Crop Protection 136 (2020) 105228

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Evaluating doses of SPLAT® verb to protect lodgepole pine trees and stands from mountain pine beetle

Christopher J. Fettig [a,*], Brytten E. Steed [b], A. Steven Munson [c], Robert A. Progar [d], Agenor Mafra-Neto [e]

[a] *Pacific Southwest Research Station, USDA Forest Service, 1731 Research Park Drive, Davis, CA, 95618, USA*
[b] *Forest Health Protection, USDA Forest Service, 26 Fort Missoula, Missoula, MT, 59804, USA*
[c] *Forest Health Protection, USDA Forest Service, 4746 South 1900 East, Ogden, UT, 84403, USA*
[d] *Sustainable Forest Management Research, USDA Forest Service, 201 14th Street SW, Mailstop 1115, Washington, DC, 20250, USA*
[e] *ISCA Technologies Inc., 1230 Spring Street, Riverside, CA, 92507, USA*

ARTICLE INFO

Keywords:
Dendroctonus ponderosae
Pinus contorta
Tree protection
Verbenone

ABSTRACT

Several formulations of the antiaggregation pheromone verbenone (4,6,6-trimethylbicyclo [3.1.1]hept-3-en-2-one) are registered to protect pine trees and forest stands from mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), but failures in efficacy are not uncommon. In 2011–2013, a novel formulation of (−)-verbenone was developed (SPLAT® Verb, 10.0% (−)-verbenone by weight, EPA Reg. No. 80286−20, ISCA Technologies Inc., Riverside, CA, USA) for protecting individual lodgepole pine, *Pinus contorta* Dougl. ex Loud., and small stands of *P. contorta* from mortality attributed to *D. ponderosae*. We evaluated the efficacy of lower doses of SPLAT® Verb than previously considered for tree protection on the Beaverhead-Deerlodge National Forest, Montana, USA, 2014–2015. In an individual tree study, *D. ponderosae* pressure was not sufficient to adequately challenge the treatments as only 56.7% of the untreated controls died. However, if one additional tree in the untreated control had died, all verbenone treatments (30 g, 50 g, and 70 g of SPLAT® Verb/tree, and one 7-g verbenone pouch/tree) would have been considered efficacious. In a small-scale stand study, fewer *P. contorta* were colonized by *D. ponderosae* on 0.41-ha experimental plots treated with 2.5 kg and 3.5 kg of SPLAT® Verb [250 g and 350 g of (−)-verbenone, respectively] and the 7-g verbenone pouch [50 pouches/plot, 350.0 g of (−)-verbenone/plot] compared to the untreated control. No significant difference was observed between 1.5 kg of SPLAT® Verb [150 g of (−)-verbenone] and the untreated control. Fewer *P. contorta* were killed by *D. ponderosae* on experimental plots treated with 1.5 kg, 2.5 kg and 3.5 kg of SPLAT® Verb and the verbenone pouch compared to the untreated control. No other significant differences were observed among treatments. Collectively, these data suggest that lower doses of SPLAT® Verb can be used for tree protection than previously considered, and at a substantial cost savings that could make this strategy economically viable for area-wide management of *D. ponderosae*.

1. Introduction

Mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), is the most important forest insect pest in North America where it colonizes several hosts, including lodgepole pine, *Pinus contorta* Dougl. ex Loud. (Negrón and Fettig, 2014). While *D. ponderosae* is native to western North America and an important part of the ecology of conifer forests of the region, extensive levels of tree mortality associated with outbreaks may impact many ecological goods and services (Morris et al., 2017). Recent outbreaks of *D. ponderosae* have been particularly severe and long lasting with >27 million ha impacted throughout western North America (British Columbia Ministry of Forests, Lands, and Natural Resource Operations, 2013). Research needs have been prioritized to address these concerns by teams of forest entomologists, forest health specialists, land managers and social scientists, and include development of effective biopesticides to mitigate undesirable levels of tree mortality in an environmentally-friendly manner (Negrón et al., 2008; Morris et al., 2017).

* Corresponding author. 1731 Research Park Drive, Pacific Southwest Research Station, USDA Forest Service, Davis, CA, 95618, USA.
E-mail address: chris.fettig@usda.gov (C.J. Fettig).

https://doi.org/10.1016/j.cropro.2020.105228
Received 1 April 2020; Received in revised form 10 May 2020; Accepted 15 May 2020
Available online 19 May 2020
0261-2194/Published by Elsevier Ltd.

