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Verbenone Plus Reduces Levels of Tree Mortality Attributed to Mountain Pine Beetle Infestations in Whitebark Pine, a Tree Species of Concern

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Abstract

In western North America, recent outbreaks of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, have been severe, long-lasting and well-documented. We review previous research that led to the identification of Verbenone Plus, a novel four-component semiochemical blend [acetophenone, (*E*)-2-hexen-1-ol + (*Z*)-2-hexen-1-ol, and (–)-verbenone] that has been demonstrated to inhibit the response of a closely-related bark beetle species, western pine beetle, *D. brevicomis* LeConte, to attractant-baited traps and trees. In this study, we evaluate the efficacy of Verbenone Plus for protecting stands of whitebark pine, *Pinus albicaulis* Engelm., a species of concern being considered for listing as a threatened and endangered species, from mortality attributed to *D. ponderosae* infestations in the central Sierra Nevada, California, USA. The experimental design was completely randomized with two treatments (untreated control, Verbenone Plus) and four replicates (0.4-ha square plots) per treatment. A total of 450 trees were killed by *D. ponderosae*, 377 were *P. albicaulis* and 73 were lodgepole pine, *P. contorta* Dougl. ex Laws. Significantly fewer pines (*P. albicaulis* and *P. contorta*) and *P. albicaulis* (only) were killed by *D. ponderosae* on Verbenone Plus-treated plots compared to the untreated control. On average, there was ~78% reduction in tree mortality attributed to Verbenone Plus. We discuss the implications of these and other results to the development of Verbenone Plus as a semiochemical-based tool for tree protection.

Keywords: Acetophenone; *Dendroctonus ponderosae*; Non-host angiosperm volatiles; Pest management; *Pinus albicaulis; Pinus contorta*

Introduction

Recent outbreaks of mountain pine beetle, *Dendroctonus ponderosae* Hopkins, have been severe, long-lasting and well-documented [1]. For example, since 2001 >25 million ha of forest have been impacted by *D. ponderosae*. *Dendroctonus ponderosae* ranges throughout British Columbia and Alberta, Canada, most of the western USA, into northern Mexico, and colonizes several pine species, most notably, lodgepole pine, *Pinus contorta* Dougl. ex Loud., ponderosa pine, *P. ponderosa* Dougl. ex Laws., sugar pine, *P. lambertiana* Dougl., limber pine, *P. flexilis* E. James, western white pine, *P. monticola* Dougl. ex D. Don, and whitebark pine, *P. albicaulis* Engelm.[2]. Episodic outbreaks of this notable pest are a common occurrence, but the magnitude and extent of recent outbreaks have exceeded the range of historic variability, and have occurred in areas where *D. ponderosae* outbreaks were once rare (e.g., *P. albicaulis* forests) or previously unrecorded (e.g., jack pine forests, *P. banksiana* Lamb.) [1-4].

Pinus albicaulis is a wide-ranging tree species in western North America that grows at the highest elevations (Figure 1), often in association with other conifers [5]. In the last decade, extensive levels of tree mortality have occurred across much of the range of *P. albicaulis* and have been attributed to climatic changes and elevated populations of *D. ponderosae* [3,6], and white pine blister rust infections caused by a non-native invasive fungi [7]. Scientists speculate that under continued warming, the loss of *P. albicaulis* may be imminent in some areas. To that end, the U.S. Fish and Wildlife Service announced in 2011 that it determined *P. albicaulis* warranted protection under the Endangered Species Act, but that adding the species to the Federal List of Endangered and Threatened Wildlife and Plants was precluded by the need to address other listing actions of higher priority [8]. Accordingly, the U.S. Fish and Wildlife Service has added *P. albicaulis* to the list of candidate species eligible for protection, and will continue to review its status on an annual basis [8].

