

EFFICACY OF VERBENONE FOR PREVENTING INFESTATION OF
HIGH-VALUE LODGEPOLE PINE STANDS BY THE MOUNTAIN PINE BEETLE

Richard F. Schmitz

ABSTRACT: Results from experimental deployment of the mountain pine beetle (MPB) (Dendroctonus ponderosae Hopkins) semiochemical verbenone, an antiaggregative pheromone component, suggest it may be useful for preventing or suppressing MPB infestations in high-value lodgepole pine stands (Pinus contorta var. latifolia Dougl.). Field measures of the response of MPB to funnel traps baited with the standard MPB lure (trans-verbenol, exo-brevicomin, and myrcene) in Utah showed a 98 percent reduction in MPB trapped when verbenone was added. When used experimentally to protect 1-ha lodgepole pine stands in Idaho from further MPB infestation, the treated stands had an average reduction of 48.6 percent in the number of infested trees. Comparable findings have resulted from similar tests conducted in Canada.

INTRODUCTION

There is an obvious need for environmentally acceptable suppression strategies that protect high-value lodgepole pine stands, such as those in travel influence zones, campgrounds, and riparian areas, from infestation by the mountain pine beetle (Dendroctonus ponderosae Hopkins) (MPB). This need has prompted investigations to determine how semiochemicals like verbenone might be used to suppress MPB populations. As a result, the effectiveness of suppression strategies utilizing synthetic semiochemicals to manipulate dispersing mountain pine beetle populations is being field tested to determine how these natural compounds should be deployed to prevent infestation of high-hazard stands (Amman and others, in press; Borden and others 1983; 1987; Lindgren and others, in press). To that end, representatives of the U. S. Environmental Protection Agency (EPA) agree that semiochemicals may be preferable to conventional pesticides in the management of insect pests and encourage their development and use (Booth 1988).

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Richard F. Schmitz is Principal Entomologist, Intermountain Research Station, Forest Service, U.S. Department of Agriculture, Ogden, UT.

Earliest efforts to exploit semiochemicals for suppression purposes concentrated on the attractive components that guide flying beetles to suitable hosts. As a result, most field tests were designed to evaluate the most effective deployment of these attractive elements to lure and concentrate beetles in stands targeted for harvesting. Results revealed that beetle populations attracted to baited stands often "spilled over" into surrounding unbaited stands that land managers intended to protect (Furniss 1972). At the same time, field tests to evaluate the function of each newly isolated component in a specific pheromone bouquet revealed that bark beetle pheromone systems contained an anti-aggregative component (Borden 1982). In general, these components appear to function as a mask that terminates response to the attractive elements, thereby ensuring that the density of attack does not exceed the threshold for optimum brood survival (Borden and others 1987).

SOURCE AND EFFECTIVENESS OF MOUNTAIN PINE BEETLE ANTIAGGREGATIVE COMPONENTS

Verbenone was identified and first isolated from the mountain pine beetle pheromone complex, using the hindguts of newly emerged and feeding female MPB, by Pitman and others (1969). It was also identified from air passed over emergent male/female pairs (Rudinsky and others 1974). The first evidence that verbenone had antiaggregative properties resulted from laboratory and field bioassays that showed (-)-verbenone inhibited MPB response to selected host- and beetle-produced volatiles (Ryker and Yandell 1983). Additionally, four other pheromone components isolated from the MPB pheromone have at times exhibited anti-aggregative properties. These include endo- and exo-brevicomin released by attacking males (Libbey and others 1985; Rudinsky and others 1974; Ryker and Rudinsky 1982), frontalin produced by feeding males (Libbey and others 1985; Ryker and Libbey 1982), and verbenone and pinocarvone produced by feeding beetles of both sexes (Libbey and others 1985).

