MCH-Based Semiochemical Repellents for Protecting Engelmann Spruce Trees From *Dendroctonus rufipennis* (Coleoptera: Curculionidae)

Jackson P. Audley,1,5 Christopher J. Fettig,1 A. Steven Munson,2 Darren C. Blackford,2 Leif A. Mortenson,3 and Agenor Mafra-Neto4

1Pacific Southwest Research Station, USDA Forest Service, 1731 Research Park Drive, Davis, CA 95618, USA, 2Forest Health Protection, USDA Forest Service, 4746 South 1900 East, Ogden, UT 84403, USA, 3Pacific Southwest Research Station, USDA Forest Service, 2480 Carson Road, Placerville, CA 95667, USA, 4ISCA Incorporation, 1230 Spring Street, Riverside, CA 92507, USA, and 5Corresponding author, e-mail: jackson.audley@usda.gov, jackson.audley@gmail.com

Subject Editor: Kamal Gandhi

Received 24 September 2021; Editorial decision 27 November 2021

Abstract

Spruce beetle, *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae), is a lethal pest of spruce trees in North America. Despite decades of research, a semiochemical repellent that consistently and effectively protects spruce trees remains elusive. We evaluated the efficacy of 3-methyl-2-cyclohexen-1-one (MCH) in a proprietary, volatile compound release technology (SPLAT) alone and with two adjuvants, *Acer* kairomone blend (AKB) and acetophenone + green leaf volatiles (PLUS) to protect individually treated *Picea engelmannii* Parry ex. Engelm. (Pinales: Pinaceae), and *Pi. engelmannii* within 11.3-m radius of the individually treated trees from colonization and mortality attributed to *D. rufipennis* in western Wyoming. Ninety-one *P. engelmannii* were baited with frontalin and randomly assigned to one of seven treatments (*n* = 13): 3.5 g of MCH applied as SPLAT MCH (SPLAT3.5), 3.5AKB, 3.5PLUS, 7 g of MCH applied as SPLAT MCH (SPLAT7), 3.5AKB, 3.5PLUS, and baited control (bait only). All repellents except SPLAT3.5 and SPLAT7 significantly reduced colonization of individually treated *P. engelmannii* compared to the baited control. 3.5PLUS, 7AKB, and 7PLUS reduced colonization most effectively, and all repellents significantly reduced mortality of individually treated *P. engelmannii* compared to the baited control. All repellents also significantly reduced colonization and mortality of neighboring *P. engelmannii*.

Key words: 3-methyl-2-cyclohexen-1-one, acetophenone, *Acer* kairomone blend, green leaf volatiles, spruce beetle

The spruce beetle, *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae), is the most significant pest of mature spruce (*Picea* spp.) in North America (Furness and Carolin 1977, Holsten et al. 1999, Fettig et al. 2021a). In recent decades, several states in the western United States have experienced significant *D. rufipennis* outbreaks. Currently, outbreaks are ongoing in Alaska (USDA Forest Service 2020), Colorado (Colorado Department of Natural Resources 2020), and Utah (Utah Department of Natural Resources Forestry, Fire & State Lands 2019). In Wyoming an ongoing outbreak on the Bridger-Teton National Forest peaked in 2015 when 39,000 ha of Engelmann spruce, *Picea engelmannii* Parry ex. Engelm. (Pinales: Pinaceae), were impacted (USDA Forest Service 2019). The frequency and extent of *D. rufipennis* outbreaks in western North America have increased in recent decades (Jenkins et al. 2014) and climate change will likely exacerbate future outbreaks (Bentz et al. 2010).