Pinus albicaulis plays a major ecological role in the functioning of high elevation ecosystems, surviving conditions that are often too cold, too dry and too windy for many other tree species [5]. *Pinus albicaulis* is considered a keystone species in the subalpine environment, stabilizing soils, moderating and regulating runoff, and facilitating the establishment and survival of other species [5,9]. Due to the slow growth and maturation of *P. albicaulis*, and the unique ecological services this species provides, protection of *P. albicaulis* from *D. ponderosae* is desirable, but challenging. Development of environmentally-friendly (e.g., biopesticides) and portable methods of tree protection are needed given the remote and sensitive nature of the subalpine environments where *P. albicaulis* persists.

Verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) is an anti aggregation pheromone of *D. ponderosae*, western pine beetle, *D. brevicomis* LeConte, and southern pine beetle, *D. frontalis* Zimmerman [10], and is produced by auto-oxidation of the host monoterpene α-pinene via the intermediary compounds *cis*- and *trans*-verbenol [11], by the beetles themselves [12], and/or through degradation of host material typically by microorganisms associated with bark beetles [13-15]. Because of its behavioral activity, as demonstrated in

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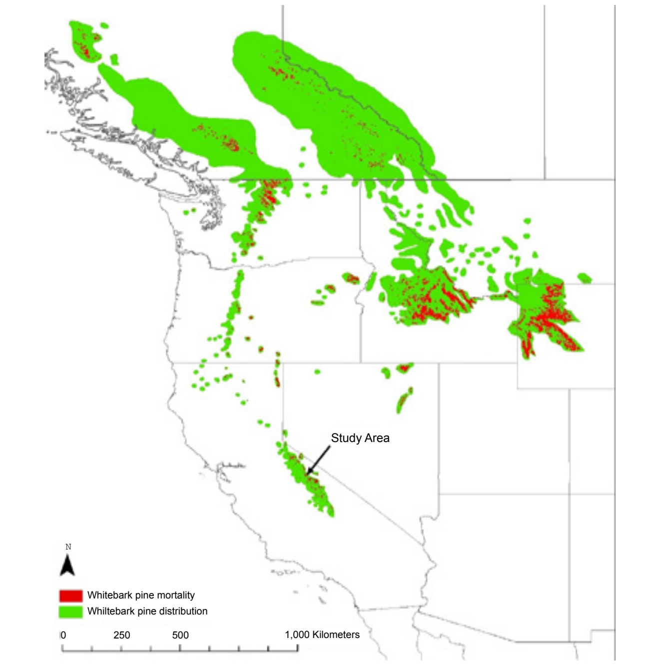

Figure 1: Distribution of *Pinus albicaulis* (green) based on Critchfield and Little [41] with areas of tree mortality (red) based on aerial survey data indicating polygons containing *P. albicaulis* killed by *Dendroctonus ponderosae* during 2007-2011.

numerous trapping bioassays, verbenone has been evaluated as a tool for mitigating coniferous tree mortality due to bark beetle infestations in western North America. Efforts have concentrated on individual tree [e.g., 16-18] or small-scale (e.g., <4 ha) stand protection, primarily from *D. ponderosae* [e.g., 19-22]. Results have been favorable, but inconsistent. Negative results have been linked to photoisomerization of verbenone to behaviorally inactive chrysanthenone [23]; inconsistent or inadequate release [24]; rapid dispersal of verbenone [25]; and/or limitations in the range of inhibition of verbenone [26], particularly when *D. ponderosae* populations were high [19-21]. A lack of efficacy may also be due to the complexity of the host selection process, which involves other visual and olfactory cues produced by hosts, non hosts and competing species [27]. Verbenone was first registered by the U.S. Environmental Protection Agency (licensed for sale and distribution) in December 1999 to control *D. frontalis* in southern forests. Since then, the label has been expanded to include *D. ponderosae* and *D. brevicomis* in forests, recreational and municipal settings, and in rights of way and other easements. Verbenone is generally deployed in individual passive release devices (pouches) by hand application to the tree bole or in bead, flake and sprayable formulations by ground or aerial application [10].

