

# *trans*-Verbenol and Alpha-Pinene: Their Utility in Manipulation of the Mountain Pine Beetle<sup>1</sup>

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## ABSTRACT

Synthetic *trans*-verbenol containing 6% *cis*-verbenol and 1% unknowns combined with alpha-pinene was highly attractive to in-flight mountain pine beetle, *Dendroctonus ponderosae* Hopkins. Alpha-pinene was the most active of the terpenes tested with *trans*-verbenol. Myrcene showed intermediate activity, while limonene and 3-carene apparently were ineffective in eliciting a response. As behavior-regulating chemicals, *trans*-verbenol and alpha-pinene were effective in manipulating the host selection patterns of *D. ponderosae*. When 725 mature white pines, distributed over eight 40-acre plots in northern Idaho, were baited with the 2 materials, 133 or 18% were attacked heavily and subsequently killed. Beetle attacks were noted on an additional 15% of the baited trees, but these were aborted without tree mortality. Prior

to baiting, all trees were sprayed to a height of about 20 ft with a 2.3% solution of lindane. The mortality of sprayed trees suggested that lindane was not effective under the conditions of this study. Only 21 of the estimated 7200 unbaited mature pines within the boundaries of the study plots were mass attacked, and 11 of these trees were within 10 to 15 ft of baited mass-attacked trees.

Paperboard cylinders, about 8 ft × 10 in., covered with a tacky substance and baited with *trans*-verbenol and alpha-pinene appeared effective as a means of deadtrapping *D. ponderosae*. An abnormally high attack density could not be induced on a white pine baited with a massive amount of *trans*-verbenol and alpha-pinene.

Past studies (Pitman et al. 1968, Pitman and Vité 1969) revealed that synthetic *trans*-verbenol (Renwick 1967), a principal volatile constituent in the hindguts of *Dendroctonus* species (Pitman et al. 1969), in combination with host volatiles was highly attractive to field populations of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins. *Trans*-verbenol in the absence of host odors shows no apparent attractive properties in field or laboratory bioassays. In the presence of host volatiles, however, synthetic *trans*-verbenol was competitive to sources of natural attraction, i.e. *D. ponderosae* females feeding in a freshly cut pine billet (Pitman and Vité 1969). By using beetles in tethered flight<sup>3</sup> in the laboratory it was found that *trans*-verbenol with alpha-pinene attracted beetles of both sexes.

The competitiveness of synthetic *trans*-verbenol and the host's volatiles to natural attractant sources prompted this study on the field manipulation of *D. ponderosae* during the summers of 1968 and 1969. Previous studies on *Dendroctonus* manipulation (Gara et al. 1965, McCambridge 1967) utilized pheromones from natural sources. This paper reports on a large-scale field manipulation of *D. ponderosae* with synthetic and natural chemical messengers.

**MATERIALS AND METHODS.**—*Chemicals.*—Synthetic pheromones were provided by Battelle Memorial Institute, Columbus, Ohio, and varied from high purity to crude depending upon the requirements of the experiment. In the principal experiment, where manipulation of *D. ponderosae* was attempted over 320 acres, the purity of *trans*-verbenol was 93% with 6% *cis*-verbenol and 1% of unknown composition. Purity for this test was obtained through short-path distillation followed by thin-layer chromatography.

Crude *trans*-verbenol was composed of 84% *trans*-verbenol, 14% *cis*-verbenol, and 2% unknown. An intermediate grade, also tested, consisted of ca. 86% *trans*-verbenol, 13% *cis*-verbenol, and 1% unknown. All terpenes were technical grade and were obtained from K and K Laboratories, Plainview, N. Y. In 2 previous studies (Pitman et al. 1968, Pitman and Vité 1969) high purity *trans*-verbenol (>99.5%) was used to establish the material's biological activity. The cost of such purity, however, precluded its use in later large-scale tests.

