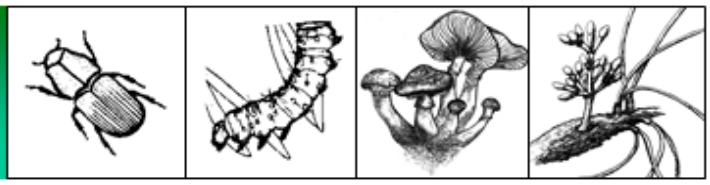
Forest Health Protection



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Effects of Verbenone and Other Repellents on Protecting Ponderosa and Lodgepole Pines from Mountain Pine Beetle-caused Mortality

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Abstract

Verbenone has been used extensively in the west to protect pine stands against mountain pine beetle (MPB) attacks. The level of protection has varied when evaluated in studies or used operationally. These variable results have been linked to variations in host stand conditions and beetle pressure.

It has been suggested that oxidized host compounds other than verbenone may also be important components of the natural anti-aggregation bouquet for MPB. Carveol, is a commercially available monoterpene alcohol derived from limonene, a common monoterpene found in pine oleoresin. We demonstrate carveol is a repellant semiochemical for MPB for the first time.

We evaluated verbenone in conjunction with other promising repellents (a green leaf volatile blend {GLV}, carveol and a proprietary ConA analog {ConA}) in individual tree tests in both ponderosa and lodgepole pine forests. In a second study, we evaluated the effectiveness of the standard verbenone 7 g pouch in an area-wide treatment test in ponderosa pine stands.

Standard 7 g verbenone pouches and 7.5 g carveol pouches protected greater than 80% of treatment trees at all three sites evaluated in individual tree tests in ponderosa pine stands. In lodgepole pine stands, low-dose verbenone in conjunction with ConA and carveol protected greater than 90% of trees in an individual tree test with high mountain pine beetle pressure (>60% mortality). In area-wide treatments in ponderosa pine stands, verbenone had a significant negative effect on percent of trees mass attacked at all three study locations. Results of this study also showed that mean tree diameter was negatively correlated with beetle attacks (P=0.02) and mean stand basal area was positively correlated with beetle attacks (P=0.058).

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INTRODUCTION

Verbenone (4, 6, 6-trimethyl-bicyclo [3.1.1] hept-3-en-2-one) is a known anti-aggregation pheromone of mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins. It has been used extensively in Montana and Idaho and other parts of the west to protect high-value pine trees and stands from losses due to MPB.

Verbenone pouches and other repellents, including green leaf volatiles (GLVs), have been evaluated for their effectiveness at protecting whitebark, ponderosa and lodgepole pines against MPB attacks (Borden et al. 2003, Gibson and Kegley 2004, Bentz et al. 1989, Bentz et al. 2005, Negron et al. 2006, Kegley and Gibson 2009, Sturdevant et al. 2009, Fettig et al. 2012). Although many of these studies showed verbenone and GLVs were effective in reducing MPB attacks in lodgepole and whitebark pines, the level of protection varied greatly.

Several studies have been conducted evaluating the effectiveness of using verbenone to protect individual trees or stands of ponderosa pine. The studies conducted have shown either no protection or inconsistent results (Bentz et al. 1989, Gibson & Kegley 2004, Kegley & Gibson 2009, Negron et al. 2006). The reasons cited for this were varying doses of verbenone used, varying stand conditions, and varying or lack of beetle pressure.

The goals of this study were to: 1) evaluate the effectiveness of using a 7 g verbenone pouch (current standard pouch) deployed at 30 pouches per acre to protect stands of ponderosa pine in Montana and Idaho, and 2) evaluate carveol that showed promise in trap shut-down experiments (Wakarchuk, personal communication) and may ultimately result in better efficacy and lower product costs. In this study the compounds carveol and ConA were evaluated alone and in conjunction with low-dose verbenone.

Studies were conducted during 2012 and 2013 using verbenone and different combinations of repellents at several locations in Montana and Idaho to protect ponderosa and lodgepole pine in area-wide and individual tree tests.

METHODS

2012 Studies

Ponderosa Pine Study Sites–2012: Individual tree and area protection studies were conducted at three field locations: Lake Como (46.081°N, -114.25°W; 4580 ft. elevation) and Chaffin Creek (45.94°N, -114.18°W; 5080 ft. elevation), Bitterroot National Forest, Montana; and Sheep Creek (45.45°N, -119.91°W; 6294 ft. elevation), Salmon-Challis National Forest, Idaho. Cooperators on each participating forest suggested drainages that would be good potential study sites based on accessibility and MPB activity. We confirmed current MPB activity for each area using ADS data and a site visit. Each treatment area had to have current MPB activity within 1 mile of a plot and a sufficient number of available live trees to test. We selected plots that were close but not within current groups of MPB-caused tree mortality. 2012 ADS data showed that Lake Como had scattered MPB activity in ponderosa pine stands and also several larger groups of 100-150 MPB-attacked trees within a mile of the treatment stands. Chaffin Creek also had small groups of MPB-attacked ponderosa pines and groups of 50-300 trees within a mile of treatment stands (figure 1). MPB was at outbreak levels in both study areas. MPB activity was declining at Sheep Creek in 2012 but was still at outbreak levels (greater than one MPB-infested tree per acre).

For the area test, stands were selected that were composed of approximately 50% or greater ponderosa pine. In addition, lodgepole pine, grand fir, and aspen were found at Chaffin Creek; Douglas-fir, lodgepole pine, grand fir, and subalpine fir were found at Lake Como; and lodgepole pine (one tree) at Sheep Creek. Stand densities varied across the three study sites and were lowest for Sheep Creek plots (Table 1).