Female *D. ponderosae* initiate colonization of *P. contorta* in a behavioral sequence mediated by aggregation pheromones (Pitman et al., 1968, 1969; Ryker and Libbey, 1982) and host kairomones (Renwick and Vité, 1970; Borden et al., 1987; Miller and Lindgren, 2000). These pioneering females are subsequently joined by males and other females, and mass attack ensues enabling *D. ponderosae* to overwhelm host tree defenses. During the later stages of host colonization, increasing amounts of the anti-aggregation pheromone verbenone (4,6,6-trimethylbicyclo [3.1.1]hept-3-en-2-one) are produced (Pitman et al., 1969; Rudinsky et al., 1974), which discourage additional colonization of the target tree. Today, verbenone is synthesized commercially and used to protect high-value trees and forest stands from mortality attributed to *D. ponderosae* and other species of bark beetles. Treatments are applied annually in late spring or early summer prior to initiation of the flight period of *D. ponderosae* to protect trees in seed orchards, residential settings, developed recreation areas (e.g., USDA Forest Service campgrounds and ski areas) and administrative sites, and to protect trees genetically resistant to white pine blister rust within forested areas. Several formulations of verbenone are registered in Canada and the USA, but failures in efficacy are not uncommon (*see* Progar et al., 2014; Seybold et al., 2018). Failures have been linked to photoisomerization of verbenone to chrysanthenone (Kostyk et al., 1993), inconsistent or inadequate release of verbenone (Bentz et al., 1989), rapid dispersal of verbenone (Gibson et al., 1991; Negrón et al., 2006); and/or limitations in the range of inhibition of verbenone (Miller, 2002), particularly when *D. ponderosae* populations are high.

Fettig et al. (2015) developed a novel formulation of verbenone (SPLAT® Verb; 10.0% (−)-verbenone by weight, EPA Reg. No. 80286–20, ISCA Technologies Inc., Riverside, CA, USA) for protecting *P. contorta* from mortality attributed to *D. ponderosae*. SPLAT® Verb is a "matrix-type" diffusion controlled-release device (but temperature dependent), which releases verbenone at rates suitable to provide significant reductions in levels of *P. contorta* mortality (Mafra-Neto et al., 2013). Rather than a single release device, like the commonly-used verbenone pouch (Seybold et al., 2018), SPLAT® Verb is a flowable, biodegradable emulsion that allows the user to adjust the size of each release point (dollop) according to desired distributions and doses in the field. The inert ingredients are certified as food safe by the U.S. Environmental Protection Agency (EPA), and SPLAT® Verb was granted organic production status by the U.S. Department of Agriculture. SPLAT® Verb was registered by the EPA for use in forestry in August 2013, and first applied commercially in 2014.

To date, research has demonstrated the effectiveness of SPLAT® Verb for protecting individual *P. contorta* and sugar pine, *P. lambertiana* Dougl. (Fettig et al., 2015, 2016), and stands of *P. contorta* from mortality attributed to *D. ponderosae* (Fettig et al., 2015). Most studies have examined doses of ~70 g of SPLAT® Verb/tree for individual tree protection, and ~3.5 kg of SPLAT® Verb/0.41 ha for small stand protection, which are recommended as operational treatments in the western USA (U.S. Department of Agriculture, Forest Service, 2012). The label recommends 75–115 g of SPLAT® Verb/tree, and 2–5 kg/0.41 ha for stand protection. Furthermore, the label indicates not to exceed 10 kg of SPLAT® Verb/0.41 ha/yr (ISCA Technologies Inc., 2016). The objective of our research was to determine the efficacy of lower doses of SPLAT® Verb for protecting *P. contorta* from mortality attributed to *D. ponderosae*.