Recent field tests in British Columbia (Borden and others 1987), using two release rates of endo-brevicomin, failed to confirm the concentration-dependent multifunctional attractive and antiaggregative qualities reported by Ryker and Rudinsky (1982). Similarly, field tests in Oregon (Libbey and others 1985) failed to substantiate the multifunctional properties of exo-brevicomin, confirming its antiaggregative qualities at high

release rates but failing to demonstrate attractive properties at low rates. When used as a tree bait, it elicits a host-specific response. On western white pine, *Pinus monticola* Dougl. ex. D. Don, it inhibited MPB attack (McKnight 1979; Pitman and others 1978), but on lodgepole pine, *P. contorta* var. *latifolia* Dougl., attack was enhanced (Borden and others 1983; McKnight 1979). Field tests of frontalinalin in Oregon revealed it had antiaggregative effects at high concentration (Rudinsky and others 1974) but induced attack on lodgepole pine in Idaho (Chatelain and Schenk 1984). Trapping experiments in which pinocarvone was added to MPB lure reduced the catch by 50 percent (Libbey and others 1985).

ROLE OF VERBENONE IN HOST COLONIZATION

Results to date suggest the primary antiaggregative semiochemical that regulates MPB response to its host is (-)-verbenone. It has been recorded from three sources: (1) female beetles (Pitman and others 1969), (2) auto-oxidation of alpha pinene to cis- and trans-verbenol, then to verbenone (Hunt and others 1988; Lindgren and Borden, these proceedings), and (3) oxidation of cis- and trans-verbenol by microorganisms (primarily yeasts) associated with the beetle (Hunt and Borden, in press; Lindgren and Borden, these proceedings).

A complete understanding of the interaction between pheromone components that regulate MPB host selection behavior requires all components be identified and tested at appropriate concentrations in the field. To date, 33 semiochemicals have been isolated from the beetle (Lindgren and Borden, these proceedings), and they often elicit conflicting responses from the beetle, depending on test concentrations and methods of deployment (Lindgren and Borden, these proceedings). The following conceptual model proposed by Borden and others (1987) summarizes what is known about the sources of verbenone, the onset of production in relation to the sequence of attack, and its probable role in regulating the duration and density of attack.

At the onset of attack by female MPB, volatiles (including the host monoterpenes alpha-pinene and myrcene together with female-produced trans-verbenol) attract additional beetles to the tree. As males reach the tree, they release exo-brevicomin, which initially attracts primarily females, thereby enhancing the level of attraction. As additional males colonize the tree, concentrations of exo-brevicomin increase and are augmented by the male-produced antiaggregant, frontalinalin. Simultaneously, concentrations of the aggregative components, trans-verbenol, and the host monoterpenes begin to decline. At this stage in colonization, it is believed verbenone levels produced by (1) auto-oxidation of the host monoterpene, alpha pinene, to cis- and trans-verbenol and then to verbenone, and (2) by conversion of cis- and trans-verbenol by microorganisms, reach concentrations that deter additional beetles from attacking the focus tree. The effect of these antiaggregants is to limit attacks to a density that ensures survival of the ensuing brood.

VERBENONE FIELD TESTS

Reducing Response to Attractive Traps

During the summer of 1986, entomologists from the Intermountain Research Station, in cooperation with personnel from Phero Tech Inc., Vancouver, BC, conducted tests in the Wasatch National Forest in Utah to compare the number of MPB attracted to the standard MPB lure (trans-verbenol, exo-brevicomin, and myrcene), with and without verbenone (Schmitz and McGregor, in press).

Methods--The MPB lure contained trans-verbenol, exo-brevicomin, and myrcene eluted at 2 mg/24 h, 0.2 mg/24 h, and 18 mg/24 h at 25 °C, respectively. Verbenone was contained in the standard plastic bubble cap and released at 5 mg/24 h/capsule at 25 °C. The test was conducted in a mature lodgepole pine stand surrounded by stands in which MPB populations were building to outbreak levels. The eight test blocks were 30 m square and were separated from one another by 30-m intervals. Funnel traps were hung at each of the four corners of a block. The four treatments--MPB lure, MPB lure with verbenone, verbenone alone, and empty trap--were randomly assigned to each of four positions. Effectiveness of verbenone as an antiaggregant was assessed by the number of MPB caught by treatment.