Table 1. Stand densities of all species at 2012 ponderosa pine study sites

Location	Basal Area (ft^2/acre)	Trees/acre
Chaffin Creek	70-97	93-208
Lake Como	68-138	73-147
Sheep Creek	48-77	31-151

Fig. 1. Ponderosa pine mortality caused by mountain pine beetles at Chaffin Creek, Bitterroot National Forest, Montana-2012.

<u>Ponderosa Pine Individual-tree Protection–2012</u>: At each study site, individual ponderosa pine trees (90 at Lake Como and Chaffin Creek and 60 at Sheep Creek) 12-30 inches in diameter at 4.5 feet above ground (d.b.h.) with no sign of MPB infestation were selected along transects, spaced at a minimum of 132 feet, and randomly assigned treatments. Basal area surrounding each treatment tree, using the treatment tree as the center of a variable radius plot, and d.b.h. were measured for each treatment and control tree at all three study sites.

Table 2. Treatments applied to individual ponderosa pine trees at each study site in 2012. All products were provided by Synergy Semiochemical Corporation.

TREATMENTS	
Lake Como:	1. two 7 g verbenone pouches, n=30
2. three component inhibitor mix (7.6 g carveol pouch, 7.5 g green leaf volatile (GLV) pouch, two 1 g MCH double bubbles, n=30	
3. control, n=30	
Chaffin Creek:	1. two 7 g verbenone pouches, n=30
	2. two 7.6 g carveol pouches, n=30
	3. control, n=30

TREATMENTS	
Sheep Creek:	1. two 7 g verbenone pouches, n=30
	2. control, n=30

Commercial MPB pheromone baits (11 mg exo-brevicomin flexlure, 190 mg trans-verbenol bubble) were stapled to the north side of a non-host tree or wooden stake located 5-10 feet from each experimental tree. Treatments were stapled to experimental trees about 6 feet above ground on north side of tree. Treatments were deployed the 3rd week in June.

Ponderosa Pine Area Protection–2012: Ten, one-acre square, fixed area plots were installed at each study site. Plots were paired in blocks with similar stand conditions, with two chains (132 ft.) between plots within blocks and greater than 0.1 miles between treatment blocks.

Figure 2. One of the stands on the Bitterroot NF used for the area-wide protection study.

Treatments were randomly assigned within treatment blocks and were the same at all three study sites: 1) control (no anti-aggregation chemicals), and 2) 30 7 g. verbenone pouches (Synergy Semiochemical corp.) applied on a grid (grid points were separated by 37 feet and release devices were stapled to north side of tree closest to grid point or to wooden stake if no trees were available within 5 feet of grid point) (fig. 2). For the verbenone treatment plots, an additional line of verbenone pouches were placed as a buffer extending 37 feet from plot perimeters. One MPB bait was stapled to a wooden stake at center of each plot. Anti-aggregate treatments and MPB baits were hung mid- to late-June (Lake Como 6/12-6/13; Sheep Creek 6/20-6/22; Chaffin Creek 6/26-7/2).

For both individual and area-wide treatment studies all ponderosa pine greater than 5” d.b.h. were examined for MPB attack in September or October (Lake Como, 9/5-9/7; Chaffin Creek, 9/10-9/12; and Sheep Creek, 10/3-10/4). Trees were considered mass attacked if boring dust or pitch tubes surrounded most of the root collar or bole, and/or the phloem and sapwood were discolored and beetle galleries and larval mines were visible following bark removal. Attacks confined to ½ or less of the tree circumference were recorded as strip attacks. Trees with large pitch tubes with little or no red, oxidized frass were marked as pitch-outs (i.e., unsuccessful attacks). Trees

with no evidence of bark beetle attacks were recorded as no attacks. A subsample of trees were evaluated the following year to confirm tree status-live versus dead.

2012 Statistical Analysis

Ponderosa Pine Individual-tree Protection–2012: Numbers of trees with no beetle attack, mass attack, strip attack, and pitch-outs were summarized in a contingency table (Table 5). Separate analyses were conducted at each site using Pearson Chi-Square test to determine significant differences in the type of MPB attack between treatments (alpha = 0.05). In addition, we used a binomial test that is the established standard for testing the efficacy of pesticides for tree protection from bark beetle-caused mortality (Shea et al. 1984, Haverty et al. 1985, 1998, Fettig et al. 2006a, 2006b, DeGomez et al. 2006, Grosman et al. 2010). This test requires two criteria to evaluate treatment efficacy: 1) trees had to be challenged by bark beetles, and 2) trees did not die from bark beetle attack during the course of the study. Sufficient bark beetle pressure to test the efficacy of treatment is considered when ≥60% of the control trees die (sufficient bark beetle pressure is typically created using beetle pheromone baits to draw beetles to trees). When the sufficient bark beetle pressure criterion is met a binomial test with the null hypothesis (H_o): tree survival ≥90% is applied to the treatment(s). With a sample size of 30 (alpha = 0.05%) this null is rejected when greater than 6 trees are killed by bark beetles. For this test we considered all mass attacked trees as dead and all other designations (strip attack, pitch out, and no attack) as live trees. This high level of protection (90% survival) used in evaluating individual tree protection by pesticides provides what we consider a very stringent test of the efficacy of anti-aggregation pheromone treatments employed in our study.