Fig. 1 shows the principal method of dispensing *trans*-verbenol and alpha-pinene on baited trees. Small-bore polyethylene tubing<sup>4</sup> with a wall thickness of 0.008 in. was charged with the 93% *trans*-verbenol in a mixture of 60 parts *trans*-verbenol and 40 parts alpha-pinene at a rate of 40 mg/ft of tubing. Over a period of 31 days, the rate of *trans*-verbenol loss per foot of tubing averaged ca. 30 µg/hr under laboratory conditions. Alpha-pinene evaporated very rapidly from the tubes and was nearly exhausted after 7 days. To obtain the release of alpha-pinene over a longer period, 15-ml Boston round bottles filled to 1/3 capacity were used. The rate of release under laboratory conditions ranged from 2 to 3 mg/hr. Polyethylene tubes 10 cm × 6.3 mm also were used; when filled with 0.6 ml alpha-pinene and closed with teflon plugs the rate of loss was ca. 2.8 mg/hr.

Glass capillary tubes 0.4 mm ID × 9 cm nearly filled with *trans*-verbenol were used during many of the individual field bioassays. With this type of dispenser the rate of *trans*-verbenol evaporation was ca. 5 mg/hr during the 1st few hours of deployment. Activity of individual terpenes was examined by placing 1 g of material in a 50-ml beaker which was covered with perforated aluminum foil.

*Olfactometers.*—The field olfactometers were of vertical and horizontal configurations (Pitman and Vité 1969) and used the lower bait chamber of a standard field olfactometer (Vité et al. 1963). Fitted to the bait chamber was either a vertical 13-in. diam

<sup>1</sup> Coleoptera: Scolytidae.

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<sup>3</sup> P. R. Hughes and G. B. Pitman. A method for observing and recording the flight behavior of tethered bark beetles in response to chemical messengers. (in manuscript).

<sup>4</sup> P. E. 90 Intramedic Polyethylene Tubing, Clay Adams, Parsippany, N. J.

× 10-ft muslin sleeve or 2 horizontal 13-in. × 8-ft perforated sheet-metal cylinders. Beetles responding to muslin sleeves were removed by hand, while those responding to the metal cylinders dropped into a lower tray to which 1-qt glass jars were attached. Paper-board cylinders ca. 8 ft long × 10 in. diam coated with a tacky material<sup>1</sup> also were used. The cylinders were supported on stakes driven into the ground (Fig. 2). Two 8-ft lengths of attractant-laden polyethylene tubing were attached to nails driven into opposite sides, at the top and bottom, of the cylinder. Beetles that responded to cylinders were trapped quickly in the tacky material; they were counted later and preserved in 70% ethanol for subsequent determination of sex.

**Study Location.**—Field research was conducted primarily at 2 sites near the Headquarters operation of Potlatch Forests, Inc., in northern Idaho. One site of study was situated near Lightning Point, ca. 20 mi northwest of Headquarters. The white pine stand in this area was characterized by 140- to 180-yr-old trees with an average DBH of ca. 24 in. and a stocking approaching ca. 75 ft<sup>2</sup> basal area/acre. The *D. ponderosae* infestation was of major proportion, with the

<sup>1</sup> Stikem Special, Michel and Pelton Co., 5743 Landregan, Emeryville, Calif.

1968 infestation accounting for an average of ca. 2 ft<sup>2</sup> basal area/acre. This figure was obtained by a 100% cruise of 320 acres, in which each tree was tallied by apparent cause and year of mortality back to 1967.

The 2nd area of study was adjacent to the Jaype mill site near Pierce, Idaho. The site is surrounded mostly by a 2nd growth stand of white pine. The probable source of *D. ponderosae* in this area was from the few brood trees contained in the cut logs stacked around the mill. During the winter of 1968–69, a salvage logging of 1968 brood trees was performed in the stands next to the mill. Snow was still visible under the log decks in mid-July, which fact probably accounted for the long protracted *D. ponderosae* flight noted in this area during the summer of 1969.