2. Materials and methods

2.1. Individual tree study

This study was conducted on the Wisdom Ranger District, Beaverhead-Deerlodge National Forest, Montana, USA (tree 1 = 45° 26.787′ N, 113° 35.873′ W, tree 150 = 45° 25.773′ N, 113° 37.529' W; ~2150 m elevation) during 2014–2015. Surrounding stands had a mean live tree (≥12.7 cm dbh, diameter at 1.37 m in height) density of 18.0 m^2/ha of basal area (cross-sectional area of trees at 1.37 m in height) of which 87.2% was *P. contorta*. Engelmann spruce, *Picea engelmannii* Parry ex Engelm., and subalpine fir, *Abies lasiocarpa* (Hooker) Nuttall, were also present. Fettig et al. (2016) reported that 28.8% of *P. contorta* in this area were killed by *D. ponderosae* during 2011–2013.

One hundred fifty (150) *P. contorta* were selected and 30 were randomly assigned to each of five treatments:

(1) SPLAT® Verb applied at 30 g/tree [3.0 g of (−)-verbenone/tree] as four ~7.5-g dollops to the tree bole (at cardinal directions) at ~2.5 m in height using a caulking gun (mean dbh ± SEM = 19.8 ± 0.4 cm)
(2) SPLAT® Verb applied as above at 50 g/tree [5.0 g of (−)-verbenone/tree] as four ~12.5-g dollops (20.6 ± 0.7 cm)
(3) SPLAT® Verb applied as above at 70 g/tree [7.0 g of (−)-verbenone/tree] as four ~17.5-g dollops (~29 mg/d at 18.7 °C) (19.8 ± 0.4 cm)
(4) one 7-g (−)-verbenone pouch (EPA Reg. No. 56261–6, Contech Inc., Delta, BC, Canada) (~50 mg/d at 20 °C) stapled to the tree bole at ~2.5 m in height on the north aspect (20.0 ± 0.4 cm)
(5) untreated control (20.0 ± 0.7 cm)

All treatments were applied June 21–22, 2014 prior to *D. ponderosae* flight. Minimum dbh for inclusion in the study was 16.5 cm as *P. contorta* ≥15 cm dbh are considered most susceptible to colonization by *D. ponderosae* (Shore and Safranyik, 1992). There were no significant differences in tree dbh among treatments ($F_{4,\ 145} = 0.3$; $P = 0.88$). Experimental trees were separated by > 100 m. One bait consisting of *trans*-verbenol (~1.2 mg/d) and *exo*-brevicomin (~0.3 mg/d) (Contech Inc.) was stapled to the bole of each tree at ~2 m in height on the north aspect to enhance attraction to *D. ponderosae*. Baits were removed September 15, 2014 after *D. ponderosae* flight ceased.

Verbenone treatments were considered to have sufficient *D. ponderosae* "pressure" (i.e., a relative measure of population density based on recent levels of tree mortality) if ≥ 60% of the untreated control trees died from colonization by *D. ponderosae*. Mortality was based on the presence of crown fade on July 18, 2015. Needles on trees colonized by *D. ponderosae* fade ~8–10 months after trees are mass attacked. In unusually dry years, foliage may fade within a few weeks of mass attack (Gibson et al., 2009). Treatments were considered efficacious when <7 trees (of 30) died as a result of *D. ponderosae* colonization while ≥60% of the untreated control trees died. These criteria were established based on a sample size of 22–35 trees/treatment and the test of the null hypothesis, Ho: S (survival ≥ 90%). These parameters provide a conservative binomial test (α = 0.05) to reject Ho when more than six trees die. The power of this test, that is the probability of having made the correct decision in rejecting Ho, is 0.84 (Shea et al., 1984).

2.2. Small-scale stand study

This study was conducted on the Wisdom Ranger District, Beaverhead-Deerlodge National Forest, Montana (plot 1 = 45° 12.212′ N, 113° 30.393′ W, plot 25 = 45° 12.769′ N, 113° 30.536' W; ~2210 m elevation) during 2014–2015. The experimental design was completely randomized with five treatments and four replicates (0.41-ha square plots) per treatment. Experimental plots were separated by > 70 m. Mean live tree density (±SEM) was 21.4 ± 1.2 m^2/ha of basal area of which ~92.1% was *P. contorta* with the remainder Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, *A. lasiocarpa* and *Pi. engelmannii*. Approximately 16% of *P. contorta* had been colonized and killed by *D. ponderosae* during 2012–2013.

