Verbenone Inhibits Attraction of *Ips pini* (Coleoptera: Curculionidae) to Pheromone-Baited Traps in Northern Arizona

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Subject Editor: Kamal Gandhi

Received 27 May 2020; Editorial decision 29 July 2020

Abstract

Recent outbreaks of engraver beetles, *Ips* spp. De Geer (Coleoptera: Curculionidae; Scolytinae), in ponderosa pine, *Pinus ponderosa var. scopulorum* Engelm. (Pinales: Pinaceae), forests of northern Arizona have resulted in widespread tree mortality. Current treatment options, such as spraying individual *P. ponderosa* with insecticides or deep watering of *P. ponderosa* in urban and periurban settings, are limited in applicability and scale. Thinning stands to increase tree vigor is also recommended, but appropriate timing is crucial. Antiaggregation pheromones, widely used to protect high-value trees or areas against attacks by several species of *Dendroctonus* Erichson (Coleoptera: Curculionidae; Scolytinae), would provide a feasible alternative with less environmental impacts than current treatments. We evaluated the efficacy of the antiaggregation pheromone verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) in reducing attraction of pine engraver, *I. pini* (Say), to funnel traps baited with their aggregation pheromone in two trapping assays. Treatments included 1) unbaited control, 2) aggregation pheromone (bait), 3) bait with verbenone deployed from a pouch, and 4) bait with verbenone deployed from a flowable and biodegradable formulation (SPLAT Verb, ISCA Technologies Inc., Riverside, CA). Unbaited traps caught no beetles. In both assays, baited traps caught significantly more *I. pini* than traps with either formulation of verbenone, and no significant difference was observed between the verbenone pouch and SPLAT Verb. In the second assay, we also examined responses of *Temnochila chlorodia* (Mannerheim) (Coleoptera: Trogositidae), a common bark beetle predator. Traps containing verbenone pouches caught significantly fewer *T. chlorodia* than the baited control and SPLAT Verb treatments. We conclude that verbenone shows promise for reducing tree mortality from *I. pini*.

Key words: 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one, pine engraver, repellent, tree protection

Recent drought events, in combination with bark beetle (Coleoptera: Curculionidae; Scolytinae) outbreaks, have led to widespread ponderosa pine, *Pinus ponderosa* Dougl. ex Laws. (Pinales: Pinaceae), mortality in the western United States (Negrón et al. 2009, Fettig et al. 2019). In contrast to mortality events in other western states, recent mortality of *P. ponderosa var. scopulorum* Engelm. in Arizona has been attributed primarily to the pine engraver, *I. pini* (Say), and Arizona five-spined ips, *Ips lecontei* Swaine (Coleoptera: Curculionidae; Scolytinae), generally considered secondary beetles, as opposed to more aggressive *Dendroctonus* spp. Erichson (Coleoptera: Curculionidae; Scolytinae) (Negrón et al. 2009, Kolb et al. 2016, USDA Forest Service 2018). Climate change models project that drought events will become more frequent and severe in the southwestern United States (Williams et al. 2013), and tree mortality from the combination of water stress, increased ambient temperatures, and bark beetles is projected to increase accordingly (McDowell et al. 2008, Bentz et al. 2010).

Many bark beetles use aggregation pheromones to elicit mass attacks for successfully attacking and killing trees. Antiaggregation pheromones may subsequently signal that the host resource is fully occupied (Seybold et al. 2018). Pheromone cues are also exploited by bark beetle predators as kairomones for locating their prey (Zhou et al. 2001, Aukema and Raffa 2004). Land managers and scientists have successfully used these semiochemicals as tools for bark beetle monitoring and control (Seybold et al. 2018). Aggregation pheromones are widely used in traps to monitor seasonality, relative abundance, and distributions (Seybold et al. 2018). Although antiaggregation formulations are currently only available
for a limited number of systems, and efficacy is mixed (Progar et al. 2014), they are of great value to land managers and can be effective for tree protection (Amman et al. 1989, Ross and Daterman 1995, Seybold et al. 2018). The two antiaggregation pheromones commercially available are verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) and MCH (3-methylcyclohex-2-en-1-one). MCH has been used successfully against Douglas-fir beetle, *Dendroctonus pseudotsugae* (Hopkins) (Coleoptera: Curculionidae; Scolytinae) (Ross and Daterman 1995, Ross and Wallin 2008), but results are more variable for reducing spruce beetle, *Dendroctonus rufipennis* (Kirby) (Coleoptera: Curculionidae; Scolytinae) attacks (Holsten et al. 2003, Hansen et al. 2016). Both verbenone and MCH have been used separately and in combination with other inhibitors, including nonhost volatiles (Huber and Borden 2001; Fettig et al. 2012a,b; Hansen et al. 2016), and are available in different formulations and release devices: bubble caps and pouches stapled to trees, plastic impregnated flakes which can be broadcast around an area, or in a flowable and bio-degradable formulation called SPLAT (Specialized Pheromone and Lure Application Technology) deployed to the tree bole (Mafra-Neto et al. 2013, Gillette and Fettig 2020).