Ponderosa Pine Area Protection–2012: The study design was a completely randomized block replicated at three sites. A dependent variable, % mass attacked, was calculated for each plot using the counts of mass attacked ponderosa pine trees at the end of the experiment and the count of live un-attacked ponderosa pine trees at the beginning of the experiment. Using the General Linear Method (SAS 9.3), we tested the effect of treatment and block (no interaction term due to blocking) on the dependent variable % mass attacked. Site was also included in the model, with treatments and blocks nested within site. Shea et al. 1984 stringent efficacy test was not used in the area-wide analysis because of the anticipated level of variability that is inherent in area-wide studies.

To further explore the potential effects that stand level variables may have on MPB-caused ponderosa pine mortality, we analyzed the plot data for correlations. Using Person's Correlation Coefficient (SAS 9.3) the independent variables trees per acre (TPA), basal area (BA), stand density index (SDI) and mean ponderosa pine d.b.h. were each analyzed separately for correlations with % mass attacked for control plots only.

2013 Studies

Lodgepole Pine Study Sites–2013: Two different individual tree protection studies were conducted at two locations: Wyman Creek (N45.56063; W -113.13379), Beaverhead-Deerlodge National Forest and Skeleton Creek (N47.96234; W -115.89627), Kootenai National Forest. Study sites were selected in primarily pure lodgepole pine stands with moderate to high MPB activity in area. Forest cooperators suggested potential study areas based on accessibility and current MPB activity. 2012 ADS data showed moderate to high levels of MPB-caused tree mortality in both areas.

Lodgepole Pine Individual Tree Protection: At each study site, individual lodgepole pine trees 8-15 inches d.b.h. with no sign of MPB infestation were selected along transects, spaced at a minimum of 132 feet, and randomly assigned treatments (30 in each treatment, Table 3).

Table 3. Treatments applied to individual trees at each study site. All products were provided by Synergy Semiochemical Corporation.

TREATMENTS	
Lake Como:	1. two 7 g verbenone pouches, n=30
2. three component inhibitor mix (7.6 g carveol pouch, 7.5 g green leaf volatile (GLV) pouch, 2 1 g MCH double bubbles , n=30	
	3. control, n=30
Chaffin Creek:	1. two 7 g verbenone pouches, n=30
	2. two 7.6 g carveol pouches
	3. control, n=30
Sheep Creek:	1. two 7 g verbenone pouches, n=30
	2. control, n=30

Commercial MPB pheromone baits were stapled to the north side of a non-host tree or wooden stake located 5-10 feet from each experimental tree. Treatments were stapled to experimental trees about 6 feet above ground on north side of tree (fig. 3). Treatments were deployed the 3rd week in June. Treatment release devices and MPB baits were removed and tree status was recorded early September. All trees were rated as mass attack, strip attack, pitch out, or no attack. Basal area surrounding each treatment tree, using the treatment tree as the center of a variable radius plot, and d.b.h. were measured for each treatment and control tree at all three study sites.

On September 24-25 and October 24-25, 1/50-acre fixed-area plots were installed at each lodgepole pine single-tree protection tree, with the treatment trees serving as the center of the plot. Tree species, d.b.h. and bark beetle damage class were recorded for each tree greater than five inches in diameter.

Fig. 3. Verbenone bubble caps, carveol, and ConA lodgepole pine treatment.

2013 Statistical Analysis

As was done in the 2012 individual tree protection studies, both Pearson Chi-Square tests, to determine differences in attack patterns, and binomial tests, to determine the efficacy of the treatments for preventing tree mortality, were used (see 2012 Statistical Analysis). With data collected on the fixed-area plots, percentage of trees mass attacked by MPB in the treatment year was calculated for each plot. This data violated normality assumption; therefore, differences in percent lodgepole mass attacked due to treatment was analyzed with the non-parametric test Kruskal-Wallis Test.

RESULTS

Ponderosa Pine Individual-tree Protection–2012: Trees in the individual-tree protection study ranged from 10 to 27.3 inch d.b.h and a variable radius plot surrounding the individual trees ranged from 20 to 240 ft^2/acre BA (Table 4).

Table 4. Comparison of tree diameter and basal area means in ponderosa pine individual tree protection studies at each site-2012

		DBH (in)		BA (ft^2/ac)	
Site	Treatment	Mean DBH*	Range	Mean BA*	Range
Lake Como	Control	16.4 (0.5)	11.5-21.8	103.3 (7.3)	40-240
	Inhibitor Mix	15.7 (0.7)	10.0-27.3	112.7 (4.4)	60-180
	Verbenone	16.1 (0.7)	10.0-27	113.3 (7)	60-220
Chaffin Creek	Control	16.2 (0.6)	12.2-23.4	78.3 (6.6)	40-160
	Carveol	17.2 (0.6)	12.8-25.0	82.1 (5.5)	40-160
	Verbenone	16.8 (0.7)	12.0-27.3	85.3 (6.8)	40-180
Sheep Creek	Control	16.6 (0.7)	11.0-26.0	88.5 (7.3)	20-160
	Verbenone	16.1 (0.6)	12.0-24.0	73.5 (6.2)	20-160

***Standard error of mean in parentheses, no significant differences found among treatments when tested at each site**

Both verbenone and carveol protected equal to or greater than 80% of treatment trees at Chaffin Creek. Chaffin Creek was the only site that experienced ≥60% MPB-caused mortality (mass attacked = 63%; Table 5) in the control, meeting the criterion for sufficient MPB pressure set by Shea et al. (1984). At the Lake Como site, >60% of the control trees were attacked by MPB but only 33% (10 trees) were mass attacked by MPB. This level of MPB activity did not meet the ≥60% criterion, but we considered this to be active enough to merit analysis with Pearson Chi-Square test.

