

Advances in Semiochemical Repellents to Mitigate Host Mortality From the Spruce Beetle (Coleoptera: Curculionidae)

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Abstract

We tested 3-methyl-2-cyclohexen-1-one (MCH) and novel semiochemicals as potential spruce beetle (*Dendroctonus rufipennis* Kirby) (Coleoptera: Curculionidae, Scolytinae) repellents over multiple years in Utah and Colorado trapping bioassays. MCH is a known spruce beetle repellent and our testing revealed *Acer* kairomone blend (AKB) and isophorone plus sulcatone as repellents. We subsequently tested these semiochemicals for area and single tree protection to prevent spruce beetle attacks at locations in Utah, Colorado, Wyoming, New Mexico, and Alaska. Individual tree protection trials found MCH–AKB provided significant protection against spruce beetle attacks in the southern Rocky Mountains but not in Alaska. Adding sulcatone or doubling MCH–AKB pouches did not further enhance protection. A degree of protection was extended to spruce at least 10 m distant from the repellents, including in Alaska. Tree diameter was not a significant covariate among treated trees but was positively correlated with the probability of infestation for surrounding spruce. In area protection trials, spruce in control plots were 2.4 times more likely to be in a higher severity attack class compared with spruce in plots treated with MCH–AKB pouches deployed at 30 sets per hectare. Tree diameter had a significant, positive relationship to the probability of infestation. We found MCH–AKB to offer a high degree of protection against beetle attack in Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) (Pinales: Pinaceae) (*Picea engelmannii* Parry ex Engelm.) (Pinales: Pinaceae), especially for single tree protection (66% of control trees were strip- or mass-attacked compared with 6% of repellent-treated trees). AKB requires registration and labeling, however, before this economical and environmentally benign semiochemical can be used operationally.

Key words: pheromone, bark beetles, Engelmann spruce, MCH, *Dendroctonus rufipennis*

The spruce beetle, *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae, Scolytinae), is an eruptive forest insect and the major disturbance agent of North American spruce (Holsten et al. 1999). Periodic outbreaks of spruce beetle are capable of killing most of the mature spruce (*Picea* spp.) (Pinales: Pinaceae) over extensive, susceptible landscapes (Dymerski et al. 2001, DeRose and Long 2007). Beginning in the 1990s, multiple western states have experienced the largest outbreaks in their respective states' histories (Jenkins et al. 2014). Ongoing outbreaks are affecting spruce forests in Alaska, Idaho, Wyoming, Utah, New Mexico, and Colorado, the latter with

an estimated 0.8 million ha infested (<https://foresthealth.fs.usda.gov/portal/Flex/FPC>; Colorado State Forest Service 2017).

Management strategies have been categorized as “indirect control”, which reduces tree and stand susceptibility to beetle-caused mortality via vegetation management, and “direct control”, which reduces or manipulates the local beetle population via fire, insecticides, removal of infested trees, or semiochemicals (Wood et al. 1985, Fettig et al. 2014). Among the indirect methods, semiochemical repellents are the most cost-effective and have negligible environmental concerns. 3-Methyl-2-cyclohexen-1-one (MCH),

for example, has been shown to significantly reduce Douglas-fir beetle-caused (*D. pseudotsugae* Hopkins) Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Pinales: Pinaceae) mortality at a cost of about US\$200 per hectare (semiochemicals only) for 1 yr of protection (Ross et al. 2015, Brookes et al. 2016), and semiochemicals are considered environmentally benign (Gillette and Munson 2009, Strom and Clarke 2011).

Semiochemical repellants have been used to successfully disrupt attacks by *Dendroctonus* species on live, standing host trees at scales ranging from individual trees to small stands (i.e., <4 ha; Ross and Wallin 2008, Progar et al. 2014, Seybold et al. 2018). MCH was identified as an anti-aggregant of the spruce beetle (Rudinsky et al. 1974) and was found to reduce trap captures (Kline et al. 1974, Furniss et al. 1976, Holsten et al. 2003) and attacks on downed host material and stumps (Rudinsky et al. 1974, Lindgren et al. 1989). Although MCH is registered for use against spruce beetle, its efficacy as a tree protectant has had mixed success, especially against higher beetle population levels (Werner and Holsten 1995, Holsten et al. 2003, Ross et al. 2004, Hansen et al. 2017). The only other known spruce beetle semiochemical useful for tree protection is a non-host blend of maple volatiles, “*Acer* kairomone blend” (AKB). AKB comprises linalool, β -carophyllene, and leaf alcohol, and can be used as an MCH adjuvant for enhanced protection against beetle attack (Hansen et al. 2017).