Semiochemical-based interruption of host colonization and aggregation behaviors can be an effective management tool for protecting trees from bark beetle attack (Silverstein 1981, Borden 1997, Progar et al. 2014, Seybold et al. 2018, Huber et al. 2021, Ross 2021). Unlike insecticide bole treatments or systemic insecticides, semiochemical repellents confer little to no harm to nontarget organisms, including humans, and are usually easier and less costly to implement (Seybold et al. 2018). For *D. rufipennis*, the antiaggregation pheromone 3-methyl-2-cyclohexen-1-one (MCH) was discovered by Rudinsky et al. (1974) and is registered in the United States and Canada to protect spruce trees from colonization by *D. rufipennis* and Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Pinales: Pinaceae), trees from
colonization by Douglas-fir beetle, *D. pseudotsugae* Hopkins. While very effective for protecting *Ps. menziesii* from *D. pseudotsugae* (Ross 2021), studies on the efficacy of MCH for protecting spruce from *D. rufpennis* exhibited mixed results (Ross et al. 2015, Seybold et al. 2018). For instance, MCH successfully reduced the number of *D. rufipennis* drawn to infested spruce logs (Kline et al. 1974), felled spruce trees (Lindgren et al. 1989), baited multiple-funnel traps, and standing live spruce trees (Holsten et al. 2003). Conversely, MCH did not protect standing live spruce trees (e.g., Ross et al. 2004, Hansen et al. 2017). Explanations for varied results include too low or inconsistent doses and/or release rates of MCH and declining efficacy with increasing *D. rufpennis* populations. These explanations, which are frequently cited in the literature, remain untested hypotheses and other ecological factors may better explain, or at least contribute to, observed inconsistencies. In the cases above and in other studies (Seybold et al. 2018), MCH was released from passive plastic release devices (e.g., bubble capsules) or in one instance a micro-infusion pump (Holsten et al. 2003).

Hansen et al. (2019) found an *Acer* kairomone blend (AKB; linalool [47.5%], β-caryophyllene [42%], and (Z)-3-hexanol [10.5%]) to be an effective adjuvant to MCH in reducing *D. rufpennis* trap catches. A combination of MCH + AKB protected *P. engelmannii* more effectively than MCH or AKB alone (Hansen et al. 2017). MCH + AKB also protected neighboring *P. engelmannii* up to 10 m from treated spruce trees in the Intermountain West; however, the same treatments did not protect white spruce, *P. glauca* (Moench) Voss, from *D. rufpennis* in Alaska (Hansen et al. 2019).

Recently, a biodegradable formulation of MCH was developed (SPLAT MCH, ISCA Inc., Riverside, CA) for protecting *Ps. menziesii* from *D. pseudotsugae* (Foote et al. 2020). The biodegradable nature of SPLAT makes it an attractive release device compared to plastic release devices for individual tree or area-wide protection especially in remote forest settings, as return trips are unnecessary to retrieve SPLAT MCH (Mafra-Neto et al. 2014). Plastic MCH bubble capsules should be retrieved resulting in additional labor and travel costs. An alternative 'PLUS' adjuvant formulation (acetophenone, and (E)-2-hexen-1-ol + (Z)-2-hexen-1-ol) successfully increased the efficacy of the antiagregation pheromone verbenone against western pine beetle, *D. brevicomis* LeConte, in ponderosa pine, *P. ponderosa* Dougl. ex Laws. (Pinales: Pinaceae) (Fettig et al. 2012a) and mountain pine beetle, *D. ponderosae* Hopkins, in whitebark, *P. albicaulis* Engel., and lodgepole, *P. contorta* Dougl. ex Loud., pines (Fettig et al. 2012b, Fettig and Munson 2020). In this study, we tested the efficacy of SPLAT MCH with and without AKB and PLUS for protecting *P. engelmannii* from colonization and mortality by *D. rufpennis*.

**Materials and Methods**

This study was conducted on the Bridger-Teton National Forest, Wyoming, 2019–2021 (43.81° N, 110.20°–110.23° W; elev. 2443-2693 m). Ninety-one uninfested *P. engelmannii* ≥30 m apart with a diameter at breast height (dbh) of ≥25.4 cm were selected in mid-June 2019 and assigned to one of seven treatments (*n* = 13; Table 1) in a completely randomized design. We determined unattacked status by confirming lack of pitch tubes and/or boring dust from *D. rufipennis* attacks on the lower (~3 m) bole. A 0.041-ha circular plot (11.3-m radius) was established around each treated tree with the pith of the tree serving as the center point of the plot (following protocols used in Foote et al. 2020). Every live tree (≥12.7 cm dbh, including the treated tree) within the plot was measured and inspected for colonization by *D. rufipennis*. There were (means ± SEMs) 432.6 ± 19.0 live trees per ha and 26.8 ± 1.2 m²/ha of basal