Verbenone is derived from degradation of the ubiquitous conifer resin constituent α-pinene and is increasingly recognized as a general bark beetle repellent (Seybold et al. 2006, 2018; Blomquist et al. 2010). Although the majority of recent research has focused on the efficacy of verbenone in *Dendroctonus*, several Ips systems have also been investigated (Paine and Hanlon 1991, Lindgren and Miller 2002, Seybold et al. 2018). For instance, in forests of lodgepole pine, *Pinus contorta* Doug. ex Loud. (Pinales: Pinaceae), in British Columbia, Canada, verbenone (Miller et al. 1995, Lindgren and Miller 2002) or verbenone with isopinol (Devlin and Borden 1994) was effective in reducing trap catches of *Ips latidens* (LeConte) (Coleoptera: Curculionidae; Scolytinae) and *I. pini*. In Norway, European spruce beetle, *Ips typographus* (L.) (Coleoptera: Curculionidae; Scolytinae), trap catches were reduced by verbenone and verbenone with isopinol (Bakke 1981) in stands of Norway spruce, *Picea abies* L. (Karst) (Pinales: Pinaceae). Verbenone and trans-conophorin reduced northern spruce engraver, *Ips perturbatus* (Eichhoff) (Coleoptera: Curculionidae; Scolytinae), colonization of white spruce, *Picea glauca* (Moench) Voss (Pinales: Pinaceae), slash in interior Alaska (Fettig et al. 2013a). Research has been conducted on the effects of verbenone on other western bark beetle species attacking *P. ponderosa* (e.g., Livingston et al. 1983, Negrón et al. 2006, Fettig et al. 2009), but little research has been conducted on the efficacy of verbenone in preventing attacks by *Ips* spp. in *P. ponderosa* forests in the southwestern United States (DeGomez et al. 2008b).

Although thinning is recommended for minimizing the overall risk of bark beetle infestations in conifer forests (Fettig et al. 2007a), current options for protecting small stands or individual high-value *P. ponderosa* from *Ips* spp. during outbreaks are limited to insecticides and supplemental watering (Kegley et al. 1997). Neither of these strategies are feasible across large areas or in forest settings. In addition, insecticidal sprays can pose an environmental hazard and have restricted uses (Fettig et al. 2013b). A pheromone-based tree protection strategy would be of value to forest managers and property owners (DeGomez et al. 2008a). The primary objective of this study was to determine the effects of two formulations of verbenone on the response of *I. pini* to pheromone-baited traps in northern Arizona. This represents an initial step in determining the suitability of verbenone as a candidate for protection of *P. ponderosa* from *I. pini* in northern Arizona. A second objective was to determine the response to verbenone of a common bark beetle predator, *Temnochila chlorodis* (Mannerheim) (Coleoptera: Trogositidae).

### Materials and Methods

Two trapping assays were conducted on the Coconino National Forest (~24 km northeast of Flagstaff, Arizona; 35° 17’ 55.81”; 111° 25’ 14.49”; ~1950 m above sea level) in the *P. ponderosa*, pinyon pine, *Pinus edulis* Engelm. (Pinales: Pinaceae), and juniper, *Juniperus* spp. (Pinales: Cupressaceae), transition zone. In 2018, USDA Forest Service aerial detection surveys documented several stands of *P. ponderosa* with tree mortality attributed to bark beetles in this area (USDA Forest Service 2018). The Flagstaff area experiences annual average precipitation of 58.8 cm, half of which arrives in the form of mid- to late-summer rains and half as winter precipitation (U.S. Climate Data 2020). During our 2019 study, temperatures were below average in May (4.5°C below normal high temperature) and precipitation was 665% above average, whereas June–August were 0.6–2.3°C above average high temperatures and each month had below average precipitation (37% of average precipitation combined).

We installed 40 five-unit funnel traps in two lines of 20 traps. Treatments were completely randomized and 10 replicates of four treatments were evaluated: 1) *I. pini* bait (racemic ipsdienol; release rate ~150–250 µg/d at 20°C; 93% purity) and lanierone (release rate ~10 µg/d at 20°C; 99% purity), 2) 7-g of verbenone pouch (75% (–)); release rate ~70 µg/d at 20°C; 98% purity) + bait, 3) 70 g of SPLAT Verb (80% (–); 10% a.i., total combined release rate ~114 mg/d at 19°C; >93% purity) + bait, and 4) unbaited control. Funnel traps, baits, and verbenone pouches were purchased from Synergy Semichemicals Corp. (Delta, British Columbia, Canada), and SPLAT Verb was donated by ISCA Technologies Inc. (Riverside, CA). The baits and verbenone pouches were affixed to the middle funnel on the five-unit funnel traps (Synergy Semichemicals Corp.). SPLAT Verb was deployed using four evenly dispersed, ~17.5-g dollops on the lids of the traps. Each trap was ~25 m from any other trap and ~2 m horizontally from the outer edge of the crown of any *P. ponderosa* or *P. edulis*. On 16 May 2019, the Maroon Fire was ignited by lightning near our study site. The Coconino National Forest determined that this ignition should be used to benefit the landscape and the fire was allowed to burn. On 23 May 2019, we relocated 20 of the traps, so no traps would be within the fire perimeter resulting in four lines of 10 traps.