**Plot Design of Manipulation Study.**—A stand of mature white pine was selected near Lightning Point in early spring of 1969, and 8 plots of 40 acres each were laid out in a semicheckerboard fashion. Within each 40-acre plot, white pines were selected on 2-chain centers (132 ft), giving a potential of 100 test trees/plot. The criterion for selection was that the tree had to be mature and the deviation from each 2-chain center could be no more than 66 ft. If a nonhost was the only tree within the prescribed space it was not baited. Each selected tree was coded for a grid identification and plotted on a map for rapid referral.

In mid-April, each marked white pine was sprayed to a height of 18–20 ft with a 2.3% solution of lindane and water, in an attempt to avoid tree mortality. At this time the snow depth a few feet from the tree base ranged from 1 to 3 ft. Some 2 weeks prior to the main 1969 *D. ponderosae* flight (June 15), the 725 sprayed trees were baited with polyethylene tubing containing a 60:40 mixture of 93% pure *trans*-verbenol and alpha-pinene, and 2–4 polyethylene containers of alpha-pinene. The average baited tree received 6.2 ft of tubing.

After the peak flight terminated, all baited trees were examined for beetle attacks. Activity was characterized as few (1–10), moderate (11–99), or heavy (100 or more) when the visible attacks were within a height of ca. 20 ft. Prior to the 2nd generation's flight in late summer all baited trees were tallied for beetle attacks. At that time, a total cruise of all mature white pine was made in 7 adjacent unbaited 40-acre plots.

Within each baited 40-acre plot, a central 20-acre square was delineated, and the heavily attacked trees were counted in both the peripheral and central areas. A 20-acre square cannot be positioned within a 40-acre square containing 100 trees on 2-chain centers so that each area includes an equal number of trees. Therefore, positioning of the inner 20-acre area was accomplished by assigning a number from 1 through 4 to each corner of the plot, consulting a table of random numbers and moving the inner square to the upper right corner, lower left corner, etc. depending upon the digit encountered.

**RESULTS.**—Of the terpenes examined, alpha-pinene, a prominent component of the white pine terpene fraction, was most effective in combination with *trans*-verbenol (Table 1). Camphene, found in very small quantities in white pine (Hanover 1966), and myrcene also were active, but to a lesser degree. Beta-pinene, the principal monoterpene of white pine

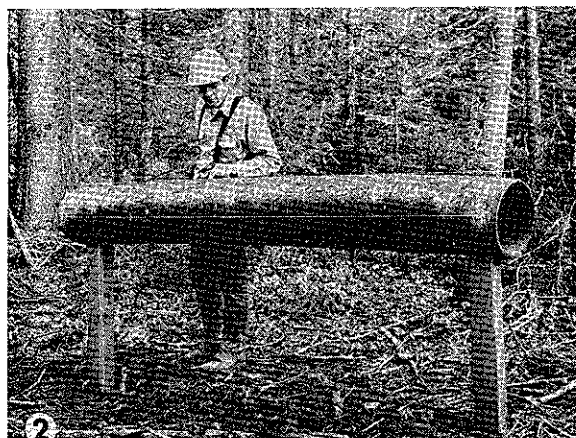
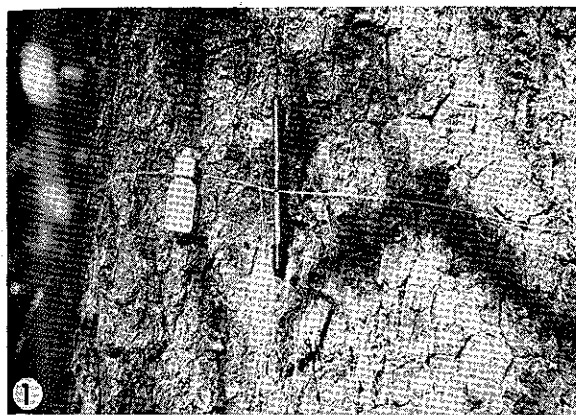


FIG. 1.—System for dispensing *trans*-verbenol (small-bore polyethylene tubing) and alpha-pinene (0.5 oz polyethylene Boston round bottles).

FIG. 2.—*D. ponderosae* trap consisting of a paperboard cylinder covered with a very tacky substance and baited with *trans*-verbenol and alpha-pinene.

