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Individual-Tree Tests of Verbenone Flakes, Verbenone Pouches, and Green-Leaf Volatiles to Protect Lodgepole Pines from Mountain Pine Beetle Attack

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INTRODUCTION

We have long known that the bark beetle-produced pheromone, verbenone (trimethyl-bicyclo-heptenone), can limit damage to pines by scolytid bark beetles (Clarke et al. 1999, Skillen et al. 1997, Syracuse Environmental Research Associates, Inc. 2000). It has also been shown that in some cases the addition of "green leaf volatiles" (GLVs) can increase the efficacy of verbenone in protecting host trees (Wilson et al. 1996; Borden et al. 2003; Huber et al. 2003; Kegley and Gibson 2009).

Studies using 5 to 7.5-gram verbenone pouches stapled to trees prior to beetle flight have shown promising results in protecting pines from mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (MPB) attack (Borden et al. 2003, Bentz et al. 2005, Kegley et al. 2003, Kegley and Gibson 2004, 2009). However, there have been operational failures in protecting trees with verbenone pouches during extremely high beetle populations (Progar 2005, Gibson 2009).

Several pheromone companies have developed dispersible verbenone formulations. One of these, Hercon Environmental's Disrupt MicroFlake®, a pheromone-releasing plastic flake, has been used for years in the gypsy moth slow-the-spread program (Sharov et al. 2002) and more recently with scolytid pheromones (Gillette et al. 2006, 2009a, 2009b). The laminated flake is much smaller than the pouch with the active ingredient contained inside a hard plastic reservoir.

Gillette et al. (2006) conducted individual-tree protection tests in lodgepole pine that showed verbenone flakes, when applied with sticker to the trunks, provided nearly complete protection from MPB attack. The flakes were registered for forestry applications in January, 2008. It has been suggested that verbenone flakes, with multiple points of elution, may have greater efficacy than verbenone pouches. In a side-by-side trial, we tested the efficacy of verbenone flakes, pouches, and a combination of verbenone and two GLVs (a hexenol/hexanol blend) pouches in protecting individual lodgepole pines from MPB.

METHODS

We conducted individual tree tests in a lodgepole pine (LPP)-dominated forest located near Fourth of July campground in the Pioneer Mountains, Beaverhead-Deerlodge National Forest, south of Wise River, Montana (T2S R12W Sec.14). MPB populations were building in this area but had not yet caused extreme tree mortality.

A total of 120 LPP, at least 8 inches in diameter-at-breast-height (DBH) and located at least two chains apart, were included in the test. Four different treatments were randomly assigned to each tree. There were 30 trees in each treatment. Treatments applied to individual trees were:

1. Hercon verbenone flakes (100-grams of flakes with 15% active ingredient) (FLK)
2. Two 7-gram Contech verbenone pouches (2PC)
3. One Synergy 7.5-gram verbenone pouch and two 10-gram GLV pouches (PGL)
4. Control (no pouches or flakes) (CTR)

Treatments were applied on June 9-10, 2009. Immediately after application, a standard MPB tree bait (aggregation pheromone) (Synergy Semiochemical Corp.) was placed 5-10 feet from each treated tree.

Flakes were applied to each tree using the PODDS (Pheromone On Demand Delivery System) applicator, developed by Hercon (Fig. 1), to which was attached a 5-pound nitrogen tank to provide propulsion of 80-100 psi. Flakes were mixed with sticker and thickening materials according to the following prescription: 563 ml Micro-Tac II, 188 ml Micro-Tac, 22.5 grams guar gum, and 200 grams flakes per bottle. Mixing was done in a 1000-ml bottle using a mixing bit attached to a cordless drill (Fig 2). Each bottle contained enough material to treat two trees. The device delivered the flake matrix in a "shotgun" pattern—globs of material with each squirt rather than a continuous stream. Material was applied at three or four different heights, as evenly as possible, on four sides of each tree between the base and approximately 20 feet up the bole (Fig. 3a).