**Table 1.** Semiochemicals for each treatment, the corresponding release rate and source, Bridger-Teton National Forest, Wyoming, 2019

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Semiochemicals</th>
<th>Release rates</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPLAT3.5</td>
<td>SPLAT MCH (3.5 g a.i.)</td>
<td>147 mg/d at 26°C</td>
<td>ISCA</td>
</tr>
<tr>
<td>3.5AKB</td>
<td>SPLAT MCH (3.5 g a.i.) + AKB</td>
<td>60 mg/d at 23°C</td>
<td>ISCA</td>
</tr>
<tr>
<td>3.5PLUS</td>
<td>SPLAT MCH (3.5 g a.i.) + Acetophenone + GLV</td>
<td>103 mg/d at 20°C</td>
<td>ISCA</td>
</tr>
<tr>
<td>SPLAT7</td>
<td>SPLAT MCH (7 g a.i.)</td>
<td>294 mg/d at 26°C</td>
<td>ISCA</td>
</tr>
<tr>
<td>7AKB</td>
<td>SPLAT MCH (7 g a.i.) + AKB</td>
<td>60 mg/d at 23°C</td>
<td>ISCA</td>
</tr>
<tr>
<td>7PLUS</td>
<td>SPLAT MCH (7 g a.i.) + Acetophenone + GLVs</td>
<td>20 mg/d at 20°C</td>
<td>ISCA</td>
</tr>
<tr>
<td>All</td>
<td>Frontalin</td>
<td>0.12 mg/d at 20°C</td>
<td>ISCA</td>
</tr>
</tbody>
</table>

*R. Fettig, ISCA Inc., Riverside, CA.*

*Release rate determined gravimetrically 18 August to 17 September 2021 in our laboratory.*

*Synergy Semiochemical Corp., Delta, BC, Canada.*
area, of which 61.1 ± 2.5% was *Pi. engelmannii*, 30.7 ± 2.1% was subalpine fir, *Abies lasiocarpa* (Hooker) Nuttall, 7.3 ± 1.4% was *P. contorta*, and 1.0 ± 0.3% was *Pse. menziesii*.

To challenge the effectiveness of repellent treatments, each treated tree (*N* = 91) was baited on the north face of the bole with the aggregation pheromone frontal (Product 3300, Synergy Semiochemical Corp., Delta, BC, Canada; Table 1). All treated trees (except for the baited control) received 3.5 g or 7 g of MCH applied as 35 or 70 g of SPLAT MCH, a flowable emulsion containing 10% MCH (Foote et al. 2020). We applied two 17.5-g dollops (one on each of the north and south faces of the bole) for SPLAT3.5 and four 17.5-g dollops (one on each cardinal face) for SPLAT7. Four additional treatments combined AKB or PLUS to each of the two doses of SPLAT MCH (Table 1). For 3.5AKB and 7AKB, AKB pouches were stapled to the north face of the bole. For 3.5PLUS and 7PLUS, acetophenone pouches were stapled to the north face and GLVs pouches were stapled to the south face of the bole. All semiochemicals were applied at ~2 m in height on 18–20 June and trees were assessed for colonization by *D. rufipennis* on 9–13 September 2019. Due to COVID-19, planned assessments of tree mortality in 2020 were delayed until 22–23 June 2021. Tree mortality was based on the presence (live) or absence (dead) of green needles on *Pi. engelmannii* colonized by *D. rufipennis* in 2019 (Fettig et al. 2021b).