At Chaffin Creek, where we met the ≥60% mortality criterion, there was a significant difference among beetle attack classification due to treatment (Chi-squared = 20.2, $P < 0.01$). Both verbenone and carveol had similar levels of MPB-caused mortality with 17% and 20% mass attacked trees, respectively, while the control had 63% mass attack trees (Table 5, Fig. 4). Therefore, both passed the binomial test (verbenone, P=0.17; carveol, P=0.07) and provided protection not significantly different than 90% protection.

At Lake Como, there was no significant difference in attack classification among treatments (Chi-squared = 10.1, P = 0.12; Table 5, Fig. 5). Verbenone, however, did have the largest number of trees not attacked and the fewest

mass attacked trees, with only 3 trees killed by MPB. If we consider live versus dead trees at Lake Como, 27 verbenone treated trees (18 no attack + 9 pitch-out) were alive at the end of the experiment while 20 control were still alive.

Sheep Creek had low MPB activity with only 4 of the 60 trees (3 verbenone treated, and 1 control (Table 5)) killed by MPB. Because of low MPB activity at Sheep Creek, no statistical analysis was conducted with these data.

Table 5. Mountain pine beetle attacks in 2012 individual tree protection ponderosa pine by site.

Site	Treatment	No Attack	Pitch Out	Strip Attack	Mass Attack
Lake Como	Verbenone	18 (60%)	9 (30%)	0	3 (10%)
	Inhibitor Mix*	9 (30%)	12 (40%)	1 (3%)	8 (27%)
	Control	10 (33%)	10 (33%)	0	10 (33%)
Chaffin Creek	Verbenone	21 (70%)	4 (13%)	0	5 (17%)
	Carveol	20 (67%)	3(10%)	1 (3%)	6 (20%)
	Control	9 (30%)	2 (7%)	0	19 (63%)
Sheep Creek	Verbenone	25 (83%)	2 (7%)	0	3 (10%)
	Control	27 (90%)	2 (7%)	0	1 (3%)

*1 carveol, 1 GLV, 2 MCH super bubbles

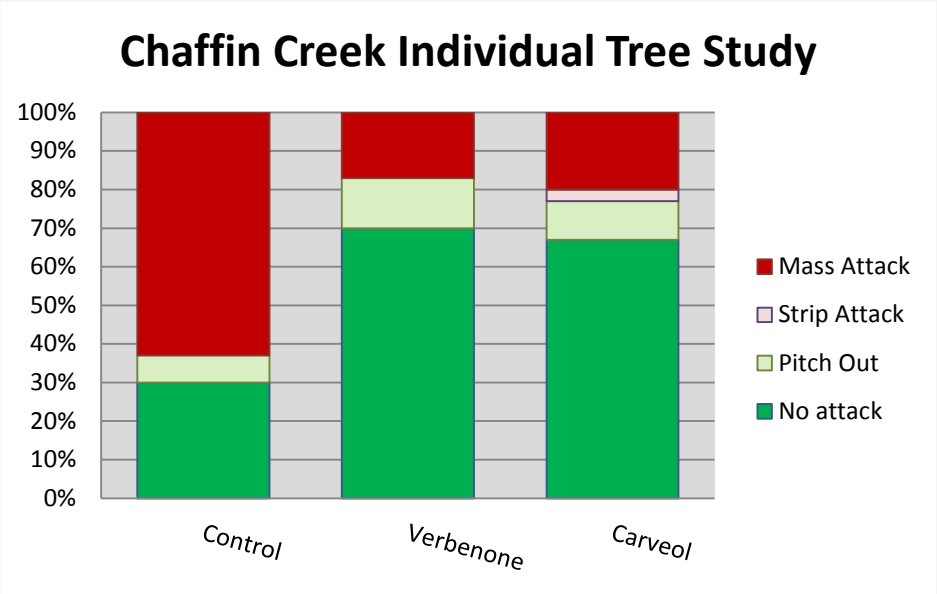

Figure 4. Percent of trees attacked and not attacked for control and treatment trees at Chaffin Creek-2012.

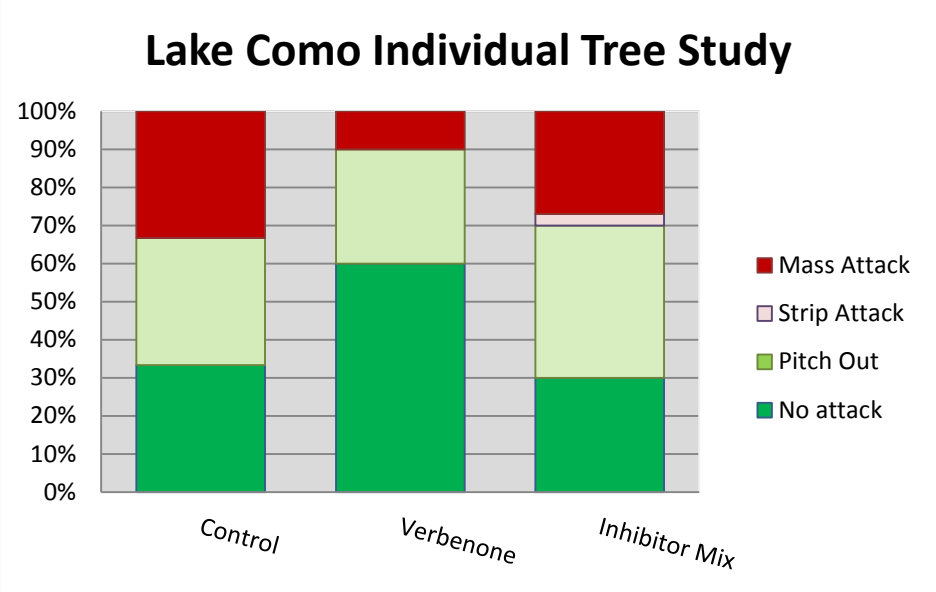

Figure 5. Percent of trees attacked and not attacked for control and treatment trees at Lake Como-2012.