Figure 1. PODDS used to spray flakes on individual lodgepole tree boles.

Figure 2. Mixing verbenone flakes with sticker and thickener in 1000-ml bottle.

Verbenone and GLV pouches were stapled to the north side of treated trees about six feet high on the bole (Fig. 3b & c).

Treatments were evaluated on Sept. 29, 2009 after MPB flight. DBH was measured on each treated tree. Trees were rated as mass-attacked, strip-attacked, pitched out, and not attacked as determined by the abundance of pitch tubes and frass, and by removing bark to expose beetle galleries. "Mass-attack" is defined as a tree successfully attacked by beetles and killed. "Strip-attack" is a tree successfully attacked on a portion of its circumference but not killed. A "pitch out" is a tree unsuccessfully attacked and "no attack" is a tree without any attacks.

The project area, near Fourth of July Campground, was partially thinned sometime within the past decade. Despite recognizing differences in stand composition (unthinned to thinned), we elected to conduct the test in the area, noting the need to measure stand characteristics and attempt to account for treatment effects as a function of stand parameters.

In order to compare treatment-area differences, we established 20 variable-radius (10 BAF) plots throughout the project area—10 in each of the "thinned" and "unthinned" portions of the area.

Plots were established on 3-chain centers along a randomly selected transect, running north to south in each area. On each plot, we recorded DBH and a damage code for each "in" tree (LPP and live non-host), equal to or greater than 5 inches DBH Damage codes were:

- "0" for a live tree; "1" for natural or unknown mortality
- "2" for a tree attacked and killed by MPB in 2009
- "3" for a tree attacked and killed by MPB in 2008
- "4" for older MPB-caused mortality
- "5" for a current-year pitchout
- "6" for a current-year strip-attack
- "7" for a previous-year strip-attack
- "8" for current secondary bark beetle attack
- "9" for older secondary bark beetle-caused mortality.

Data analyzed with the FINDIT analysis program (Bentz 2000) enabled us to determine total trees and basal area per acre, and amount of current and past MPB activity for each of the slightly dissimilar portions of the area.
During the evaluation, we also tallied all live and currently attacked LPP in a 20-foot diameter "plot." Treatment trees served as plot centers and were included as plot trees. This was done to further illustrate differences between "unthinned" and "thinned" portions of the project area and to estimate beetle pressure at each tree.

STATISTICS

The binary responses (yes or no) for mass attack, strip attack, mass+strip attack, pitch-out and all attacks combined were modeled as a Logit response from the family of the Generalized Linear Models (McCulloch and Searle, 2001) regressed on four treatment levels (CTR, FLK, 2PC and PGL) crossed with thinning treatment (thinned/ unthinned).

Logit model

$$\log\left(\frac{p_{ij}}{1-p_{ij}}\right) = SxT_{ij}$$

Where *S* is the semiochemical treatment with *i* CTR, FLK, 2PC or PGL, and *T* is the thinning treatment with *j*= thinned or unthinned. p_{ij} is the probability of a tree being attacked. The parameters were estimated with the SAS GENMOD procedure (SAS 2009). The Bonferrroni approach was used for testing the pairwise comparisons.

Figure 3. Trees treated with Hercon verbenone flakes (FLK) (a), Contech 7-gram verbenone pouches (2PC) (b), and Synergy 10-gram glv and 7.5-gram verbenone pouches (PGL) (c).

RESULTS

Stand Data

Total basal area in the unthinned area was 119 square feet per acre compared to 86 feet per acre in the thinned area (Table 1). Most of the basal area is lodgepole pine. Other tree species in the stand included spruce, subalpine fir, and Douglas-fir. In the current outbreak, there were 86 lodgepole pine per acre (34%) mass attacked in the unthinned stand compared to 21 (12%) in the thinned stand.