Statistical analyses (R Statistical Software [version 3.6.3] via RStudio [Version 1.2.5033, R Core Team 2020]) were conducted on proportions of trees, with data presented as percentages for reader clarity. The efficacy of repellents was determined by comparing colonization (strip attacks = evidence of successful entry into the tree based on presence of boring dust in pitch tubes and around the base of the tree) on less than the full circumference of the bole plus mass attacks = evidence of successful entry around the complete circumference of the bole) and mortality rates to the baited controls. Pairwise, two-sample tests for equality of proportions (prop.test, base package) were used for comparisons among individual trees. Mean proportions of *Pi. engelmannii* were compared among treatments using a generalized linear model with a quasibinomial distribution with a log link (lme4 package). Treatment, mean dbh, and mean percent *Pi. engelmannii* were considered as covariates in each model. Final model selection was informed based on likelihood ratio tests (Zuur et al. 2009). Following model fit, differences among means were determined by least squares means (lsmeans) tests with a Tukey adjustment (emmeans package). Additional comparisons within the data were made using the non-parametric Kruskal–Wallis test (kruskal.test) and multiple means comparison (dunn.test) with a Bonferroni correction (stats package).

### Results and Discussion

#### Individual Trees

The dbh (mean ± SEM) of treated *Pi. engelmannii* was 26.6 ± 4.8 cm, which did not differ among treatments ($\chi^2 = 11.96, df = 6, P = 0.06$; Table 2). All repellents except SPLAT3.5 and SPLAT7 significantly reduced colonization (strip attack + mass attack) of *Pi. engelmannii* compared to the baited control ($\chi^2 = 6.1, df = 1, P < 0.01$ for all comparisons; Fig. 1A). 3.5PLUS, 7AKB, and 7PLUS reduced colonization the most with three (23%), two (15%), and two (15%) colonized trees respectively (Figs. 1A and 2). A more detailed breakdown of colonization (Fig. 2) more clearly reveals the additional repellency conferred by each adjuvant AKB and PLUS, especially when added to SPLAT7. Despite differences in colonization rates, all repellents significantly reduced mortality of *Pi. engelmannii* compared to the baited control ($\chi^2 = 15.8, df = 1, P < 0.001$ for all comparisons; Fig. 1B). Based on Hansen et al. (2017, 2019) we expected MCH + AKB to outperform SPLAT MCH alone (SPLAT3.5 and SPLAT7) but this was only the case in preventing *D. rufipennis* colonization and not mortality of *Pi. engelmannii*. It should be noted that *D. rufipennis* colonization (e.g., strip attacks) will usually entice further attacks in subsequent years and could result in mortality of treated trees in the absence of additional mitigation. Interestingly, we did not observe this during our study as mortality among treated spruce remained ≤15% even though our assessment of mortality was delayed a year. It could be that the *D. rufipennis* population declined in 2020 and subsequent colonization pressure was reduced explaining the discrepancy between colonization and mortality (Fig. 1A and B). Thus, despite significant reductions in treated tree mortality, the greater colonization rate observed for SPLAT3.5 and SPLAT7 should be taken into consideration prior to adoption for operational use.

#### Circular Plots

No significant differences were observed in mean (±SEM) dbh of *Pi. engelmannii* within 0.041-ha plots among treatments ($\chi^2 = 0.4, df = 6, P = 0.99$; Table 2). Mean (±SEM) percent of *Pi. engelmannii* within these plots differed among treatments ($\chi^2 = 13.00, df = 6, P = 0.04$) and ranged from 49.3 ± 6.5% for SPLAT7 to 77.3 ± 5.2% for the baited control (Table 2). We acknowledge that greater host density is positively correlated with greater levels of bark beetle-caused tree mortality, including *D. rufipennis* (Jenkins et al. 2014); however, it is unlikely variations in *Pi. engelmannii* density within 0.041-ha plots exerted much influence on our results as only one difference was observed among treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ddbh cm (mean ± SEM)</th>
<th>Ddbh cm (mean ± SEM)</th>
<th>Percent <em>Pi. engelmannii</em> (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPLAT3.5</td>
<td>24.6 ± 4.1a</td>
<td>36.3 ± 2.0a</td>
<td>39.5 ± 6.5ab</td>
</tr>
<tr>
<td>3.5AKB</td>
<td>27.2 ± 5.1a</td>
<td>37.1 ± 2.4a</td>
<td>63.6 ± 5.8ab</td>
</tr>
<tr>
<td>3.5PLUS</td>
<td>26.4 ± 4.8a</td>
<td>36.7 ± 2.0a</td>
<td>56.5 ± 6.2ab</td>
</tr>
<tr>
<td>SPLAT7</td>
<td>23.6 ± 4.1a</td>
<td>35.3 ± 0.9a</td>
<td>49.3 ± 6.3b</td>
</tr>
<tr>
<td>7AKB</td>
<td>27.4 ± 4.6a</td>
<td>36.2 ± 2.2a</td>
<td>33.2 ± 5.7ab</td>
</tr>
<tr>
<td>7PLUS</td>
<td>29.0 ± 5.3a</td>
<td>35.8 ± 2.0a</td>
<td>64.4 ± 8.2ab</td>
</tr>
<tr>
<td>Baited control</td>
<td>28.2 ± 5.3a</td>
<td>39.4 ± 1.8a</td>
<td>77.3 ± 5.2a</td>
</tr>
</tbody>
</table>