Verbenone has been found ineffective for protecting individual *P. ponderosa* [18,28] and *P. ponderosa* stands [29] from *D. brevicomis* infestations. As a result, based on the semiochemical-diversity hypothesis [30], Shepherd et al. [27] suggested that synthetic verbenone should be deployed with other beetle-derived or non host cues that more accurately reflect the complexity of the olfactory environment in forests. In the context of pest management, a diverse array of chemical cues and signals may disrupt bark beetle searching more than high doses of a single semiochemical (e.g., verbenone) or even mixtures of semiochemicals intended to mimic one type of signal (e.g., antiaggregation pheromones), because they represent heterogeneous stand conditions to foraging insects [27, 30]. Fettig et al. [31] reported that combinations of bark volatiles [benzaldehyde, benzyl alcohol, (*E*)-conophthorin, guaiacol, nonanal, and salicylaldehyde], three green leaf volatiles [(*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*)-2-hexen-1-ol], or the nine compounds combined did not affect the response of *D. brevicomis* to attractant-baited traps. However, when the bark and green leaf volatiles were combined with (−)-verbenone, they reduced trap catches to levels significantly below that of verbenone alone. A nine-component blend [benzyl alcohol, benzaldehyde, guaiacol, nonanal, salicylaldehyde, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol and (−)-verbenone] reduced trap catch by 87% compared to the attractant-baited control [31]. Based on this work, Fettig et al. [32] were first to demonstrate the successful application of a semiochemical-based tool for protecting *P. ponderosa* from mortality attributed to *D. brevicomis*. Additional research confirmed the effect [33], but initial blends were complex and likely not feasible for operational use.

Fettig et al. [34] further examined the response of *D. brevicomis* to several blends of non host angiosperm volatiles and (−)-verbenone in attractant-baited traps in hopes of improving the efficacy of their 9-component blend, and to reduce the number of components involved. Their research resulted in development of a novel four-component blend [acetophenone, (*E*)-2-hexen-1-ol + (*Z*)-2-hexen-1-ol, and (−)-verbenone; Verbenone Plus] that has been demonstrated to inhibit the response of *D. brevicomis* to attractant-baited traps and trees in several studies [28, 34]. The objective of this study was to determine the effectiveness of Verbenone Plus for protecting *P. albicaulis* from mortality attributed to *D. ponderosae* in California, USA.

Materials and Methods

This study was conducted at June Mountain Ski Area, Inyo National Forest, California, USA (37.75°N, 119.06°W; 3,012-m elevation) (Figure 1), 2010. Site selection was based on reports indicating that *D. ponderosae* infestations were causing substantial levels of tree mortality in this area (B. Bulaon, unpubl. data) and subsequent field visits. The experimental design was completely randomized with two treatments and four replicates (0.4-ha square plots) per treatment. Treatments included: (1) untreated control and (2) Verbenone Plus [acetophenone, (*E*)-2-hexen-1-ol + (*Z*)-2-hexen-1-ol and (−)-verbenone] [34] (Table 1). Plots were located in stands with a mean stand density of 48.7 m^2/ha of which ~65% was *P. albicaulis* with the remainder *P. contorta* (Table 2).

Semiochemicals were hand-applied in a ~10.6 by 10.6 m grid (50 U/plot) to the nearest tree at ~2 m in height on 10 June 2010 and remained throughout the seasonal flight period of *D. ponderosae* [35]. Treatments were removed and plots assessed for *D. ponderosae*

Semiochemical	Source*	Purity (%)	Release device	Release rate (mg/d)**
Acetophenone	Sigma-Aldrich	99	Contech 15 ml poly-ethylene bottle	18.0 (20ºC)
(*E*)-2-Hexen-1-ol (*Z*)-2-Hexen-1-ol	Bedoukian	97	Contech pouch (1:1 blend)	50.0 (20ºC)
Verbenone [77%-(-)]	Contech	97	7-g Contech pouch	50.0 (20ºC)

*Sigma-Aldrich = Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada; Bedoukian = Bedoukian Research Inc., Danbury, Connecticut, USA; Contech = Contech Enterprises Inc., Delta, British Columbia, Canada.
**Reported by manufacturer of release device and measured in the laboratory at specified temperature.

Table 1: Description of semiochemicals, release devices and release rates used in tree protection studies in *Pinus albicaulis* stands, June Mountain Ski Area (37.75°N, 119.06°W; 3, 012-m elevation), Inyo National Forest, California, USA, 2010.