	Unthinned	Thinned
Total Trees/Acre	273	191
Total Basal Area/Acre	119	86
LPP Trees/Acre	254	182
LPP Basal Area/Acre	110	80
Live LPP (>5")/Acre	164	157
2009 MPB Attacks/Acre	47	11
2008 MPB Attacks/Acre	27	3
Older MPB Attacks/Acre	12	7
Total MPB Attacks/Acre	86	21

Table 1. Project area parameters: "Unthinned" compared to "thinned" (from 10 FINDIT plots in each "unthinned" and "thinned" portion). "MPB Attacks" are beetle-killed trees and do not include "strip-attacks" or "pitchouts."

The majority (67) of the treatment trees occurred in the unthinned area and 53 occurred in the thinned part of the stand. Of those in the unthinned area, 46% were mass attacked; 21% were mass attacked in the thinned area.

Treatment Tree Plot Data

In the 20-foot diameter plots surrounding each treatment tree, there was an average of 3.5 live lodgepole pines in the unthinned area and 1.9 in the thinned. There were no mass attacks in 28% of plots in the unthinned part of the stand and 75% of plots in the thinned area. In the unthinned part of the stand, 69% of plots had at least one mass attack, whereas only 28% of plots in the thinned part experienced at least one mass attack.

	Unthinned	**Thinned**
Total Treatment Trees	67	53
MPB Mass-Attacks (Treatment tree) (%)	31 (46%)	11 (21%)
MPB Strip-Attacks (Treatment tree)	6	3
MPB Pitchouts (Unsuccessful attacks)(Treatment tree)	9	8
No attacks on Treatment tree	21	31
No mass-attacks on Treatment tree, but mass-attacks on "plot" trees.	50%	10%
Avg. No. Live LPP per "plot" (20 ft diameter)	3.5	1.9
"Plots" with no mass-attacks by treatment	19 2PC(5); PGL(6); FLK (8); CTR (0)	40 2PC(12); PGL(11); FLK(12); CTR(5)
"Plots" with at least 1 mass-attack	46 (69%)	15 (28%)
Total "Plot" trees	238	105
"Plot" trees mass-attack (%)	95 (40%)	23 (22%)

Table 2. Project area parameters: "Unthinned" compared to "thinned" (from 20-foot-diameter plot surrounding each of 120 treatment trees—treatment tree was plot center and was included as plot tree).

Treatment Trees

Pheromone-treated trees were protected from mass attack 70-83% of the time while 77% of untreated control trees were killed (Figure 4). All three treatments were significantly different from controls ($p<0.001$) but not from each other. Of all the treatments, the flake treatment (FLK) had the least amount of mass attacks (17%) but the greatest amount of pitchouts (20%) and strip attacks (23%). The 2-PC treatment had the greatest amount of mass attacks (30%) and 10% pitchouts. The PGL treatment had 20% mass attacks, 7% strip attacks, and 10% pitchouts.

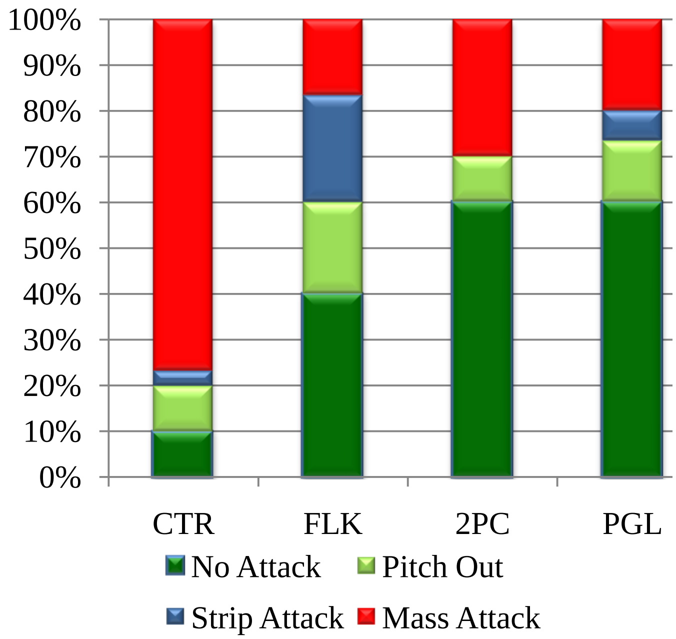

Figure 4. Percent trees with no attack, pitch outs, strip attack or mass attack by treatment. CTR =Control; FLK =Flake; 2PC=2-verbenone pouches; PGL=2 GLV pouches + 1 verbenone pouch.