Treatments included:

(1) SPLAT® Verb applied at 1.5 kg/plot [150.0 g of (−)-verbenone/plot] to individual tree boles (both hosts and nonhosts) as 17.5-g

dollops at ~2 m in height on the north aspect using a caulking gun in a ~7.9 by 7.9-m grid
(2) SPLAT® Verb applied as above at 2.5 kg/plot [250.0 g of (−)-verbenone/plot] in a ~5.8 by 5.8-m grid
(3) SPLAT® Verb applied as above at 3.5 kg/plot [350.0 g of (−)-verbenone/plot] in a ~4.9 by 4.9-m grid
(4) 7-g verbenone pouches [50 pouches/plot, 350.0 g of (−)-verbenone/plot, Contech Inc.] stapled to the tree bole at ~2 m in height on the north aspect in a ~9.1 by 9.1-m grid
(5) untreated control

All treatments were applied June 24–26, 2014 prior to *D. ponderosae* flight. Verbenone treatments were applied to achieve as uniform coverage as possible accounting for the natural distribution of vertical substrates (tree boles) to which dollops and pouches were attached. One tree bait (Contech Inc.) was attached to the tree nearest to the center of each plot (regardless of species or size) at ~2 m in height on the north aspect to enhance *D. ponderosae* attraction. Baits were removed September 16–18, 2014 after *D. ponderosae* flight ceased.

Prior to treatment, stand structure and composition was determined by census of live trees (≥12.7 cm dbh) on each experimental plot June 23–25, 2014. There were no significant differences in tree dbh ($F_{4,\ 20} = 0.9$; $P = 0.47$) or basal area ($F_{4,\ 20} = 1.0$; $P = 0.45$) among treatments. At the same time, all infested trees ≥12.7 cm dbh were identified and tagged, and were excluded from statistical analyses (i.e., these trees were colonized and/or killed prior to implementation of treatments). A census was conducted of each plot for trees colonized by bark beetles on September 16–18, 2014. However, tree mortality was based on the presence of crown fade on August 4–6, 2015. A one-way analysis of variance (treatment) was performed on the proportion of trees successfully colonized (strip and mass attacks, Gibson et al., 2009) and killed by *D. ponderosae* using $\alpha = 0.05$ (SigmaStat Version 12.0, Systat Software Inc., San Jose, CA, USA). Data were tested for normality using the Shapiro-Wilk test and transformed.

3. Results and discussion

3.1. Individual tree study

In total, 45 *P. contorta* were successfully colonized (strip and mass attacks) by *D. ponderosae*, 20 at densities sufficient to cause tree mortality within ~1 yr. The untreated control had the highest number of *P. contorta* colonized and killed (29 and 17, respectively), while 50 g and 70 g of SPLAT® Verb had the fewest (3 and 0, respectively, for both treatments). *Dendroctonus ponderosae* pressure was not sufficient to adequately challenge the treatments as only 56.7% of the untreated controls died. As stated earlier, treatments are considered efficacious when <7 trees die as a result of *D. ponderosae* colonization while ≥60% of the untreated control trees die (Shea et al., 1984). If one additional tree in the untreated control had died, all verbenone treatments would have been considered efficacious (Table 1). This experimental design (Shea et al., 1984) is most often used to evaluate the efficacy of insecticides to protect conifers from bark beetles in the western USA and provides a very conservative test of efficacy (Fettig et al., 2013). While it is increasingly used to evaluate the efficacy of semiochemical repellents, semiochemicals are expected to provide lower levels of tree protection than many of the insecticides registered for this application (Progar et al., 2014).

In a study conducted in Wyoming, USA, SPLAT® Verb applied at 70 g to individual *P. contorta* resulted in complete tree protection while 93.3% mortality occurred in the untreated controls (Fettig et al., 2015). In addition, significantly fewer *P. contorta* were killed by *D. ponderosae* within 0.041-ha circular plots surrounding *P. contorta* treated with SPLAT® Verb compared to the untreated control (Fettig et al., 2015). The authors concluded that the high levels of tree protection observed in their study were attributed to the release of verbenone from multiple

Table 1
Efficacy of verbenone treatments for protecting individual *Pinus contorta* (n = 30/treatment) from colonization and mortality attributed to *Dendroctonus ponderosae*, Beaverhead-Deerlodge National Forest, Montana, USA, 2014–2015.

