Comparisons of Efficiency of Two Formulations of Verbenone (4, 6, 6-trimethylbicyclo [3.1.1] hept-3-en-2-one) for Protecting Whitebark Pine, Pinus albicaulis (Pinales: Pinaceae) From Mountain Pine Beetle (Colopetera: Curculionidae)

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Abstract

Whitebark pine, Pinus albicaulis Engelm., is a subalpine tree endemic to western North America. This species provides multiple ecosystem services and is suffering widespread mortality from mountain pine beetle, Dendroctonus ponderosae Hopkins. Verbenone is a pheromone produced as D. ponderosae feed, and high air concentrations of verbenone deter D. ponderosae from colonizing trees. Synthetic verbenone has been formulated into products used to prevent D. ponderosae from colonizing trees. We compared the ability of verbenone pouches and SPLAT Verb to protect individuals and small stands of P. albicaulis. With individual trees in Montana, all treated trees survived regardless of verbenone formulation and rate, whereas untreated trees suffered 70 and 90% mortality in 2015 and 2016. In plot experiments in California from 2015 to 2017, and Oregon from 2015 to 2018, verbenone was applied to trees spaced ~10 m apart, and survival of small (12.7–23 cm DBH = diameter at 1.37 m height), medium (23.1–33 cm DBH) and large (>33 cm DBH) trees was compared. In California, where >80% of untreated trees survived, pouches increased survival ~2 to 3% and SPLAT Verb increased survival ~4 to 7% regardless of tree size. In Oregon, verbenone pouches and SPLAT Verb performed similarly on medium and small trees, but large trees had greater survival when treated with SPLAT Verb (~93%) than pouches (~82%). Compared to verbenone pouches, SPLAT Verb appears to better protect P. albicaulis from D. ponderosae.

Key words: semiochemical, SPLAT Verb, tree protection, verbenone pouch

Likely due to increasing temperatures, populations of mountain pine beetle, Dendroctonus ponderosae Hopkins, have been expanding latitudinally and in elevation. Over the last 15 yr in the western United States, this insect has killed billions of trees on millions of hectares (Bentz et al. 2010, Negrón and Fettig 2014, Fettig et al. 2020a). Among the trees most impacted by D. ponderosae is whitebark pine, Pinus albicaulis Engelm. Pinus albicaulis is the only subalpine pine in many areas (Jewett et al. 2011), and it reduces runoff and soil erosion and produces seeds bears, birds and other wildlife rely on as food (Tomback 1982). In some areas, D. ponderosae and
white pine blister rust (Cronartium ribicola Dietrich, Pucciniaceae: Cronartiaceae) have reduced P. albicaulis densities >90% (Kendall and Arno 1990), which has prompted consideration for protection under the U.S. Endangered Species Act (Federal Register 2011). Pinus albicaulis is projected to suffer additional >50% reductions between 2014 and 2027 due to interacting effects of D. ponderosae, blister rust and fire (Mahalovich 2013, Krist et al. 2014).

Synthetic verbenone is an important tool for protecting P. albicaulis and other pines from D. ponderosae. Verbenone is a pheromone produced through oxidation of a-pinene by microbes in the beetle’s gut and in beetle galleries (Hunt and Borden 1990, Progar et al. 2014, Seybold et al. 2018). Synthetic verbenone has been formulated into products that are applied to tree boles to inhibit D. ponderosae colonization. Verbenone studies have been promising for P. albicaulis and lodgepole pine, Pinus contorta Douglas ex Loudon (Pinales: Pinaceae). In several studies, ~90% of individually treated P. albicaulis survived D. ponderosae outbreaks compared to 0–40% survival of untreated trees (Kegley et al. 2003, Kegley and Gibson 2004, Gillette et al. 2006, Kegley and Gibson 2009). Other P. albicaulis studies have been less encouraging, with ~70% survival for treated trees compared to 10–30% survival for untreated trees (Kegley and Gibson 2009, Perkins et al. 2015). In addition to individual tree studies, verbenone has been applied to evenly spaced trees within plots to protect both treated trees and neighboring untreated trees. Survival in treated versus untreated plots, respectively, was 95 versus 75% (Amman et al. 1989) and 30 versus 10% (Progar 2005) for P. contorta, and 96 versus 93%, 92 versus 85%, and 80 versus 30% for P. albicaulis (Bentz et al. 2005, Gillette et al. 2012). In contrast to these positive results for P. albicaulis and P. contorta, verbenone failed to protect ponderosa pine (Pinus ponderosa Lawson & C. Lawson (Pinales: Pinaceae) from D. ponderosae (Negrón et al. 2006), perhaps because greater heat, radiation, and wind in less dense stands of this tree species caused verbenone to disperse at concentrations below those that would inhibit D. ponderosae colonization.