The estimated probabilities of the different types of attack for the four semiochemical treatments are shown in figures 5-6. The letters on the graphs designate significant differences between treatments. The control treatment was significantly different from all semiochemical treatments for probability of mass attack and mass and strip attacks combined. The semiochemical treatments were not significantly different from each other.

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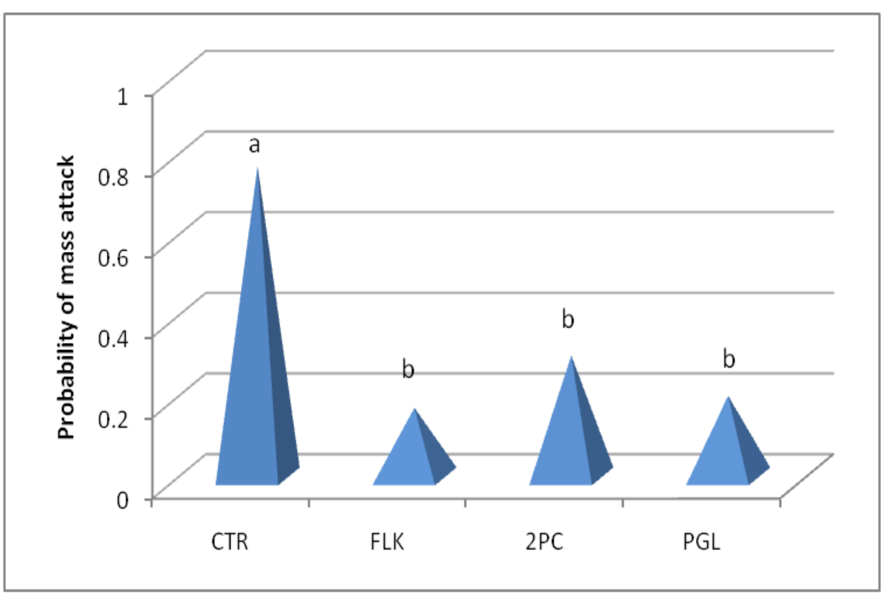


Figure 5. Probablility of mass attack by treatment. CTR=Control; FLK=Flake; 2PC=2 verbenone pouches; PGL=2 glv pouches + 1 verbenone pouch.