Treatment	Dose[a] (g)	Number of *P. contorta* colonized	Number of *P. contorta* killed
SPLAT® Verb	3	4	2
SPLAT® Verb	5	3	0
SPLAT® Verb	7	3	0
Pouch	7	6	1
Control	0	29	17

[a] (−)-verbenone; SPLAT® Verb is 10.0% (−)-verbenone by weight.

points per tree (e.g., *see* Gillette et al., 2006), and the larger zone of inhibition provided by SPLAT® Verb compared to other formulations of verbenone (Miller, 2002; Fettig et al., 2015). Similar results were obtained when the study was repeated the following year in Montana (Fettig et al., 2016). In a study conducted in California, USA, 40, 70 and 100 g of SPLAT® Verb were effective for protecting individual *P. lambertiana* from mortality attributed to *D. ponderosae* (Fettig et al., 2016), including very large trees (maximum = 223.1 cm dbh) that were stressed by a recent wildfire. During this study, 72% of *P. lambertiana* in the control treatment died (Fettig et al., 2016). Similarly, it appears likely that 30, 50 and 70 g of SPLAT® Verb are effective for protecting *P. contorta* from mortality attributed to *D. ponderosae* (Table 1), although further investigations are needed under higher levels of beetle pressure. As a comparison to SPLAT® Verb, two verbenone pouches (14 g of verbenone) are recommended for protecting individual *P. contorta* (Kegley and Gibson, 2009; Kegley et al., 2010).

3.2. Small-scale stand study

In total, 168 *P. contorta* were successfully colonized (strip and mass attacks) by *D. ponderosae*, 75 at densities sufficient to cause tree mortality within ~1 yr. The untreated control had the highest number of *P. contorta* colonized and killed (121 and 63, respectively), while 2.5 kg of SPLAT® Verb had the fewest (3 and 0, respectively). A significant treatment effect was observed for the percentage of *P. contorta* colonized ($F_{4,\ 20} = 5.7$, $P = 0.003$) and killed ($F_{4,\ 20} = 7.5$, $P < 0.001$) by *D. ponderosae*. Fewer *P. contorta* were colonized on experimental plots treated with 2.5 kg and 3.5 kg of SPLAT® Verb and the verbenone pouch compared to the untreated control while no significant difference was observed between 1.5 kg of SPLAT® Verb and the untreated control (Fig. 1). Fewer *P. contorta* were killed by *D. ponderosae* on experimental plots treated with 1.5 kg, 2.5 kg and 3.5 kg of SPLAT® Verb and the verbenone pouch than the untreated control (Fig. 1). No other significant differences were observed among treatments.

In a study conducted in Idaho, USA, Fettig et al. (2015) demonstrated that SPLAT® Verb applied at 3.5 kg/0.41 ha was effective for protecting stands of *P. contorta* from mortality attributed to *D. ponderosae*, and that SPLAT® Verb was more effective than the verbenone pouch applied at the same dose. Our results indicate doses as low as 1.5 kg of SPLAT® Verb/0.41 ha yield suitable levels of tree protection. However, we observed no significant differences among SPLAT® Verb treatments, regardless of dose, and the verbenone pouch. This is despite the pouch being applied at a higher dose than two of the SPLAT® Verb treatments (Fig. 1). It is of note that levels of tree colonization and mortality within the untreated controls in Fettig et al. (2015) and our study were similar, suggesting similar beetle pressure in both experiments.