Bark beetles rely on chemoreception to locate suitable hosts and overcome host defenses via pheromone-mediated mass-attack (Seybold et al. 2018). Understanding the ecological functions of various semiochemicals allows for the development of tools which can disrupt beetle behavior. In nature, tree odors are rarely pure compounds but rather dynamic blends which change with the tree's physiological state over time. Host terpene blends constitute the primary attraction of bark beetles to suitable hosts while non-host and unsuitable host tree odors tend to repel bark beetles. Host terpene content is also part of the constitutive tree defense system and several terpenes show some level of toxicity to bark beetles, their symbionts, and aerobic organisms in general. Monoterpenes are frequently detoxified via oxidation and some of the terpene oxidation products are well-known semiochemicals like *cis*- and *trans*-verbenol, myrtenol, and verbenone (Seybold et al. 2006).

Ecologically, it makes sense that bark beetles can recognize not only host terpene ratios but also their oxidation products because these are indicators of the host's physiological state and therefore suitability for colonization. Pure, unoxidized terpenes are likely to be associated with healthy vigorous host trees, whereas early-stage terpene oxidation products signal a host tree with compromised defenses. Highly oxidized, late-stage products are found in high concentrations on dead or dying host trees that are no longer suitable for colonization. The mountain pine beetle (*D. ponderosae* Hopkins) repellent verbenone is an example of this concept because it is one of the late-stage α -pinene oxidation products, while the early-stage oxidation products, *cis*- and *trans*-verbenols are highly attractive. Interestingly, the oxidation of spruce beetle pheromone attractants 3-methyl-2-cyclohexen-1-ol (seudenol) and 1-methyl-2-cyclohexen-1-ol (MCOL) produces MCH which changes the ecological signal from attraction to repellency. Recently MCH has been identified as an oxidation product of limonene (D. Wakarchuk, unpublished data), one of several Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) monoterpenes common to the pine family along with α - and β -pinene, 3-carene, myrcene, β -phellandrene, and santene (Mardarowicz et al. 2004). Each of these monoterpenes has numerous oxidation products, some of which may be behaviorally active for spruce beetles.

Synergy Semiochemicals Corp. (Delta, B.C., Canada) has produced a library of over 500 oxidized monoterpene products, representing

a broad range of conifer terpene metabolites. A subset of these were screened for antennal reactivity using spruce beetles collected from Utah (B. Sullivan, US Forest Service, Southern Research Station, Pineville, LA; unpublished data). Our goals were to test antennally active compounds in trapping bioassays to quantify attraction or repellency relative to a standard baited trap, including candidate compounds other than oxidized monoterpenes. Based on those results, we then conducted field trials of the most promising repellents to determine their efficacy as single tree and area protectants against spruce beetle attack. Our results inform resource managers of semiochemical-based management options for spruce beetle in the western United States.

Methods

Trapping Bioassays

The western Uinta Mountains in northeastern Utah have had epidemic spruce beetle populations since 2010 (DeBlander et al. 2010), providing suitable beetle populations for field testing semiochemical attraction or repellency. We tested candidate semiochemicals during the adult flight period (June–July) from 2013 to 2015 on the Heber-Kamas and Evanston-Mountain View Ranger Districts, Uinta-Wasatch-Cache National Forest. Additional assays were conducted during 2017 in the Uinta Mountains and at two Colorado locations, Hidden Valley, Rocky Mountain National Park (epidemic population phase; >5 infested stems per hectare; Bentz and Munson 2000, Hansen et al. 2006a) and near Guanella Pass, Clear Creek Ranger District, Arapahoe-Roosevelt National Forest (building population phase; 1–5 infested stems/ha).