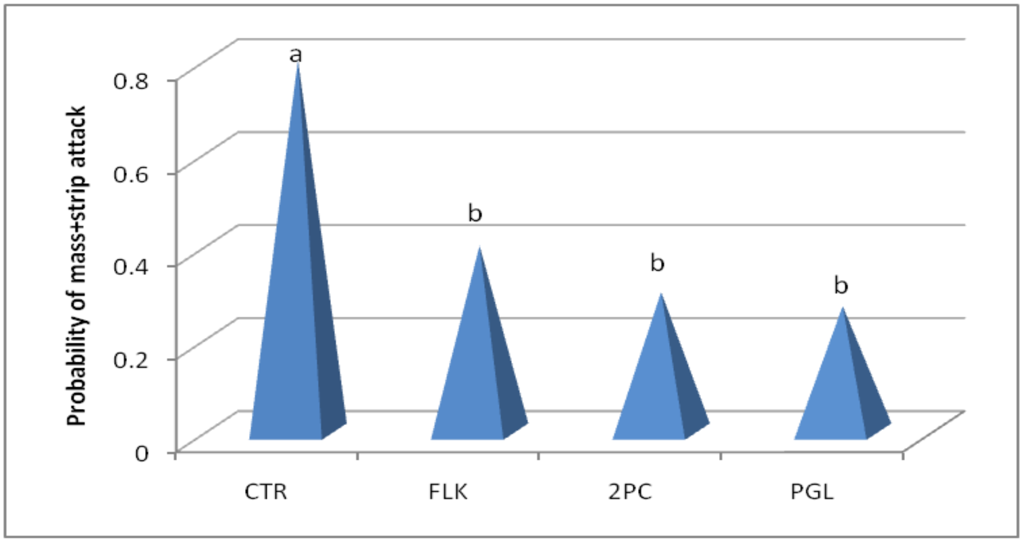
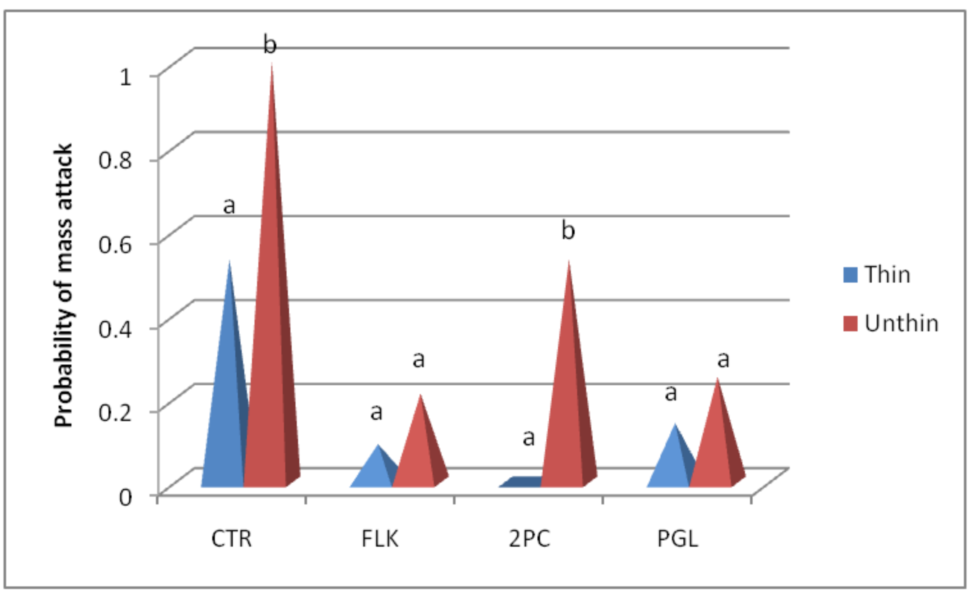


Figure 6. Probablility of mass and strip attack combined by treatment. CTR =Control; FLK =Flake; 2PC=2-verbenone pouches; PGL=2- GLV pouches + 1-verbenone pouch.

Thinning Effects

The estimated probabilities of the different types of attack for the four semiochemical treatments crossed with the two stocking levels are shown on figures 7-8. The letters on the graphs show pairwise comparisons, and the significance of ratio between thinned and unthinned at each semiochemical treatment. The overall effect of thinning was not significant. However, for the control and 2-PC treatments, there was a significant difference in probability of mass attack and mass and strip attacks combined between trees in thinned and unthinned areas of the stand.

Figure 7. Probability of mass attack by treatment in thinned and unthinned areas. CTR =Control; FLK =Flake; 2PC=2-verbenone pouches; PGL=2-GLV pouches + 1-verbenone pouch.