4. Conclusions

The effectiveness of SPLAT® Verb to protect individual *P. contorta*

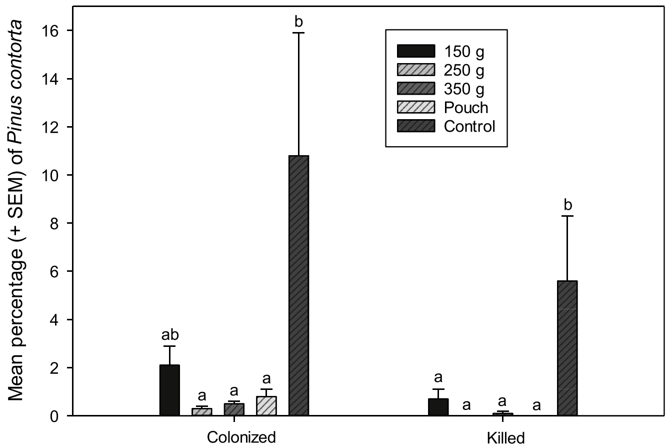

Fig. 1. Mean percentages (+SEM) of *Pinus contorta* colonized and killed by *Dendroctonus ponderosae* on 0.41-ha square plots, Beaverhead-Deerlodge National Forest, Montana, USA, 2014–2015. Means (+SEM) followed by the same letter within each group (colonized and killed) are not significantly different (Tukey's HSD; $P > 0.05$). SPLAT® Verb treatments are given in grams of (−)-verbenone; SPLAT® Verb is 10.0% (−)-verbenone by weight.

and small stands of *P. contorta* from mortality attributed to *D. ponderosae* has been demonstrated in several studies conducted under conditions when SPLAT® Verb, and other similar treatments, would be used operationally (Fettig et al., 2015, 2016; current study). To that end, during our study several USDA Forest Service campgrounds in nearby areas were treated with verbenone and/or insecticides to limit levels of tree mortality. Our data suggest that lower doses of SPLAT® Verb (e.g., 1.5 kg/0.41 ha) can be used to protect stands than previously considered or recommended on the label (ISCA Technologies Inc., 2016). This would result in substantial cost and labor savings (e.g., ~50%, depending on labor). Furthermore, dollops of SPLAT® Verb biodegrade within ~1 yr of application, and do not need to be retrieved from the field as do other verbenone products formulated in plastic releasers (e.g., pouch). This could result in additional cost and labor savings.

Significant research and development remains to be conducted concerning SPLAT® Verb, including evaluating efficacy for protecting whitebark pine, *P. albicaulis* Engelm., a species being considered for listing as a threatened and endangered species in the USA due to the impacts of *D. ponderosae*, white pine blister rust and climate change (Federal Register, 2015). *Pinus albicaulis* grows at high elevations and often in steep and rugged terrain where development of an environmentally-friendly, portable and biodegradable method of tree protection is of priority. A related product (SPLAT® MCH, ISCA Technologies Inc.) has been developed and shows promise for protecting *Ps. menziesii* from mortality attributed to Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins. *Dendroctonus pseudotsugae* is the most important insect pest of *Ps. menziesii*, and similar to SPLAT® Verb, SPLAT® MCH exploits our understanding of the beetle's antiaggregation pheromone, 3-methylcyclohex-2-en-1-one, in a biodegradable formulation (Foote et al., 2020). In a recent assessment of the state of knowledge and development concerning semiochemical methods for control of bark beetles in western North America, Gillette and Fettig (2020) concluded future progress depends on, among other factors, development of less expensive methods of deployment and improvements in release device performance (to include development and refinement of biodegradable releasers, such as SPLAT®).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Christopher J. Fettig: Conceptualization, Methodology, Investigation, Formal analysis, Resources, Writing - original draft, Supervision, Project administration, Funding acquisition. **Brytten E. Steed:** Methodology, Investigation, Writing - review & editing, Project administration. **A. Steven Munson:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Robert A. Progar:** Investigation, Data curation, Writing - review & editing. **Agenor Mafra-Neto:** Conceptualization, Writing - review & editing, Funding acquisition.

Acknowledgments

We thank R. Cruz, V. DeBlander, L. Dunning, C. Hayes and J. Neumann (Forest Health Protection, USDA Forest Service), J. DeVillier and M. Barrett (Pacific Northwest Research Station, USDA Forest Service), and L. Mortenson (Pacific Southwest Research Station, USDA Forest Service) for technical assistance. In addition, we thank the Beaverhead-Deerlodge National Forest for providing access to study sites. This research was supported, in part, by a collection agreement (14-CO-11272139-018) from ISCA Technologies Inc. to the Pacific Southwest Research Station.

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides in the United States must be registered by appropriate State and/or Federal agencies before they can be recommended.

References

Bentz, B.J., Lister, C.K., Schmid, J.M., Mata, S.A., Rasmussen, L.A., Haneman, D., 1989. Does Verbenone Reduce Mountain Pine Beetle Attacks in Susceptible Stands of Ponderosa Pine? RN-RM-495. U.S. Department of Agriculture, Forest Service, Ogden, UT.