Table 1.—Field response of *D. ponderosae* to olfactometers baited with synthetic *trans*-verbenol and various host terpenes. Jaype mill, near Pierce. 1968.

Date	Experimental treatment		Total responses	Average response <sup>c</sup>	Range	Sex ratio ♂:♀
	Pheromone <sup>a</sup>	Terpene <sup>b</sup>				
July 25	<i>trans</i> -Verbenol	Alpha-pinene	103	12.8	2-28	1:0.5
	<i>trans</i> -Verbenol	Beta-pinene	8	1	0-3	1:1
July 26	<i>trans</i> -Verbenol	Alpha-pinene	101	16.8	5-26	1:0.8
	<i>trans</i> -Verbenol	Camphene	70	11.7	2-22	1:0.7
	<i>trans</i> -Verbenol	Myrcene	50	8.3	5-12	1:0.9
	<i>trans</i> -Verbenol	Nonane <sup>d</sup>	2	.3	0-1	—
July 27	<i>trans</i> -Verbenol	Alpha-pinene	93	11.6	1-34	1:0.9
	<i>trans</i> -Verbenol	3-carene	5	.8	0-2	1:0.7
	<i>trans</i> -Verbenol	Limonene	2	.3	0-1	—
	<i>trans</i> -Verbenol	Nonane	1	.2	0-1	—

<sup>a</sup> 93% *trans*-verbenol, 6% *cis*-verbenol and 1% unknown.

<sup>b</sup> Terpenes were dispensed by adding ca. 1 g to a 50-ml beaker and covering with perforated aluminum foil.

<sup>c</sup> Average based on 6 to 8 15-min intervals starting usually at 6:00 PM Pacific Daylight Time.

<sup>d</sup> Not a known constituent of white pine oleoresin (Hanover 1966).

oleoresin, was ineffective. Alpha-pinene is also the principal host constituent of the pheromone complex of *D. frontalis* Zimmerman (Renwick and Vité 1969).

*Response to trans-Verbenol of Various Purity.*—Mixtures of 93, 86, and 84% *trans*-verbenol were bioassayed under competitive conditions with horizontal olfactometers (Table 2). Average response over two to three 30-min periods varied from 19 to 46 beetles, and no significant difference was detected between the 3 purity levels of *trans*-verbenol.

Since *trans*-verbenol of purity >99.5% was not available for comparison, the material of 93% purity was tested against a natural attractant source consisting of 125 virgin *D. ponderosae* feeding in a fresh white pine billet. Competitiveness of the synthetic pheromone was assessed by positioning field olfactometers containing 6 ft of polyethylene tubing charged with 93% *trans*-verbenol at 35, 80, and 110 ft from the olfactometer containing the infested billet. At all distances, 6 ft of tubing was competitive to the natural pheromone source. During three 30-min periods, the response to the synthetic pheromone averaged 150, while the average response to the feeding females was 9.7.

*Field Manipulation with trans-Verbenol.*—Soon after initiation of the 1969 flight it became apparent that lindane would not be effective in preventing beetle attack on the 725 sprayed and baited white pines. Of these baited pines, 133 or 18% were heavily attacked and killed (Table 3). Ca. another 15% received varying levels of attack, but the beetles were mostly pitched out or in some other way the attacks were aborted; no tree mortality resulted in this group. In the eight 40-acre plots containing baited trees, only 21 heavily attacked trees were found among the 7200 potential host trees. Ten of these attacked trees did not appear to be under the immediate influence of an adjacent baited and killed tree, while 11 were within 10-15 ft of baited and heavily attacked trees. A cruise of 9 adjacent unbaited 40-acre plots revealed an average of 17 attacked and killed pines/40 acres. The average of heavily attacked trees in the 8 baited 40-acre plots was 18.1 trees/40 acres.

In examining the plot map of the baited and killed trees, it appeared that a greater number of killed trees were in the outer perimeters of the 40-acre plots. The plots were delineated into center and

peripheral areas of 20-acres each. Heavily attacked trees were counted in each area. Application of a t-test to the data yielded a highly significant value of 4.65 for 7 df; 71% of the heavily attacked trees occurred in the outer 20-acre areas.