<u>Ponderosa Pine Area Protection–2012</u>: Mean d.b.h. across all three study sites ranged between 9.8 and 12.8 inches (Table 6). Mean TPA ranged between 81.4 and 150 trees per acre across all three study sites.

Table 6. Stand characteristics for treatment plots in each of three sites in area protection test 2012. Numbers in parentheses are standard errors of the mean.

Site	Treatment	Ponderosa Pine Mean DBH (in)	Mean Trees/acre	Mean Basal Area (ft2/acre)	Mean Stand Density Index
Lake Como	Control	12.8 (1.1)	100.2 (14.6)	91.8 (9.3)	142.1 (15.3)
	Verbenone	12.8 (0.9)	110.4 (7.3)	99.5 (11.7)	156.4 (15.5)
Chaffin Creek	Control	9.8 (0.7)	128.0 (16.3)	81.4 (4.7)	139.4 (7.1)
	Verbenone	9.7 (0.2)	150.0 (17.8)	81.5(5.3)	147 (10.5)
Sheep Creek	Control	11.0 (0.9)	99.0 (14.1)	66.5 (4.5)	109.5 (8.8)
	Verbenone	12.1 (1.3)	81.4 (19.8)	59.0 (5.7)	96.8 (12.3)

The verbenone treatment had a significant effect on % mass attack (F = 7.8, P = 0.01) at all three sites; control plots had higher % mass attack than verbenone treated plots (Lake Como, Control = 7.5 % ± 3.5 (SEM), Verbenone = 1.8 % ± 0.9; Chaffin Creek, Control = 41.1% ± 7.8, Verbenone = 10.6 % ± 2.9; Sheep Creek, Control = 12.4 % ± 10.6, Verbenone = 2.5 % ± 2.5; Fig. 6).

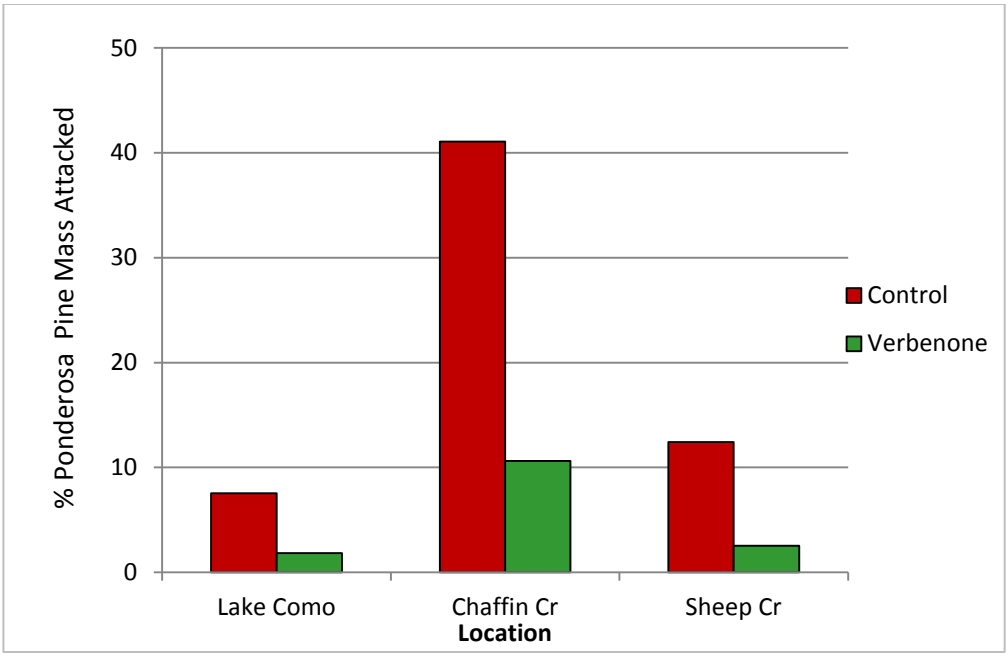

Fig. 6. Percent of trees killed by MPB in area ponderosa pine treatment vs. control plots at the three study sites.

The correlation analysis found two significant correlations at the 0.05 alpha level. Mean PP Diameter had a significant negative correlation (r = -0.52, P = 0.04) with % mass attacked. Trees in stands with an average d.b.h greater than 16" d.b.h. not attacked. Also, TPA had a positive correlation (P = 0.05) with % mass-attacked trees (Table 7).

Table 7. Results from Pearson's correlation analysis comparing stand level data for control in Area Protection Study and their corresponding percentage of ponderosa pine killed by mountain pine beetles in 2013.

		% Mass Attacked	
Stand Variable	N	Person's Coefficient	*P*-value
Mean Ponderosa Diameter	15	-0.52	0.04
Stand Density Index	15	0.23	0.40
Basal Area (all tree species)	15	0.04	0.87
Trees per acre (all tree species)	15	0.51	0.05

Lodgepole Pine Individual Tree Protection–2013: Both lodgepole pine sites met the ≥60% mortality criterion in the control trees for sufficient mountain pine beetle pressure. There were a statistically significant difference in d.b.h between treatments at Wyman Creek (Table 8). However, the average d.b.h. of trees were very similar and it is our professional judgment that the differences were not biologically significant.