Borden, J.H., Ryke, L.C., Chong, L., Pierce Jr., H.D., Johnston, B.D., Oehlschlager, A.C., 1987. Response of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: scolytidae), to five semiochemicals in British Columbia lodgepole pine forests. Can. J. For. Res. 17, 118–128.

British Columbia Ministry of Forests, Lands and Natural Resource Operations, 2013. Facts about B.C.'s mountain pine beetle. http://www.for.gov.bc.ca/hfp/mountain_pine_beetle/Updated-Beetle-Facts_April2013.pdf accessed 3 January 2020.

Federal (U.S.) Register, 2015. Endangered and threatened wildlife and plants 80, 80584–80614.

Fettig, C.J., Grosman, D.M., Munson, A.S., 2013. Advances in insecticide tools and tactics for protecting conifers from bark beetle attack in the western United States. In: Trdan, S. (Ed.), Insecticides - Development of Safer and More Effective Technologies. InTech, Rijeka, Croatia, pp. 472–492.

Fettig, C.J., Munson, A.S., Reinke, M., Mafra-Neto, A., 2015. A novel semiochemical tool for protecting lodgepole pine from mortality attributed to mountain pine beetle (Coleoptera: Curculionidae). J. Econ. Entomol. 108, 173–182.

Fettig, C.J., Steed, B.E., Bulaon, B.M., Mortenson, L.A., Progar, R.A., Bradley, C.A., Munson, A.S., Mafra-Neto, A., 2016. The efficacy of SPLAT® Verb for protecting individual *Pinus contorta*, *Pinus ponderosa*, and *Pinus lambertiana* from colonization by *Dendroctonus ponderosae*. J. Entomol. Soc. Br. Columbia 113, 11–20.

Foote, G.G., Fettig, C.J., Ross, D.W., Runyon, J.B., Coleman, T.W., Gaylord, M.L., Graves, A.D., McMillin, J.D., Mortenson, L.A., Mafra-Neto, A., 2020. A biodegradable formulation of MCH (3-methylcyclohex-2-en-1-one) for protecting *Pseudotsuga menziesii* from *Dendroctonus pseudotsugae* (Coleoptera: Curculionidae) colonization. J. Econ. Entomol. https://doi.org/10.1093/jee/toaa061.

Gibson, K.E., Schmitz, R.G., Amman, G.D., Oakes, R.D., 1991. Mountain Pine Beetle Response to Different Verbenone Dosages in Pine Stands of Western Montana. RP-INT-444. U.S. Department of Agriculture, Forest Service, Ogden, UT.

Gibson, K., Kegley, S., Bentz, B., 2009. Mountain Pine Beetle. For. Insect Dis. Leaflet 2. U. S. Department of Agriculture, Forest Service, Portland, OR.

Gillette, N.E., Stein, J.D., Owen, D.R., Webster, J.N., Fiddler, G.O., Mori, S.R., Wood, D. L., 2006. Verbenone-releasing flakes protect individual *Pinus contorta* trees from attack by *Dendroctonus ponderosae* and *Dendroctonus valens* (Coleoptera: Curculionidae, Scolytinae). Agric. For. Entomol. 8, 243–251.

Gillette, N.E., Fettig, C.J., 2020. Semiochemicals for bark beetle management: where do we go from here? Can. Entomol. (in press).

ISCA Technologies Inc, 2016. SPLAT® Verb Bark Beetle Repellent, Amended Label, p. 2.

Kegley, S., Gibson, K., 2009. Individual-tree Tests of Verbenone and Green-leaf Volatiles to Protect Lodgepole, Whitebark and Ponderosa Pines, 2004–2007. FHP Report 09–03. U.S. Department of Agriculture, Forest Service, Coeur d'Alene, ID.

Kegley, S., Gibson, K., Gillette, N., Webster, J., Pederson, L., Mori, S., 2010. Individual-tree Tests of Verbenone Flakes, Verbenone Pouches, and Green-leaf Volatiles to Protect Lodgepole Pines from Mountain Pine Beetle Attack. FHP Report 10–02. U.S. Department of Agriculture, Forest Service, Coeur d'Alene, ID.