This distribution of heavily attacked trees in the baited 40-acre plots was quite different from the natural attack pattern by *D. ponderosae*. Prior to baiting, 1 representative 40-acre plot was given a 100% cruise and each mature white pine was tallied as to its location within the plot, DBH, and condition if the tree was dead as a probable consequence of bark beetle activity; the date of mortality was fixed as 1968, 1967, or 1966 and earlier. Based on these data the spatial distribution of beetle killed trees for the 3 periods was found to follow a negative binomial. The average k value of the 3 periods was 0.70264. A negative binomial distribution of 1969 mortality also was indicated in adjacent unbaited 40-acre plots.

*Deadtrapping.*—The use of traps which destroy the insects soon after they have come in contact with the device has merit, under certain forest management practices, over the technique of baiting living trees.

Table 2.—Relative attractiveness under field conditions of *trans*-verbenol containing various levels of known and unknown impurities and alpha-pinene, Jaype mill, near Pierce. 1969.

Date	Purity of <i>trans</i> -verbenol	No. observations <sup>a</sup>	Average response	Range	Sex ratio ♂:♀
June 18	Crude	3	26.6	24-31	1:0.2
	Distilled	3	32.0	21-41	1:0.2
June 19	Distilled and TLC <sup>b</sup>	3	22.6	20-26	1:0.2
	Crude	2	44.0	26-62	1:0.6
	Distilled	2	42.0	37-48	1:0.3
July 1	Distilled and TLC	2	46.0	33-51	1:0.5
	Crude	3	20.6	6-37	1:0.4
	Distilled	3	19.0	7-30	1:0.5
	Distilled and TLC	3	21.0	15-28	1:1.3

<sup>a</sup> Each observation was for 30 min, starting usually at 6:30 PM Pacific Daylight Time.

<sup>b</sup> TLC = thin layer chromatography.

Table 3.—Effect of baiting with *trans*-verbenol and alpha-pinene 725 mature white pine systematically selected in 40-acre plots, near Lightning Point, July–Aug., 1969.

Plot <sup>a</sup> no.	No. trees baited	No. trees attacked <sup>b</sup>			Percent of baited trees			No. unbaited trees heavily attacked <sup>c</sup>
		Heavy	Moderate	Few	Heavy	Moderate	Few	
1	95	25	1	17	26.3	1.0	17.9	1
2	88	11	1	5	12.5	1.1	5.7	2
3	88	12	5	4	13.6	5.7	4.5	2
4	95	10	4	14	16.5	4.2	14.7	3
5	60	7	3	4	11.6	5.0	6.6	0
6	100	23	5	14	23.0	5.0	14.0	4
7	100	23	1	10	23.0	1.0	10.0	3
8	99	22	4	16	22.2	4.0	16.2	6

<sup>a</sup> 40 acres per plot.

<sup>b</sup> Attack classified as "heavy" when there were an estimated 100 or more visible penetrations, "moderate" when there were 10 to 99, and "few" when only 1 to 10.

<sup>c</sup> Estimate of 900 unbaited trees/plot.

The feasibility of deadtrapping *D. ponderosae* was tested by setting up 3 paperboard cylinders, 2 horizontally and 1 vertically, coated with a tacky substance and baited with *trans*-verbenol and alpha-pinene. During the 15-day period the traps were in operation in mid-September 1969 near Jaype, 921 *D. ponderosae* were caught. The daily response to the vertical trap averaged 46.0, while the average response to the 2 horizontally positioned cylinders was 130.5. Because of the difficulty in determining sex by dissection, only 520 pooled beetles were examined; the ♂:♀ ratio was 1:0.4.