Table 8. Comparison of tree diameter means in lodgepole pine individual tree protection studies at each site in 2014.

*Standard error of mean in parentheses

Site	Treatment	DBH(in)*	DBH Range	BA (ft^2/ac)*	BA Range
Skeleton Creek	Control	11.2 (0.3)	8.0-14.8	124.3(9.0)	62.5-241.2

Site	Treatment	DBH(in)*	DBH Range	BA (ft^2/ac)*	BA Range
Wyman Creek1	High Dose Carveol + ConA	10.7 (0.4)	8.2-14.8	123.7(10.5)	39.2-246.9
	Control	10.5 (0.2)	8.3-13.0	197.5(11.0)	76.5-372.1
	Verbenone +Carveol + ConA	10.9 (0.3)	8.9-14.8	209.4(11.0)	98.4-326.2
	Verbenone + ConA	10.1 (0.2)	8.1-13.0	205.2(12.7)	95.9-403.3

At Wyman Creek, less than 10% of treatment trees were mass attacked by MPB. The inhibitor compound at Skeleton Creek provided about 65% protection of individual trees (Table 9, Fig. 7).

Table 9. Mountain pine beetle attacks in lodgepole pine by site

Site	Treatment	No Attack	Pitch Out	Strip Attack	Mass Attack	Secondary Beetles
Skeleton Creek	Carveol + ConA	16 (53%)	3 (10%)	1 (3%)	10 (33%)	0
	Control	8 (27%)	1 (3%)	1 (3%)	20 (67%)	0
Wyman Creek	Low verb + ConA	16 (53%)	11 (37%)	2 (7%)	1 (3%)	0
	Low verb + ConA +Carveol	11 (37%)	11 (37%)	5 (17%)	2 (7%)	1 (3%)
	Control	2 (7%)	5 (17%)	3 (10%)	20 (67%)	0

The inhibitor compound at Skeleton Creek provided about 70% protection of individual trees (table 9, fig. 7). At Skeleton Creek we met the ≥60% mortality criterion (20 of 30 trees mass attacked and killed by MPB); therefore, the binomial test was performed. With 10 trees mass attacked and killed by MPB the Carveol/ConA treatment failed to meet the 90% protection level ($P < 0.001$, reject hypothesis that level of protection is equal to 90%). In our analysis of differences in attack patterns there was no significant difference among beetle attack classification due to treatment (Chi-square = 7.0, $P = 0.07$). However, seventy percent of control trees were mass or strip attacked by MPB (21 trees) as compared to 36% (11 trees) of treatment trees attacked (see Table 9).

At the Wyman Creek study we meet the ≥60% mortality criterion (20 of 30 control trees mass attacked and killed by MPB); therefore, the binomial test was performed. Both the Low Verb/ConA treatment (one mass attacked, $P = 0.18$) and the Low Verb/ConA/Carveol treatment (two mass attacked, $P = 0.41$) passed the binomial test and provided protection not significantly different than 90% protected. A significant difference was also found between the attack patterns due to treatment (Chi-square = 44.3, $P < 0.001$). While 24% of Control trees were free of successful MPB attacks (No Attack + Pitch Out), 90% of Low Verb/ConA and 77% of Low Verb/ConA/Carveol were free of successful MPB attacks (see Table 9).

At both the Wyman and Skeleton Creek lodgepole pine single-tree protection studies, trees within 16.7 feet of treatments trees experienced statistically different levels of MPB mass attacks due to treatment (Wyman Creek, df = 2, *Chi-square* = 19.7, $P < 0.001$; Skeleton Creek, df = 1, *Chi-square* = 5.8, $P = 0.02$). At Wyman Creek, control tree plots had the highest level of mass attacked trees (37.0% ± 4.4%), followed by low dose verbenone + carveol + ConA tree plots (16.3% ± 3.7%), a 56% reduction from the control and then low dose verbenone + ConA tree plots with the lowest level (11.9% ± 3.0%), a 67% reduction from the control. At Skeleton Creek, control tree plots

had 46.3% ± 6.4% mass attacked and carveol + ConA tree plots had 24.3% ± 5.7% mass attacked, a 47% reduction in the treatment compared to the control.