Results--A total of 1,130 MPB were trapped by the four treatments. Results by block are given in figure 1. The number and percentage caught by treatment are tabulated below:

	<u>Number</u>	<u>Percent</u>
MPB lure alone	1,083	95.8
MPB lure with verbenone	19	1.7
Verbenone alone	7	0.6
Unbaited trap	<u>22</u>	<u>1.9</u>
Total	1,130	100.0

The number of MPB responding to the MPB lure with verbenone was significantly less than to the MPB lure alone. Overall, the addition of verbenone to the synthetic MPB lure reduced the catch by 98 percent.

A field test of MPB response to synthetic semiochemicals in British Columbia revealed that when verbenone was released in funnel traps at 1 or 5 mg/24 h in the presence of the attractive synthetic MPB lure (trans-verbenol, exo-brevicomin, and myrcene), it reduced the response of males approximately 75 percent. Although not statistically significant, the reduction in female response followed a similar trend (Borden and others 1987).

Protecting Stands from Infestation

The encouraging results from the studies that used verbenone to suppress catch of MPB in traps prompted a second set of tests to determine the efficacy of verbenone for reducing MPB infestation

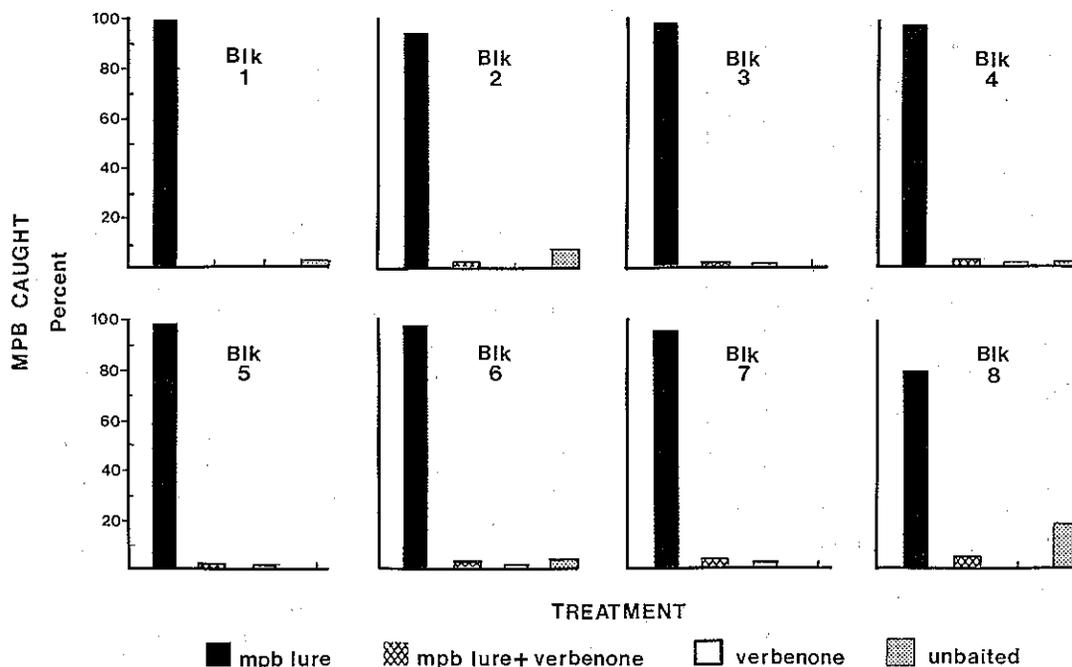


Figure 1--Number of MPB responding to funnel traps baited with synthetic MPB lure and the antiaggregant verbenone, alone and in combination, in eight test blocks, Wasatch National Forest, 1986.

in selected stands of lodgepole pine (Amman and others, in press). The test was conducted in the Sawtooth National Recreation Area, ID, while similar tests by Lindgren and others were being conducted in British Columbia, during 1987.