In each year, the reference treatment was a 12-unit funnel trap baited with a three-component lure (Synergy Semiochemicals Corp, Delta B.C., Canada; ChemTica Internacional, S.A., Santa Rosa, Costa Rica) while candidate semiochemicals were added to an otherwise similarly baited funnel trap (Table 1). Pouches containing semiochemicals were placed on the outside of the trap at the top of the third funnel from the bottom. Traps were spaced at least 50 m apart. Each year, traps were deployed in early to mid-June to capture peak beetle flight. To eliminate variance due to trap location differences, trap captures were collected and traps rotated weekly such that each treatment had at least one sampling interval at each location. In some cases, traps were deployed an extra week yielding an additional sample with each treatment at its original location. A piece of insecticide-impregnated plastic (2,2-dichlorovinyl dimethyl phosphate; Spectrum Brands, St. Louis, MO) was placed in each trap cup to prevent beetle escape and reduce losses to predatory insects such as clerid beetles (Coleoptera: Cleridae). Collections were frozen until contents could be sorted, identified, and counted.

Individual Tree Protection

From the trapping bioassay results, we selected the most promising repellents for further testing in single tree and area protection trials. For individual tree protection trials, we selected live, unattacked spruce trees spaced at least 20 m apart, averaging 25–58 m to the nearest neighboring experimental tree depending on site (Table 2). In 2017, we identified suitable Engelmann spruce at one location in Utah and two in Colorado. All trees were baited with frontalinal applied on north bole aspects to ensure treatments were challenged by spruce beetles. Treatments were control (bait only) and bait with MCH plus AKB (MCH: 1,000 mg bubble, eluting 12 mg/d at 25°C; AKB ~6.8 g active ingredient, eluting ~65 mg/d at 25°C; Synergy Semiochemicals Corp.). AKB consists of three active ingredients, linalool (47.5% of the active ingredient, by weight), β -caryophyllene

Table 1. List and sample sizes of semiochemicals tested for spruce beetle reactivity, 2013–2015 and 2017

Compound	Total traps	Replicates	Samples
2013—Utah (40.495°N, 111.081°W; 40.527°N, 111.026°W) ¹			
Verbenene	48	8	7
Piperitone		8	7
(–) <i>cis</i> -Carveol		8	7
Isophorone plus sulcatone		8	7
(–) Carvone		8	7
Reference: enhanced		8	7
2014—Utah (40.685°N, 110.923°W; 40.824°N, 110.939°W) ²			
Celery ketone	80	8	7
Pentyl furan		8	7
(+) Carvone		8	7
3-Pinene-2-ol		8	7
(+) <i>cis</i> -Carveol		8	7
(+) Limonene epoxide		8	7
Eucarvone		8	7
Carene oxide		8	7
Reference: enhanced		8	7
2015—Utah (40.685°N, 110.923°W; 40.824°N, 110.939°W) ³			
MCH (1000 mg)	110	10	6
Isophorone + sulcatone		10	6
MCH + isophorone + sulcatone		10	6
Nopol		10	6
Terpene-4-ol		10	6
Cymene-8-ol		10	6
AKB		10	6
Sulcatol		10	6
Methylbutenol		10	6
Reference: enhanced		10	6
2015—Utah (ChemTica; 40.820°N, 110.891°W) ⁴			
MCH (400 mg × 10)	24	8	6
MCH plus E2-hexen-1-ol and Z3-hexen-1-ol		8	6
Reference: standard		8	6
2017—Colorado (39.608°N, 105.725°W; 40.392°N, 105.655°W) ⁵			
MCH (reduced load and elution rate)	40	8	5
Linalool		8	5
β-Caryophyllene		8	5
Linalool + β-caryophyllene		8	5
Reference: standard		8	5
2017—Utah (A) (40.761°N, 110.880°W) ⁶			
MCH	40	8	5
Linalool		8	5
β-Caryophyllene		8	5
Leaf alcohol		8	5
Reference: standard		8	5
2017—Utah (B) (40.757°N, 110.879°W) ⁶			
MCH + linalool + β-caryophyllene	40	8	5
MCH + linalool + leaf alcohol		8	5
MCH + β-caryophyllene + alcohol		8	5
MCH + AKB		8	5
Reference: standard		8	5