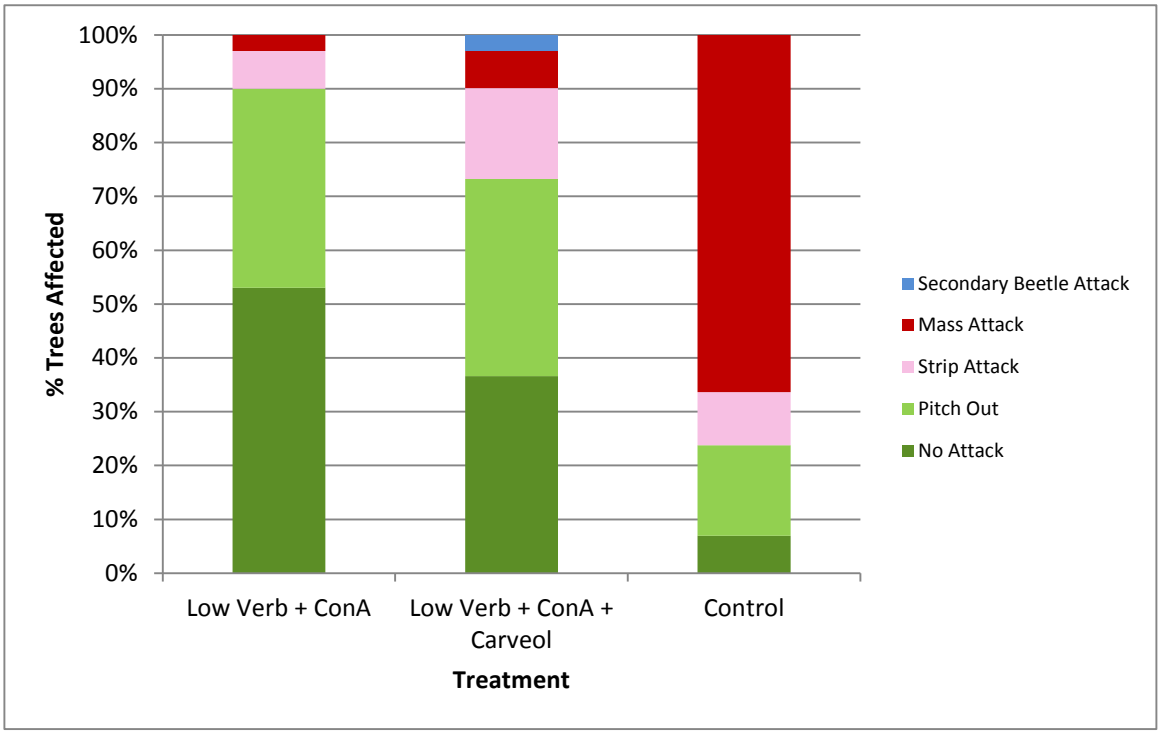

Fig. 7. Percent trees mass attack, strip attack, pitch out, or no attack for low dose verbenone + ConA, low dose verbenone + ConA + carveol, and control treatments on lodgepole pine at Wyman Creek.

DISCUSSION

Ponderosa Pine

The results of this study show that verbenone pouches can significantly reduced MPB-cause ponderosa pine mortality when applied at a rate of 30 per acre (201 grams/acre) in a grid for area protection. Verbenone reduced mortality at all three sites regardless of beetle pressure and stand characteristics. At Chaffin Creek where beetle pressure was the highest, verbenone performed the best resulting in a 74% reduction in MPB-caused tree mortality compared to untreated controls.

Our results are in contrast to previous verbenone studies. Both Negron et al. (2006) and Bentz et al. (1989) found no significant differences between verbenone treatments and control plots in ponderosa pine in the Black Hills, South Dakota and in Colorado. However, Bentz et al. (1989) used various densities of verbenone bubble capsules instead of pouches. Although they did not state the amount of verbenone in their bubble capsules, if similar to those in a Negron et al. (2006) test, capsules contained 0.8 grams of verbenone so their highest density treatment had 54 grams of verbenone /acre compared to 201 grams/acre in our study. Negron et al. (2006) tested bubble capsules as well as 5 gram verbenone pouches and concluded that higher temperatures, solar radiation, and wind movement in less dense ponderosa pine stands may disperse the pheromone, causing a decrease in its effect on insect communication. They also concluded that they did not have sufficient beetle pressure in their study. In an

area protection test in ponderosa pine in Montana, Gibson & Kegley (2004) found that 5-gram verbenone pouches placed at 20 pouches per acre and replaced mid-season had significantly less MPB attacks than control plots. However, beetle populations were low during this test.

Although we did not specifically test for the effect of stand density on MPB attacks, stand density and tree diameters were measured in area-wide protection test and, although not conclusive, correlation analysis yielded some interesting results. As average stand ponderosa pine diameter increased, % trees mass attacked in the stand decreased. We are not sure what caused this correlation but it is an interesting trend that suggests that MPB may not prefer stands with mostly large-diameter mature trees, especially when there are ample pole-sized stands to successfully breed in. The correlation analysis also showed that MPB attacks increased with increasing TPA. MPB are thought to avoid more open stands because of increased light, wind, and temperatures (Bartos and Amman 1989), and it could be hypothesized that higher levels of MPB attacks in our stands with higher TPA were due to preferred micro-climate in denser stands.

Our results show that carveol provided a similar level of protection (80% no attack, pitch out, or strip attack) as 2 standard verbenone pouches (83% no attack, pitch out or strip attack) applied to individual ponderosa pine trees at the Chaffin Creek site. At Como Lake, the inhibitor mix alone did not provide as good of protection as the verbenone treatment when applied to individual trees. However, both verbenone and the inhibitor mix protected more pines as compared to controls. Based on these preliminary tests, carveol and the inhibitor mix may provide alternatives to the verbenone alone tactic for MPB in ponderosa pine.

Lodgepole Pine

Low-dose verbenone when combined with carveol or a combination of carveol and ConA was successful in protecting individual lodgepole pines despite high beetle pressure at the Wyman Creek (67% of control trees were mass attacked by MPB). At Skeleton Creek, twice as many control trees were mass attacked (66%) as those treated with a combination of carveol and ConA without verbenone. Significantly fewer lodgepole pine were killed on 1/50th acre plots surrounding trees treated with low dose verbenone + other inhibitors or the other inhibitors alone than on plots surrounding control trees. This suggests that the range of tree protection (or beetle pheromone attraction disruption) due to treatments went beyond the tree that was treated, at least up to a 16.7 foot radius surrounding treated trees. Combining lower-dose verbenone with other potentially less expensive inhibitors may provide alternative tactics to high dose verbenone alone tree protection and warrants further testing.