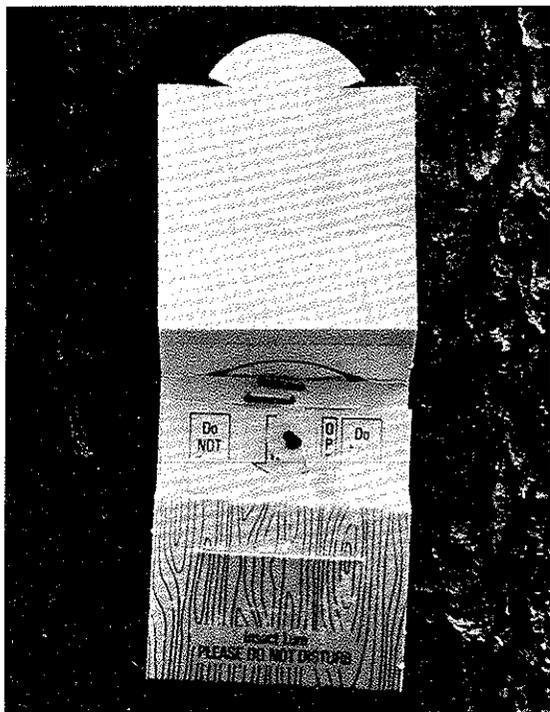
The Sawtooth National Recreation Area was selected for the study because MPB populations in that area were rapidly increasing, providing an opportunity to test the effectiveness of verbenone for preventing infestation of high-value trees in campgrounds, near administrative sites, wildlife sites, and near summer homes. Lodgepole pines 15.2 cm and larger diameter at breast height (d.b.h.) averaged 20 cm d.b.h. and 144 years old. The stand consisted of 75 percent lodgepole pine; the remainder was mostly Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) and a few quaking aspen (*Populus tremuloides* Michx.). The ratio of infested trees from 1985 to 1986 was 1:8. A survey through the main part of the infestation revealed 57 newly infested trees per hectare. Eighty percent of these were 20 cm and larger d.b.h., those sizes of trees in which MPB reproductive success is best (Cole and others 1976).

Methods--Verbenone was eluted from the standard plastic bubble cap at 5 mg/24 h/capsule at 25 °C in the presence of the MPB tree bait. Constituents of the MPB lure were the same as those used in the earlier trap tests (trans-verbenol, 2 mg/24 h; exo-brevicomin, 0.2 mg/24 h; and myrcene, 18 mg/24 h, at 25 °C). Treatments consisted of (1) MPB tree lure, (2) verbenone, (3) MPB tree lure and verbenone, and (4) check. Each treatment was applied individually to 1-hectare blocks and replicated four times. Five MPB tree baits were used in each baited block.

The cardboard containing the MPB lure was stapled 2 m above ground level on the north side of a lodgepole pine 20 cm or larger d.b.h. (fig. 2a). MPB tree lures were distributed in the center of the block and at each cardinal direction from the center, approximately 20 m from the outside boundary of the block.

Verbenone-treated blocks had 100 verbenone bubble capsules obtained from Phero Tech Inc., Vancouver, BC (chemical purity 98.6 percent; optical purity ee = (-)72 percent), spaced in a grid pattern approximately 10 m apart. The capsules were stapled to the north sides of trees 2 m above ground (fig. 2b). In the blocks treated with MPB tree bait plus verbenone, baits and verbenone bubble capsules were distributed as described for each alone. Check blocks were untreated.

All lodgepole pines larger than 15.2 cm d.b.h. were examined in each block to determine the d.b.h. and number killed for the years 1986 and 1987. Treatment effects were assessed by comparing the percentage of all lodgepole pine 15.2 cm d.b.h. and larger in each block that was infested by MPB in 1987. Because stand characteristics have been shown to affect mountain pine beetle infestation behavior (Cole and Amman 1980), stand measurements were made on five 10-factor basal area plots in each treatment block. Plots were positioned so overlap did not occur--one at block center and one in each corner. All trees 12.7 cm d.b.h., regardless of species, were tallied. These data were used to calculate percentages of trees that were lodgepole pine, average d.b.h. of lodgepole pine, stand basal area, and crown competition (Krajicek and others 1961) to be analyzed by ANOVA for differences among treatments.