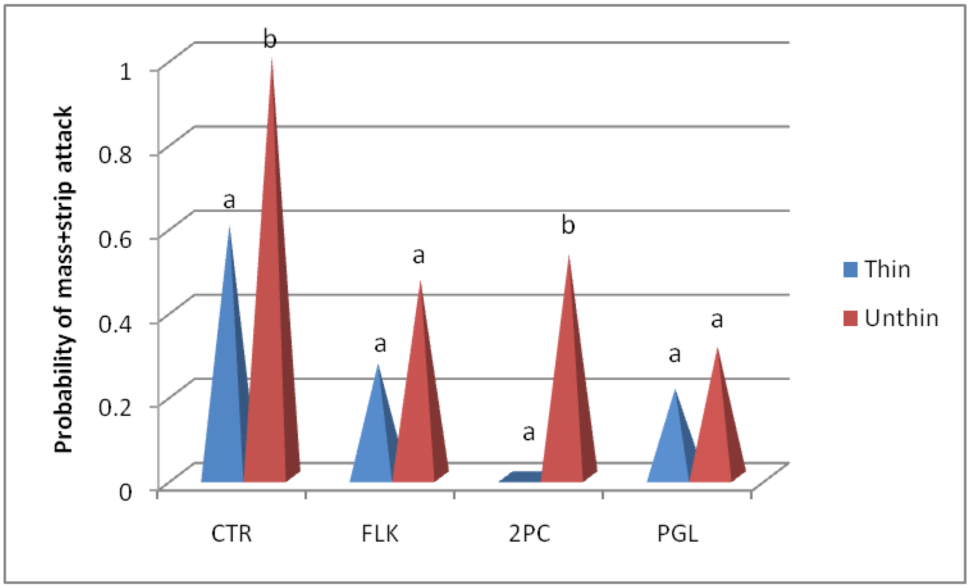


Figure 8. Probability of mass and strip attack by treatment in thinned and unthinned areas. CTR =Control; FLK =Flake; 2PC=2-verbenone pouches; PGL=2-GLV pouches + 1-verbenone pouch.

The variable DBH was tested as a covariate in the logit model, but it was not statistically significant.

DISCUSSION

One objective of this test was to compare multiple-point sources of verbenone elution (the flakes) with the more standard method of application (the pouches). Although efficacy of verbenone flakes for individual-tree protection was comparable to pouches, the application method was more laborious and time consuming. In addition, the targeted application of 15 grams of verbenone on each tree was not always met due to flake material partially missing the tree when aiming high on the bole. In spite of this, the shift seen toward fewest mass attacks but more pitchouts and strip attacks with flakes than with the other products suggests that applying the pheromone circumferentially around the bole may reduce the numbers of beetles attacking the bole, as compared to a single or two point-source releasers (pouches). This evidence may prove to be useful in the design of other new products with enhanced efficacy.

At least in this somewhat limited test, we did not achieve the level of protection from the flake application that would justify using flakes, rather than pouches. Until and unless flake technology is improved to deliver more verbenone per flake and/or a more efficient method of application, this current test suggests application of flakes is not a reasonable substitution for pouches at the single-tree level of application. Area-wide treatments, which do not employ a sticker, remain a desirable option for use of flakes, especially where access by foot is difficult.

Recent tests (Kegley and Gibson 2009), found the following registered treatments provided the best individual tree protection—generally 80% or greater—against MPB attack, when compared to untreated controls:

- Two 7.5-gram Synergy verbenone pouches (Synergy Semiochemicals, Corp.)
- Two 7-gram Contech verbenone pouches (Contech, Inc.)

Those tests also suggest that verbenone pouches combined with a GLV pouch provide equally good protection. Although not yet registered, GLV pouches are less expensive than verbenone pouches and have the potential to reduce treatment costs.

We now have supplemental results suggesting that the addition of GLV to verbenone may both enhance the effectiveness and reduce the cost of using verbenone alone. While two verbenone pouches will provide acceptable individual-tree protection from MPB attack for one season, current data suggests one verbenone pouch and two GLV pouches may provide equally good protection. Certainly, the addition of GLV to verbenone bears further evaluation.

This test also found that thinning lodgepole pine to approximately 86 square feet of basal area per acre, compared to an unthinned stand of 119 square feet of basal area per acre, reduced the probability of MPB attack even when baited with attractant pheromones. Although limited in scope, this test provides support for thinning lodgepole pine stands to reduce losses from MPB (McGregor et al. 1987).

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