Previous research has primarily tested verbenone-wetted pads sealed in slow-release polyethylene pouches (hereafter verbenone pouches) that are stapled to tree boles, though Amman et al. (1989) tested liquid verbenone in polyethylene capsules (bubblecaps), and Gillette et al. (2006, 2012) tested sprayable polymer flakes containing verbenone. A newer verbenone product, SPLAT Verb (ISCA Technologies Inc., Riverside, CA), was registered with the U.S. Environmental Protection Agency in 2013 (Mafra-Neto et al. 2014) and has proven effective in protecting P. contorta and sugar pine, Pinus lambertiana, Doug. (Pinales: Pinaceae) (Fettig et al. 2015, 2016, 2020b). SPLAT Verb is a flowable wax emulsion that is applied to tree boles with a caulk gun (Fig. 1). SPLAT Verb and verbenone pouches have proven similarly effective at protecting P. contorta from D. ponderosae (Fettig et al. 2015, 2020), and our objective was to determine if this is also true for P. albicaulis.

Materials and Methods

Experiments were conducted in the Tobacco Root Mountains of Montana (45.5544° N, −111.9744° W, elevation 2600 m), the southern Cascades of California (41.7952° N, −122.1556° W, elevation 2400 m) and the Strawberry Mountains of northeast Oregon (44.3008° N, −118.7593° W, elevation 2500 m). The Montana experiment compared survival of individually treated and untreated trees, whereas California and Oregon experiments compared survival in treated and untreated plots.

Individual Tree Experiment

The Montana site had 31 m² of basal area per hectare comprised of 63% P. albicaulis, 30% subalpine fir, Abies lasiocarpa (Hooker) Nuttall (Pinales: Pinaceae), 6.7% Engelmann spruce, Picea engelmannii Parry ex Engelmann (Pinales: Pinaceae), and 0.3% P. contorta. We selected for study 150 P. albicaulis spaced >100 m apart and >25.4 cm DBH (diameter at 1.37 m in height). Thirty trees were randomly assigned to receive 1) no verbenone (control), 2) two 7 g a.i. (active ingredient) verbenone pouches (Synergy Semiochemicals Corporation, Delta, B.C. Canada), or SPLAT Verb at 3) 5 g a.i., 4) 7 g a.i., or 5) 14 g a.i. Release rates were 50 and 28.6 mg/d for verbenone pouches and 1.75-g a.i. aliquots of SPLAT Verb, respectively (Fettig et al. 2015). All studies used the (−)-verbenone enantiomer. Verbenone pouches were stapled to north sides of trees 2 m above the ground. For 5, 7, and 14 g a.i. SPLAT Verb treatments, respectively, one 1.25 a.i., 1.75 a.i., or 3.5 a.i. (~8-cm diameter) dollop (Fig. 1) was applied to each cardinal aspect of tree boles 2 m above the ground using a caulk gun (Model X-Lite, Newborn Brothers Co., Inc., Jessup, MD). To enhance D. ponderosae attraction to P. albicaulis so we could test verbenone efficacy, we stapled pouches containing pheromone attractants [trans-verbenol (release rate: ~1.2 mg/d) and exo-brevicomin (~0.3 mg/d)] (Synergy Semiochemicals Corporation) to the north side of all study trees 2 m above the ground. Treatments were applied during the week of 16 June 2015, and tree survival (i.e., based on the absence of crown fade) was assessed the week