Kostyk, B.C., Borden, J.H., Gries, G., 1993. Photoisomerization of antiaggregation pheromone verbenone: biological and practical implications with respect to the mountain pine beetle, *Dendroctonus ponderosae* Hopkins. J. Chem. Ecol. 19, 1749–1759.

Mafra-Neto, A., de Lame, F.M., Fettig, C.J., Munson, A.S., Perring, T.M., Stelinski, L.L., Stoltman, L., Mafra, L.E.J., Borges, R., Vargas, R.I., 2013. Manipulation of insect behavior with specialized pheromone & lure application technology (SPLAT®). In: Beck, J., Coats, J., Duke, S., Koivunen, M. (Eds.), Natural Products for Pest Management. ACS Publications, Washington, DC, pp. 31–58.

Miller, D.R., 2002. Short-range horizontal disruption by verbenone in attraction of mountain pine beetle (Coleoptera: Scolytidae) to pheromone-baited funnel traps in stands of lodgepole pine. J. Entomol. Soc. Br. Columbia 99, 103–105.

Miller, D.R., Lindgren, B.S., 2000. Comparison of α-pinene and myrcene on attraction of mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: scolytidae) to pheromones in stands of western white pine. J. Entomol. Soc. Br. Columbia 97, 41–46.

Morris, J.L., Cottrell, S., Fettig, C.J., Hansen, W.D., Sherriff, R.L., Carter, V.A., Clear, J., Clement, J., DeRose, R.J., Hicke, J.A., Higuera, P.E., Mattor, K.M., Seddon, A.W.R., Seppä, H., Stednick, J.D., Seybold, S.J., 2017. Managing bark beetle impacts on ecosystems and society: priority questions to motivate future research. J. Appl. Ecol. 54, 750–760.

Negrón, 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. RN-RMRS-31. U.S. Department of Agriculture, Forest Service, Fort Collins, CO.

Negrón, J.F., Bentz, B.J., Fettig, C.J., Gillette, N.E., Hansen, E.A., Hayes, J.L., Kelsey, R. G., Lundquist, J.E., Lynch, A.M., Progar, R.A., Seybold, S.J., 2008. USDA Forest Service bark beetle research in the western United States: looking towards the future. J. For. 106, 325–331.

Negrón, J.F., Fettig, C.J., 2014. Mountain pine beetle, a major disturbance agent in US western coniferous forests: a synthesis of the state of knowledge. For. Sci. 60, 409–413.

Pitman, G.B., Vité, J.P., Kinzer, G.W., Fentiman Jr., A.F., 1968. Bark beetle attractants: *trans*-verbenol isolated from *Dendroctonus*. Nature 218, 168.

Pitman, G.B., Vité, J.P., Kinzer, G.W., Fentiman Jr., A.F., 1969. Specificity of population-aggregating pheromones in *Dendroctonus*. J. Insect Physiol. 15, 363–366.

Progar, R.A., Gillette, N.E., Fettig, C.J., Hrinkevich, K.H., 2014. Applied chemical ecology of the mountain pine beetle. For. Sci. 60, 414–443.

Renwick, J.A.A., Vité, J.P., 1970. Systems of chemical communication in *Dendroctonus*. Contrib. Boyce Thompson Inst. 24, 283–292.

Rudinsky, J.A., Morgan, B., Libbey, L.M., Putnam, T.B., 1974. Antiaggregative-rivalry pheromone of the mountain pine beetle, and a new arrestant of the southern pine beetle. Environ. Entomol. 3, 90–98.

Ryker, L.C., Libbey, L.M., 1982. Frontalin in the male mountain pine beetle. J. Chem. Ecol. 8, 1399–1409.

Seybold, S.J., Bentz, B.J., Fettig, C.J., Lundquist, J.E., Progar, R.A., Gillette, N.E., 2018. Management of western North American bark beetles with semiochemicals. Annu. Rev. Entomol. 63, 407–432.

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

Shore, T.L., Safranyik, L., 1992. Susceptibility and Risk Rating Systems for the Mountain Pine Beetle in Lodgepole Pine Stands. Info. Report BC-X-336. Forestry Canada, Pacific Forestry Centre, Victoria, BC.

U.S. Department of Agriculture, Forest Service, 2012. Using Verbenone to Protect Trees from Mountain Pine Beetle. FHP Report, p. 8.