A



B

Figure 2--Dispensers from which MPB lure and verbenone were released: (A) paper container with plastic vials containing the three-component attractive lure, (B) verbenone bubble cap with paper sunshade to limit exposure to solar radiation.

Results--A significant difference in percentages of infested trees among treatments was shown by ANOVA. Blocks having MPB baits only had significantly more mass-attacked trees than other treatments. The effect of verbenone is apparent.

The average percent of lodgepole pine infested by mountain pine beetles in blocks treated with mountain pine beetle tree baits and verbenone is shown here:

	MPB tree bait present	MPB tree bait absent
Verbenone present	7.425	0.875
Verbenone absent	24.425	3.275

Verbenone in the presence of mountain pine beetle tree bait resulted in a 2.3-fold reduction in infested trees.

An examination of the percent change in numbers of MPB-infested trees between 1986 and 1987 for the four treatments shows that only in verbenone-treated blocks did an average reduction occur (-48.6 percent). However, despite the overall reduction, an increase occurred in three of the four blocks. Check stands showed either no change or a decline in three of the four blocks, with a

large increase in the fourth block. However, the average increase was 64.7 percent from 1986 to 1987. Changes in infestation in verbenone-treated and check blocks were small when compared to baited blocks, which showed an average infestation increase of 2,575 percent. Blocks containing MPB baits and verbenone had an average infestation increase of 418.8 percent. The large difference in MPB infestation between MPB bait blocks and MPB bait plus verbenone blocks is considered due to the effect of verbenone. This suggests verbenone has considerable potential for reducing infestation of lodgepole pine stands, a conclusion also reached from tests by Lindgren and others (in press) in British Columbia.

Further, infestation differences probably were not related to differences in stand characteristics, since ANOVA failed to detect differences among treatments in percent of trees that were lodgepole pine, d.b.h. of lodgepole pine, basal area, and crown competition factor.

Discussion--Although verbenone-treated blocks had significantly fewer infested trees than blocks with MPB baits, the question remains: Would verbenone-treated stands have shown the significant reduction in MPB infestation without the accompanying source of attraction provided by

MPB baits? MPB dispersal may have been altered by the presence of pheromone baits, thereby affecting distribution among the other treatments. At this population level (3.7 infested trees per hectare in 1986), beetles from surrounding stands were probably drawn into the study blocks as indicated by the large increase in infested trees in 1987 (27.5 trees per hectare), especially in blocks containing MPB baits, which had 80.3 infested trees per hectare in 1987. How beetles would respond at higher population levels, or in the absence of the synthetic attractants, could not be deduced from the study. The investigators suggest that MPB may be attracted to the general area of verbenone-treated trees or stands and then infest trees where verbenone concentrations are low. Thus, while preventing infestation of treated trees and stands, infestation level of surrounding stands may be increased. Additional tests are under way to clarify this point.

OUTLOOK

The research results described here point to a growing understanding of the effects of semiochemicals, especially the antiaggregative components, on MPB host selection and aggregation behavior, and how they may best be integrated with existing suppression strategies once registered for operational use. Before these chemical messengers can be exploited to the fullest extent, it is likely several major gaps in our knowledge base regarding semiochemical deployment will need to be filled. These include (1) a knowledge of the structure of odor plumes and the effect topography and stand microenvironment have on their concentration and movement, (2) an understanding of the inherent differences in response between individual beetles in a population and between different population levels, (3) knowledge of the effects of environment on dispersion patterns of emerging adults, and (4) response of associated insects.

Even though this knowledge is lacking, test results obtained to date suggest that verbenone has the potential to prevent MPB infestations from reaching unacceptable levels in high-value lodgepole stands. Further, its potential for preventing rather than limiting the level of infestation would likely be enhanced if used in conjunction with synthetic MPB lures deployed to attract the beetle to traps or stands away from the area to be protected. At this point, test results suggest that verbenone has promise as an environmentally acceptable tool that can be incorporated with other strategies for preventing or suppressing MPB infestations, and therefore warrants the testing needed to determine how it can be deployed most effectively.

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