Plot	Treatment*	% Crown cover	% Slope	Mean dbh ± SE**	Basal area (m²/ha)	% *P. albicaulis****	Trees per ha	% *P. albicaulis*****
1	Untreated control	60	15	29.1 ± 3.0	76.1	79	840	94
2	Verbenone Plus	40	18	21.4 ± 2.7	17.7	100	395	100
3	Untreated control	60	25	23.5 ± 1.5	48.6	95	964	87
4	Untreated control	20	18	25.5 ± 1.6	53.1	63	914	70
5	Verbenone Plus	40	17	27.3 ± 2.4	74.7	41	988	88
6	Verbenone Plus	40	5	21.3 ± 2.1	45.5	49	939	63
7	Untreated control	40	30	24.3 ± 2.2	57.0	40	939	63
8	Verbenone Plus	20	32	21.4 ± 3.1	17.2	52	370	87

*Verbenone Plus [acetophenone, (*E*)-2-hexen-1-ol + (*Z*)-2-hexen-1-ol and (−)-verbenone] applied at 50 U/plot.
**dbh, diameter at breast height (1.37 m) in cm; SE, standard error.
***Based on basal area (cross-sectional area of trees at 1.37 m in height).
****Based on number of trees.

Table 2: Characteristics of experimental 0.4-ha plots at June Mountain Ski Area (37.75°N, 119.06°W; 3, 012-m elevation), Inyo National Forest, California, USA, 2010.

attacks 12–13 October 2010. Analyses were limited to trees successfully mass attacked by *D. ponderosae* during the treatment period. A tree was considered successfully mass attacked, and therefore killed by *D. ponderosae* if boring dust surrounded the root collar, and/or the phloem and sapwood were discolored, the bark separated readily from the sapwood, and adult (parent) galleries and larval mines were visible following bark removal. Tests of normality and equal variance were conducted to confirm data met assumptions of normality and homoscedasticity prior to analysis (SigmaStat Version 12.0, Systat Software Inc., San Jose, California, USA). The mean percentages of trees (*P. albicaulis* and *P. contorta*) and of *P. albicaulis* (only) killed by *D. ponderosae* were compared by *t*-test using α=0.05 (SigmaStat Version 12.0).

Results

Among all plots, a total of 469 trees were attacked by *D. ponderosae*. However, 19 trees exhibited strip attacks (i.e., a partial attack of the tree bole typically insufficient to cause tree mortality), and therefore were excluded from our analyses. Of the 450 trees that were killed by *D. ponderosae*, 377 were *P. albicaulis* and 73 were *P. contorta*. At the plot level, tree mortality ranged from 0 trees (plot 5, Verbenone Plus-treated) to 139 trees (plot 7, untreated control), and from 0% to 36.6% of trees, respectively. In the untreated control, levels of tree mortality exceeded 15% on all plots. For *P. albicaulis*, mortality ranged from 0 trees (plot 5, Verbenone Plus-treated) to 112 trees (plot 7, untreated control), and from 0% to 41.5% of *P. albicaulis*, respectively. A significantly lower percentage of trees (*P. albicaulis* and *P. contorta*) were killed by *D. ponderosae* on Verbenone Plus-treated compared to untreated control plots (t = -4.25, P = 0.005) (Figure 2). The effect was consistent for *P. albicaulis* as significantly fewer *P. albicaulis* died on Verbenone Plus-treated plots (t = -4.04, P = 0.007) (Figure 2). On average, there was ~78% reduction in tree mortality attributed to the application of Verbenone Plus in *P. albicaulis* stands.