Induction of a mass attack in excess of the normal density of female attacks/ft<sup>2</sup> of bark surface (7–15) was attempted through deployment of large quantities of *trans*-verbenol and alpha-pinene on a white pine. It was theorized that intraspecific competition of developing brood might be caused by massive baiting with pheromone. A white pine 22 in. DBH, ca. 500 m west of the Jaype mill was baited in mid-July 1969 on the lower 10 ft of the bole with 200 ft of attractive tubing and several alpha-pinene containers (Fig. 3). By Aug. 21, the tree had succumbed to a mass attack by *D. ponderosae*; during the period of this study no other trees in the immediate area could be found under attack.

The tree was felled and the attacks were found to extend ca. 100 ft of the tree's 160-ft height. At breast height, the attack density was 11.8 ♀/ft<sup>2</sup> of bark surface, based on five 1-ft<sup>2</sup> samples. At 15 and 30 ft, the attack density averaged 7.6 and 6.3 ♀/ft<sup>2</sup>. Eleven trees from the Lightning Point, Idaho, area, averaging 25.7 in. DBH, and baited with ca. 7.5 ft of attractive tubing and 4 containers of alpha-pinene each, sustained an attack density averaging 12.2, 8.7 and 6.9 ♀/ft<sup>2</sup> of bark surface at heights of 5, 15 and 30 ft, respectively. Brood development and emergence in the massively baited tree did not vary significantly from a tree baited with 7.5 ft of attractive tubing.

Discussion.—Large-scale implementation of *trans*-verbenol and alpha-pinene in programs of population regulation of *D. ponderosae* should occur in the near future. The reliability of these chemicals to attract in-flight beetles is high, since in a steady state of delivery and in competition with natural attractant sources these compounds induced attacks on baited white pine. This conclusion is apparent since only 21 out of an estimated 7200 unbaited potential hosts were attacked and killed, and 11 were within 10–15 ft of heavily attacked and baited trees.

*Trans*-verbenol evaporating from polyethylene tubing was much more attractive than 150 ♀ actively feeding in a fresh white pine billet. If pheromones are to be used in a bark beetle management program they must be competitive to natural sources.

In the manipulation study near Lightning Point, living trees were used to trap the responding beetles, i.e. they entered and colonized the tree. If a bark beetle management program were to incorporate this approach, it must be emphasized that follow-up measures to decimate the brood and parent adults must be taken prior to emergence. Spraying with lindane prior to baiting does not seem effective in



FIG. 3.—White pine tree baited with 200 ft of small-bore polyethylene tubing charged with *trans*-verbenol and alpha-pinene. Larger tubes and bottle contained alpha-pinene.

preventing attacks by the mountain pine beetle. Similar results were obtained (Vité and Pitman 1970) when protection of ponderosa pine from western pine beetle, *D. brevicomis* LeConte, attacks was attempted in the presence of the species' population pheromone. An alternative to using living trees in a management program would be use of sticky traps, such as the paperboard cylinders described in this study. A potential technique, requiring no further treatment after baiting, would be to induce attacks on intermediate and suppressed pines with inner bark too thin to sustain a developing brood. An approach, but rather hypothetical, would be to "short circuit" in some manner the mechanism which prevents female *D. ponderosae* from overpopulating their host. With *D. ponderosae* a massive dose of pheromone does not override the mechanism regulating the spacing or attack density.

A problem still unresolved in the implementation of bark beetle pheromones in pest suppression is the spatial configuration of the attractant traps. A grid on 132-ft centers appears impractical in terms of the difficulty in attractant deployment, plus the long distance over which population pheromones are seemingly effective. Data from the Lightning Point study indicate that beetles were being drawn to the baited trees from adjacent unbaited areas. However, the distance over which there was an active response to the attractant could not be determined. Because of the great variability of forested areas, in terms of terrain, micrometeorology, vegetation, distribution of brood trees, and other physical and biological factors, it is difficult to determine experimentally the optimum rate of pheromone delivery and distance between traps. The objective is to maximize the distance between traps and still maintain the highest possible level of trapping efficiency. A purely empirical approach, such as arbitrarily setting a delivery

rate and spacing factor to a certain degree irrespective of pest and host species and topography, may be the most realistic way to apply bark beetle pheromones.

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