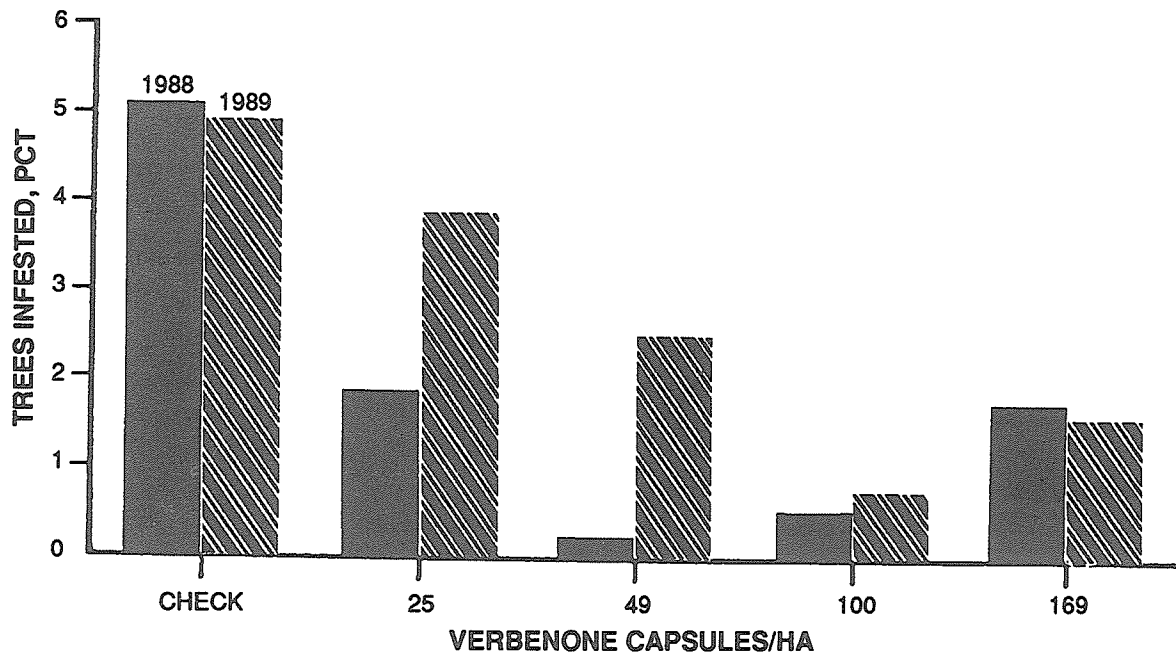


Figure 2—Percentage of lodgepole pine trees 12.7 cm and larger d.b.h. infested by MPB in plots treated with different numbers of verbenone bubble capsules in 1988 and 1989, Sawtooth National Recreation Area, ID.

appeared to do equally well in 1988, MPB infestation was unacceptably high in 1989, compared to that achieved with 100 capsules per hectare (fig. 2).

Verbenone Effect Outside Treated Plots

No significant difference occurred in the number of trees per hectare killed by MPB within the two 20-m-wide strips around verbenone-treated plots ($P > 0.58$ and 0.32). Although none of the capsule treatments differed significantly (table 1), the trend is for fewer beetle-infested trees in strips surrounding plots treated with verbenone than in check plots (fig. 3). These data show that MPB infestation is not increased around verbenone-treated plots, but rather that the verbenone effect may extend beyond boundaries of the treated plots, especially at a dosage of 169 capsules per hectare. We speculate that most of the beetles dispersed from treated plots flew beyond the two 20-m-wide strips surrounding the plots before infesting trees.

Tree Numbers

Stand characteristics are important to MPB infestation behavior (Cole and Amman 1980). In the 1988 and 1989 tests, no significant differences occurred in either the numbers of infested trees from which beetles would have emerged to infest trees during the test or in numbers of lodgepole pine available for infestation among the treated stands

(table 1). Bentz and others (1989) did not find a significant effect due to number of uninfested trees or diameter class in tests of verbenone in ponderosa pine stands.

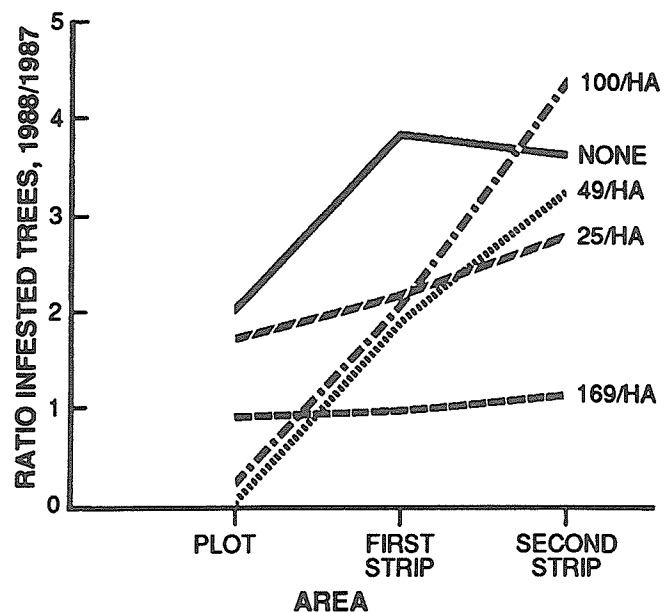


Figure 3—Ratio of 1988 to 1987 infested lodgepole pine in plots treated with different numbers of verbenone capsules and in two surrounding 20-m-wide strips.

CONCLUSIONS

These results demonstrate that MPB infestations at densities of six to 14 infested lodgepole pine per hectare are most effectively reduced by verbenone treatment at a dosage rate of 100 verbenone capsules per hectare (release rate of 5 mg per capsule per 24 hours at 25 °C). MPB infestation does not increase in 40-m-wide strips immediately surrounding verbenone-treated plots of lodgepole pine 1 hectare in size. Verbenone could be used operationally to prevent infestation, if the risk of losing some trees is acceptable.

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Five dosages of verbenone were deployed in lodgepole pine stands in 1988 and 1989 to determine optimum level for prevention of infestation by mountain pine beetle. In 1988, the least amount of beetle infestation occurred where 49 bubble capsules per hectare were deployed in a grid pattern. Each capsule released at a rate of 5 mg per capsule per 24 hours at 25 °C. The percentage of trees infested was 0.21 percent (49 capsules per hectare) compared to 5.39 percent in check stands. In 1989, least infestation was obtained with 100 capsules per hectare. Infested trees were 0.72 percent, compared to 4.93 percent in check stands. Beetle infestation did not increase in areas adjacent to verbenone-treated blocks.

KEYWORDS: *Dendroctonus ponderosae*, *Pinus contorta*, pheromone

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