Management Implications

The results of our studies show that standard 7 g verbenone pouches (Synergy Semiochemical) applied at 30 pouches per acre is effective at significantly reducing MPB-caused mortality in ponderosa pine stands across varying stand densities and beetle pressure. In the individual tree tests in ponderosa pine, verbenone alone protected between 80-90% of study trees. It is also worth noting that verbenone performed the best at the site with the highest MPB pressure.

It appears that the addition of low-dose verbenone is an important component of an anti-aggregation compound for MPB. Combining lower-dose verbenone with other potentially less expensive and effective inhibitors may provide effective tree protection at significant cost-savings and warrants further testing.

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Citations

Bentz, B; Lister, C.K.; Schmid, J.M.; Mata, S.A.; Rasumssen, L.A.; Haneman, D. 1989. Does verbenone reduce mountain pine beetle attacks in susceptible stands of ponderosa pine? Research Note RM-45, Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, 4 p.

Bentz, B.J.; Kegley, S.; Gibson, K; Thier, R. 2005. A test of high-dose verbenone for stand-level protection of lodgepole and whitebark pine from mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) attacks. J. Econ. Entomol. 98(5), 1614-1621.

Borden, J.H.; Chong, L.J.; Earle, T.J.; Huber, D.P.W. 2003. Protection of lodgepole pine from attack by the mountain pine beetle, Dendroctonus ponderosae (Coleoptera: Scolytidae) using high doses of verbenone in combination with no host bark volatiles. The Forestry Chronicle, 79 (3), 685-691.

Degomez, T.E.; Hayes, C.J.; Anhold, J.A.; McMillin, J.D.; Clancy, K.M.; Bosu.P.P. 2006. Evaluation of insecticides for protecting southwestern ponderosa pines from attack by engraver beetles (Coleoptera: Curculionidae, Scolytinae). J. Econ. Entomol. 99,393-400.

Fettig, C.J.; Allen, K.K.; Borys, R.R.; Christopherson, J.; Dabney, C.P.; Egar, T.G. Gibson, K.E.; Herbertson, E.G.; Long, D.F.; Munson, A.S.; Shea, P.J.; Smith, S.L.; Haverty, M.I. 2006a. Effectiveness of bifenthrin (Onyx®) and carbaryl (Sevin SL®) for protecting individual, high-value conifers from bark beetle attack (Coleoptera: Curculionidae, Scolytinae) in the western United States. J. Econ. Entomol. 99, 1691-1698.

Fettig, C.J.; DeGomez, T.E.; Gibson, K.E.; Dabney, C.P.; Borys R.R. 2006b. Effectiveness of permethrin plus-C (Masterline®) and carbaryl (Sevin SL®) for protecting individual, high-value pines from bark beetle attack. Arbor. Urban Forestry. 32,247-252.

Fettig, C.J.; Bulaon, B.M; Dabney, C.P.; Hayes, C.J.; McKelvey, S.R. 2012. Verbenone plus reduces levels of tree mortality attributed to mountain pine beetle infestations in whitebark pine, a tree species of concern. J. Biofertil Biopestici 3(1), 1-5

Gibson, K.; Kegley, S. 2004. Testing the efficacy of verbenone in reducing mountain pine beetle attacks in second-growth ponderosa pine. Report 04-07. Missoula, Montana. U.S. Department of Agriculture, Forest Service, Northern Region Forest Health Protection. 8 p.

Grosman, D.M.; Fettig, C.J.; Jorgenson, C.L.; Munson, A.S. 2010. Effectiveness of two synthetic insecticides for protecting western conifers from mortality due to bark beetle attack. West. J. Appl. For. 25, 181-185.

Haverty, M.I.; Shea, P.J.; Hall, R.W. 1985. Effective residual life of carbaryl for protecting ponderosa pine from attack by western pine beetle (Coleoptera: Scolytidae). J. Econ. Entomol. 78, 197-199.

Haverty, M.I.; Shea, P.J.; Hoffman, J.T.; Wenz, J.M.; Gibson, K.E. 1998. Effectiveness of esfenvalerate, cyfluthrin, and carbaryl in protecting individual lodgepole pines and ponderosa pines from attack by Dendroctonus spp. PSW-RP-237, U.S. Department of Agriculture, Forest Service. 12 p.

Kegley, S.; Gibson, K. 2009. Individual-tree tests of verbenone and green-leaf volatiles to protect lodgepole, whitebark and ponderosa pines, 2004-2007. Report 09-03. Coeur d'Alene, ID: U.S. Department of Agriculture, Forest Service, Northern Region Forest Health Protection. 12 p.

Negron, J.F.; Allen, K.; McMillin, J.; Burkwhat, H. 2006. Testing verbenone for reducing mountain pine beetle attacks in ponderosa pine in the Black Hills, South Dakota. Research Note RMRS-RN-31. Fort Collins, CO: Rocky Mountain Research Station. 7 p.

Shea, P.J.; Haverty, M.I.; Hall, R.C. 1984. Effectiveness of fenitrothion and permethrin for protecting ponderosa pine trees from attack from western pine beetle. J. Ga. Entomol. Soc. 19, 427-433.

Sturdevant, N; Gibson, K; Cramer, W. 2009. Protecting lodgepole pine from mountain pine beetle attack using verbenone and green-leaf volatiles, Rocky boys Indian Reservation, 2008. Report 09-05. Missoula, MT: U.S. Department of Agriculture, Forest Service, Northern Region Forest Health Protection, 5 p.