Discussion

This paper is the first report on the effectiveness of Verbenone Plus for protecting *P. albicaulis* from mortality attributed to *D. ponderosae*. In 2008, we examined the effect of Verbenone Plus on the response of *D. ponderosae* to attractant-baited traps in *P. contorta* stands in Utah, USA, but the experiment failed to produce meaningful results due to adverse weather conditions that hampered *D. ponderosae* flight. In that experiment, 4.9 ± 1.5 and 0.2 ± 0.1 *D. ponderosae* (mean ± SEM, n = 42) were captured in the control and Verbenone Plus treatments, respectively. Several years of research initially resulted in the development of Verbenone Plus for protecting *P. ponderosa* from mortality attributed to *D. brevicomis* [27-29,31-34], where it serves as the only effective semiochemical-based tool for tree protection in that system [28].

Limited work has occurred regarding the development of semiochemical-based tools to protect *P. albicaulis* from *D. ponderosae* infestation [17,21,36-38]. This is likely due to its limited commercial value [5], and that until recent years levels of tree mortality attributed to *D. ponderosae* in *P. albicaulis* forests were limited throughout much of the geographic range [9] (Figure 1). In the Sierra Nevada, *P. albicaulis* has experienced significant levels of tree mortality (Figure 1), and some previous attempts to protect trees from *D. ponderosae* by application of verbenone have failed (e.g., June Mountain Ski Area in 2009; B. Bulaon, unpubl. data).

Warwell et al. [39] modeled the contemporary climate profiles of *P. albicaulis* and predicted future responses to warming. They reported rapid and large-scale declines in the area occupied by *P. albicaulis*. For example, the contemporary climate profile was predicted to decline by ~70% and move upward in elevation by ~330 m by 2030. By the end of this century, the contemporary climate profile of *P. albicaulis* was projected to decline to an area equivalent to <3% of its current distribution [39]. In 2007, the Whitebark Pine Restoration Program was initiated by the USDA Forest Service with the primary goals of protecting and enhancing *P. albicaulis* populations, providing adequate

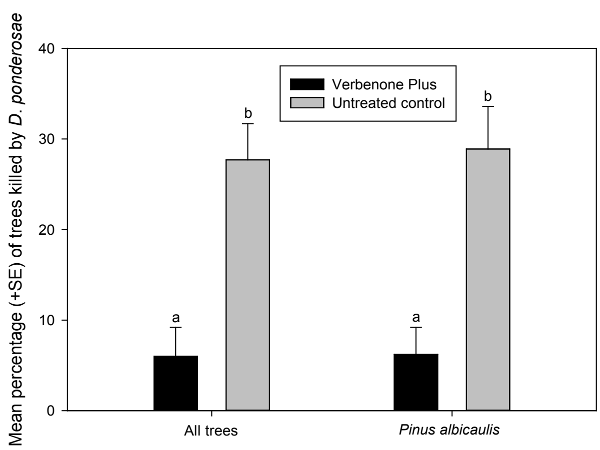

Figure 2: Mean percentage (+ SE) of trees killed by *Dendroctonus ponderosae* on 0.4-ha experimental plots treated with and without Verbenone Plus (acetophenone, (*E*)-2-hexen-1-ol + (*Z*)-2-hexen-1-ol, and (–)-verbenone) at June Mountain Ski Area (37.75°N, 119.06°W; 3,012-m elevation), Inyo National Forest, California, USA, 2010. Means followed by the same letter within groups are not significantly different (*t*-test, *P*>0.05).

regeneration, and increasing the proportion of *P. albicaulis* with natural resistance to white pine blister rust [40]. This should include maintenance and protection of mature, cone-bearing and disease-resistant trees from throughout the geographic range of *P. albicaulis* [7]. Based on our research, we suggest additional work on Verbenone Plus should concentrate on determining optimal release rates and spacing necessary to achieve adequate levels of efficacy in other areas throughout the range of *P. albicaulis* (Figure 1), and comparison of the efficacy of Verbenone to Verbenone Plus within the same *P. albicaulis* stands. Ongoing research (not presented here) indicates that Verbenone Plus is effective for protecting *P. contorta* from mortality attributed to *D. ponderosae* (C. Fettig, unpubl. data), and its efficacy compared to verbenone alone is being evaluated in that system. Such data would be useful in facilitating commercialization of Verbenone Plus (i.e., as the only effective semiochemical-based tool for *D. brevicomis*) given the recent impacts of *D. ponderosae* to forest resources.

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