Fig. 1. Verbenone pouches (A) and SPLAT Verb (B) applied to the boles of trees to prevent mountain pine beetle, Dendroctonus ponderosae Hopkins, infestation.
of 27 June 2016. Treatments were reapplied the week of 27 June 2016 after selecting new trees to replace study trees infested by *D. ponderosae* in 2015. Tree survival was reassessed the week of 10 July 2017.

**Small Stand Experiments**

The California site had 56 m$^2$ of basal area per ha comprised of 88% *P. albicaulis*, 11% red fir, *Abies magnifica* A. Murray bis (Pinales: Pinaceae), and 1% white fir, *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr (Pinales: Pinaceae). The Oregon site had 36 m$^2$ of basal area per hectare comprised of 91% *P. albicaulis*, 7% *A. lasiocarpa*, 1% *P. engelmannii*, and 1% *P. contorta*. Average DBH of *P. albicaulis* on California and Oregon plots was 31.5 and 20.6 cm, respectively. The size class distribution was more balanced at the California site with an average of 51 small, 41 medium, and 57 large DBH sized trees per study plot, whereas at the Oregon plots, there was an average of 136 small, 32 medium, and 6 large DBH sized trees. At each site, we selected 18 (3 treatments × 6 replications) 0.41-ha square plots spaced ~100 m apart. Treatments, which were randomly assigned to plots, were the following: 1) no verbenone (control); 2) verbenone pouches at 280.0 g a.i. per plot; and 3) SPLAT Verb at 280.0 g a.i. per plot. In treated plots, verbenone was applied to 40 trees separated by ~10 m using the application procedures described for the individual tree experiment, except that one pouch was applied to trees instead of two. At the beginning of the experiment, pretreatment surveys showed *D. ponderosae* had begun colonizing *P. albicaulis* at both sites, but that fewer *P. albicaulis* were colonized at the California site. To increase *D. ponderosae* attraction to plots in California, pheromone attractants [trans-verbenol (~1.2 mg/d), exo-brevicomin (~0.3 mg/d) and terpinolene (~170 mg/d) (Synergy Semiochemicals Corporation)] were used. In each plot and year, lures were affixed to a stake in the center of the plot 1.3 m above the ground. Treatments were applied during the weeks of 6 July 2015, 20 June 2016, and 26 June 2017 in California, and 13 July 2015, 16 July 2016, 17 July 2017, and 18 July 2018 in Oregon. Tree survival was determined the year following final treatment.

**Statistical Analysis**

For the individual tree experiment, the observed survival probability for treated trees was 1.0. This prevented analysis with a parametric statistical model, so means are presented along with 95% CI calculated from the standard errors. For the small stand experiments, tree diameters were partitioned into three classes for analysis: small (12.7–23 cm DBH), medium (23.1–33 cm DBH), and large (>33 cm DBH) as in Progar et al. (2013). Because the data are binary, a Bayesian probit model with uniform prior distributions was fit separately to data from each small stand experiment. To fit the model, we used a FORTRAN (Intel Corporation 2013) program that implements the Gibbs sampler described by Albert and Chib (1993). The model had terms for treatment, tree size class and treatment by tree size class interactions. Confidence intervals and significance levels were computed directly from the posterior distribution using methods of Gelman et al. (2014).