The first trapping assay was conducted 14 May–7 June 2019, whereas the second assay was conducted 19 July–29 August 2019. For both assays, we used the same postfire arrangement in terms of trap locations and randomization. In hopes of increasing trap catches in the second assay, we altered the release rate for racemic ipsdienol. In Arizona, this enantiomer has been shown to enhance attraction of *I. pini* over the racemic blend (Steed and Wagner 2008). During the second trapping assay we also tallied catches of *T. chlorodis*. Trap catches during both assays were collected every 6–10 d, and stored in a freezer until collections were identified, counted, and sorted by species and gender using available keys (Wood 1982) and voucher specimens.

Trap catches from unbaited controls were excluded from statistical analyses because of the heteroscedasticity that they cause (Reeve and Strom 2004). A test of normality was performed, and square root transformations were used when the data deviated significantly from a normal distribution. A two-way analysis of variance (treatment and sex) was performed on the number of *I. pini* captured using α = 0.05. Differences in the sex ratio of *I. pini* among treatments were analyzed using a one-way analysis of variance. If a significant treatment effect was detected, the Tukey’s multiple comparison test (Tukey’s HSD) was used for separation of treatment means.
Results
In total, 145 and 689 I. pini were collected during the first and second trapping assays, respectively. In the first assay, the ratio of males to females was 1.0; in the second assay, the ratio was 1.07. In both assays, there was no gender by treatment interaction (Assay 1: F$_{2, 14} = 0.09; P = 0.92$; Assay 2: F$_{2, 14} = 0.2; P = 0.83$), and data were pooled across genders. Unbaited traps caught no I. pini. In both assays, baited traps caught significantly more I. pini than traps with either formulation of verbenone, and no significant difference was observed between the verbenone pouch and SPLAT Verb (Fig. 1; Assay 1: F$_{2, 25} = 22.7, P < 0.001$; Assay 2: F$_{2, 25} = 19.1, P < 0.001$). In the second assay, 388 T. chlorodia were caught. Unbaited traps caught no T. chlorodia. Traps with verbenone pouches caught fewer T. chlorodia than the control and SPLAT Verb treatments (F$_{2, 27} = 8.3, P = 0.001$; Fig. 1).

Discussion
Our results indicate that verbenone inhibits attraction of I. pini to its aggregation pheromone suggesting that verbenone deserves further exploration as a potential repellent against I. pini attacks on P. ponderosa in northern Arizona. During the first assay, SPLAT Verb and the verbenone pouch reduced trap catches compared with the baited control by 82.5 and 76.7%, respectively. During the second assay, SPLAT Verb and the verbenone pouch reduced trap catches by 8.2 and 8.7%, respectively. It should be noted that our trap catches were low, particularly during Assay 1. Higher I. pini populations may respond differently. Interestingly, unlike the verbenone pouch, SPLAT Verb did not inhibit attraction of T. chlorodia to pheromone-baited traps. Previous studies have reported that verbenone was inhibitory to T. chlorodia (Erbilgin et al. 2007), attractive (Fettig et al. 2007b), or showed mixed results (Gillette et al. 2009). Our results should be interpreted with some caution as SPLAT Verb and the verbenone pouch were released from different locations on the trap, and at different rates (~114 vs 70 mg/d at 20°C, respectively) but the same dose (7 g).

Additional studies need to be undertaken prior to recommending deployment of verbenone for protecting trees from I. pini attacks. These studies should include investigating the efficacy of verbenone in preventing attacks on live P. ponderosa at different doses, in different stand structures, and with varying beetle populations. In addition, we suggest testing the efficacy of verbenone combined with nonhost volatiles or other repellents (e.g., Huber et al. 2001; DeGomez et al. 2008b; Fettig et al. 2012a,b). In the southwestern United States, widespread thinning is being conducted to restore forests and to increase forest resilience (USDA Forest Service 2020), which creates large quantities of slash. Prompt slash removal is often a hurdle for land managers. If colonization of slash by I. pini can be inhibited by using verbenone, this antiaggregation pheromone could represent an important tool in forest restoration in the southwestern United States (DeGomez et al. 2008a).

Acknowledgments
We thank Daniel DePinte (Forest Health Protection, USDA Forest Service) for assistance with field work, the Coconino National Forest for logistical support, and ISCA Technologies Inc. for donating SPLAT Verb. Funding for this project was received from the USDA Forest Service Pesticide Impact Assessment Program (FS-PIAP R3-2019-01). This article was written and prepared by U.S. Government employees on official time and it is, therefore, in the public domain and not subject to copyright.

References Cited