Means were compared using a Kruskal–Wallis (kruskal.test) test followed by a Dunn’s pairwise, multiple means comparison test (dunn.test) to identify differences among treatment groups. Within a column, different letters indicate significant differences among treatments, α = 0.05.
Fig. 1. Percent of individually treated *Picea engelmannii* colonized (strip attacks + mass attacks) (A) and killed by *Dendroctonus rufipennis* (B), and mean percent (±SEMs) of *P. englemanii* within 11.3 m of individually treated *Pi. engelmannii* colonized (C) and killed by *D. rufipennis* (D). Treatments included: 3.5 g of MCH applied as SPLAT MCH (SPLAT3.5), 3.5 + *Acer* kairomone blend (3.5AKB), 3.5 + acetophenone and (E)-2-hexen-1-ol and (Z)-2-hexen-1-ol (3.5PLUS), 7 g MCH applied as SPLAT MCH (SPLAT7), 7 + AKB (7AKB), 7 + PLUS (7PLUS) and baited control. All individually treated trees were baited with frontalin (A, B). Within each subfigure, different letters indicate significantly different means based on pairwise, lsmmeans tests (emmeans package, R Statistical Software in RStudio) α = 0.05.

Fig. 2. *Dendroctonus rufipennis* colonization of individually treated *Picea engelmannii* by treatment: 3.5 g MCH applied as SPLAT MCH (SPLAT3.5), 3.5 + *Acer* kairomone blend (3.5AKB), 3.5 + acetophenone and (E)-2-hexen-1-ol and (Z)-2-hexen-1-ol (3.5PLUS), 7 g MCH applied as SPLAT MCH (SPLAT7); 7 + AKB (7AKB); 7 + PLUS (7PLUS) and baited control. All trees were baited with frontalin.

Treatment was the only variable retained in the final model (quasibinomial, log link), and all repellents were effective for reducing colonization of *Pi. engelmannii* within an 11.3-m radius of treated *Pi. engelmannii* (z-ratio < −4.1, P < 0.001 for all; Fig. 1C). This was unexpected given that MCH alone (SPLAT3.5 and SPLAT7) was ineffective in protecting baited trees from colonization (Fig. 1A). It suggests that SPLAT MCH may have been overwhelmed by the aggregation pheromone signal from an individually treated tree (i.e., initially emanating from our baits but then also from natural sources), but that the repellency of SPLAT MCH is sufficient to...
reduce *D. rufipennis* colonization of adjacent trees void of an aggregation pheromone signal.

Similarly, all repellents significantly reduced mortality of *P. engelmannii* within 11.3 m of treated trees (\(z\)-ratio < −3.6, \(P < 0.001\) for all Fig. 1D). A positive correlation between mean dbh and percentage of *P. engelmannii* killed by *D. rufipennis* (corr. coef. = 0.27, \(t = 2.66, df = 89, P = 0.01\)) supports previous observations that *D. rufipennis* prefers large-diameter *P. engelmannii* (e.g., >35.5 cm dbh) surrounding repellent-treated trees that were not killed by *D. rufipennis*, likely due to semiochemical repellent influence.