**Results and Discussion**

In the individual tree experiment, ~70 to 90% of untreated control trees were killed by *D. ponderosae* each year, whereas no verbenone-treated trees died regardless of rate or formulation (Fig. 2). Because the 5-, 7-, and 14-g a.i. SPLAT Verb rates and 14-g a.i. verbenone pouch rate were similarly effective, the 5-g a.i. rate could be used to reduce costs. Similarly, Kegley and Gibson (2007) observed no difference in *P. albicaulis* mortality between verbenone pouch rates of 10 and 20 g a.i. per tree. In addition, Fettig et al. (2020) observed no differences in *P. contorta* mortality among SPLAT Verb rates of 150, 250, and 350 g a.i. per plot. While higher rates have not been more effective, reapplying verbenone later in the season may increase protection (Kegley and Gibson 2004, Perkins et al. 2015). An advantage of SPLAT Verb over verbenone pouches is that SPLAT Verb...
Fig. 3. Means (dots) and 95% CI (bars) quantifying survival of three size classes of whitebark pine, *Pinus albicaulis* Engelm., in 0.41-ha plots in California during 2015–2017. Treatments were SPLAT Verb, verbenone pouches and an untreated control. Within a size class, estimates with different letters significantly differ ($P < 0.05$).

Fig. 4. Means (dots) and 95% CI (bars) quantifying survival of three size classes of whitebark pine, *Pinus albicaulis* Engelm., in 0.41-ha plots in Oregon during 2015–2018. Treatments were SPLAT Verb and verbenone pouches. Within a size class, estimates with different letters significantly differ ($P < 0.05$).
can be applied at lower rates (e.g., 5 g a.i. per tree). Currently available verbenone pouches contain 6.8–7.8 g a.i. of verbenone, and the cost per g a.i. is greater for pouches than SPLAT Verb. In addition to SPLAT Verb, verbenone bubblecaps manufactured by Synergy Semiochemical are another low-dose (0.98-g a.i.) option.

In the small stand experiment in California, survival of untreated trees exceeded 80%. Verbenone pouches increased survival 2–3% and SPLAT Verb increased survival 4–7% (Fig. 3). Our study design and analyses were more sensitive to beetle impact and treatment effects because we account for tree size in our model. Treatment differences would become more pronounced when beetle caused mortality is higher. In the small stand experiment in Oregon, wildfire destroyed trees in several of the control plots that would likely bias study results as fire injured trees may be more attractive to D. ponderosae, therefore comparisons to untreated controls were not possible. For small and medium trees in Oregon, P. albicaulis survival was similar for SPLAT Verb and verbenone pouches (Fig. 4). With large trees, P. albicaulis survival was greater for SPLAT Verb (93%) than verbenone pouches (82%). Our California and Oregon results differ from results of previous studies comparing SPLAT Verb and verbenone pouches. In previous studies on P. contorta, SPLAT Verb and verbenone pouches were similarly effective (Fettig et al. 2015, 2020), whereas SPLAT Verb outperformed pouches in P. albicaulis stands in our California and Oregon experiments (Figs. 3 and 4). Our results mirror past studies showing verbenone efficacy sometimes varies with tree size (Progar 2005, Bjorklund and Lindgren 2009, Progar et al. 2013, but see Perkins et al. 2015).

In summary, in the individual tree experiment, our lowest rate of SPLAT Verb was as effective as higher rates of SPLAT Verb and verbenone pouches, and in small stand experiments, SPLAT Verb increased survival more than verbenone pouches. One mechanism potentially explaining why SPLAT Verb outperformed verbenone pouches is the distribution of verbenone doses around the tree bole. In our case, we applied 25% of the dose to each cardinal aspect of the tree, which presumably helped equalize verbenone air concentrations around the bole regardless of wind direction. Other advantages of SPLAT Verb over verbenone pouches is it is less visually obtrusive, slightly less expensive, and does not require retrieval from the field because it rapidly biodegrades (Fettig et al. 2015). However, compared to a given dose of SPLAT Verb, the same dose of verbenone in pouches is lighter to transport and slightly quicker to apply.

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