In conclusion, all repellents were effective for protecting *P. engelmannii* from mortality attributed to *D. rufipennis* (Fig. 1B and D). The high level of protection observed is surprising given the high level of mortality in the baited control (100% and 42.5% in individual trees and circular plots, respectively), which suggests a conservative evaluation of the efficacy of these treatments. Furthermore, in an unrelated study 56.7% of live *P. engelmannii* in surrounding stands were reported colonized by *D. rufipennis* in 2018 in the absence of frontal baits (Fettig et al. 2021b). While bark beetle populations can vary greatly year-to-year, this provides an indication of an outbreak population in the area the year prior to the start of this study. Generally, semiochemical repellents are deemed less effective under higher beetle populations (e.g., Progar et al. 2014, Seybold et al. 2018).

SPLAT MCH alone was as effective as the other repellents for reducing *P. engelmannii* mortality (Fig. 1B and D), but less effective for reducing colonization of individually treated trees (Fig. 1A). While several studies have evaluated different formulations of MCH and reported mixed results, this is the first to evaluate SPLAT MCH in the *D. rufipennis* system. In our study, we used higher release rates of MCH (147 and 294 mg/d at 26°C for SPLAT3.5 and SPLAT7, respectively) than in previous studies evaluating other formulations of MCH. For instance, Ross et al. (2004) reported a lack of efficacy for area-wide protection of *P. engelmannii* in Utah when using MCH bubble capsules eluting at ~7–9 mg/d at 22–25°C. Hansen et al. (2017) reported mixed results for protection of individually treated *P. engelmannii* when using bubble capsules eluting at 12 mg/d at 25°C. Holsten et al. (2003) reported MCH alone to be effective with an 87% reduction of attacks on standing live Sitka spruce, *P. sitchensis* (Bong.) Carr., in Alaska using microinfusion pumps eluting MCH at 2.6–5.0 mg/d regardless of temperature. The release rates of MCH in these and other *D. rufipennis* studies have generally been informed by research on *D. pseudotsugae* where MCH is highly effective for tree protection at low release rates (Ross et al. 2015, Ross 2021). It may be that our higher release rates of MCH explain the efficacy observed in our study, and that higher releases are needed to impart repellency sufficient to protect *P. engelmannii* from *D. rufipennis*. The low cost of MCH (e.g., ~$0.66/g, Product W336009-1KG-K, Sigma–Aldrich, Saint Louis, MO) may justify the use of high release rates.

We encourage evaluation of SPLAT MCH (with and without adjuvants) for protecting *P. glauca* from mortality attributed to *D. rufipennis* in Alaska and other locations. We also encourage further evaluation of SPLAT MCH on *P. engelmannii* in both individual-tree and area-wide treatments that may be needed to protect recreational sites, sensitive ecological zones, and rural properties. SPLAT MCH will likely be registered for use against *D. pseudotsugae* and *D. rufipennis* by U.S. Environmental Protection Agency in 2022 (A.M.N., unpublished data).

Acknowledgments

We thank J. Neumann and L. Dunning (Forest Health Protection, USDA Forest Service), S. Hamud, C. Dabney and H. Fettig (Pacific Southwest Research Station, USDA Forest Service), J. Runyon and B. Dunning (Rocky Mountain Research Station, USDA Forest Service), R. Progar (Sustainable Forest Management Research, USDA Forest Service), and C. Bernardi, R. Silva and J. Saroli (ISCA, Inc.) for technical assistance. In addition, we thank T. Stiles (Bridge-Teton National Forest, USDA Forest Service) for providing access to the study site and M. Hansen (Rocky Mountain Research Station, USDA Forest Service) and J. Moan (Alaska Division of Forestry) for thoughtful discussions. Funding for this project was provided, in part, by the USDA Forest Service Pesticide Impact Assessment Program (R10-2019-SPLAT MCH) and SERG International-A Partnership in Forest Pest Management Research. This article was written and prepared by US Government employees on official time, and it is, therefore, in the public domain and not subject to copyright.

References Cited


Scolytinae) attack in southern Rocky Mountains. J. Econ. Entomol. 110: 2140–2148.


R Core Team. 2020. R: a language and environment for statistical computing. R Core Team, Vienna, Austria. (http://R-project.org/).