Each treatment was rotated one position after each weekly sample such that each treatment had at least one interval at each location within a replicate array. All treatments included lures. The “enhanced” reference lures used frontalinal, 1-methyl-2-cyclohexen-1-ol (MCOL), and a host terpene blend. The “standard” reference lure used frontalinal, MCOL, and α-pinene. All semiochemicals were provided by Synergy Semiochemicals Corp. except for the 2015 MCH + leaf alcohols trials wherein semiochemicals were provided by ChemTica Internacional, S.A.

Table 1. Continued

¹At each location, traps were arranged in two parallel transects of 12 traps each.

²The first location had 49 traps in seven heptagons and the second had 63 traps in nine heptagons. Each array included the reference treatment and there were four additional treatments in a tangential experiment not reported here (112 total traps, results from 80 reported herein).

³Eleven total treatments, arranged in five pentagons and five hexagons at each location.

⁴Six traps in each of two parallel transects at each of two locations ~0.5 km apart. One MCH trap fell to the ground and was thus only sampled five times.

⁵Four pentagonal arrays at each location.

⁶Eight pentagonal arrays. MCH with reduced load and elution rate compared with commercial product.

(42% of the active ingredient), and leaf alcohol (Z3-hexenol; 10.5% of the active ingredient). Repellents and baits were stapled to north aspects of boles about 2 m above the ground. Each treatment was replicated 5–10 times at each of the three areas.

In 2018 trials we used six sites, ranging from Alaska to New Mexico (white spruce, *P. glauca* (Moench) Voss, in Alaska and Engelmann spruce elsewhere; Table 2). At most southern Rocky Mountains locations, frontalinal tree baits (eluting 125 µg/d at 25°C; Synergy Semiochemicals Corp.) were stapled to the tops of 1.2 m posts about 2 m upwind from treated trees. At Mill Park, Utah and Hidden Valley, Colorado, however, we did not use any baits because the local beetle populations were extreme (50–80% of total spruce basal area killed the previous year). Because of the scarcity of live spruce >25 cm diameter at breast height (dbh) at Hidden Valley, one-half of replicates planned for that location were instead deployed at Guanella Pass, Colorado. At Alaska locations, frontalinal tree baits were attached to non-host trees 3–3.7 m distant. This change in baiting protocol was made to better mimic applications wherein baits would never be included with a goal of tree protection. Treatments were control (baited or unbaited), MCH–AKB, MCH–AKB plus sulcatone, and double-dose MCH–AKB (Synergy Semiochemicals Corp.). We also tested MCH-alone in Alaska. In the case of double-dose MCH–AKB, repellent sets were applied to northeast and northwest bole faces.

Tree diameter was tested as a covariate by selecting spruce from a range of diameter classes, focused on the larger classes available at each site. Depending on the site and year, diameter classes had intervals of 5 or 7.6 cm with trees of each treatment spread equally among the classes. At most sites, size classes ranged 30–65 cm dbh, but we selected trees as small as 19 cm dbh at Hidden Valley, Colorado where few uninfested spruce exceeded 25 cm because of previous spruce beetle-caused mortality.

Treated trees were examined for attack status in September after beetle flight was completed. We also examined all trees within 10 m of the treatment tree. These data enabled investigation of any “halo effect” as well as an inclusion of covariates such as beetle pressure from nearby, recently infested spruce and density of hosts and non-hosts. Data collected included dbh, status (live, spruce beetle mass-attacked, strip-attacked, unsuccessfully attacked or “pitchout,” or other mortality), and year of attack. Year of attack was determined using characters described by Hansen et al. (2006a):

1. Current year attack—presence of boring dust and immature brood, occasionally fresh pitch tubes, on a green-needled tree;
2. Previous year attack—symptoms range from fading needles to some or most needles fallen, live beetles may still be present, especially at the root collar;

Table 2. Locations, number of treatments, number of replicates per treatment, and average distance to nearest neighboring trees for single tree protection trials to prevent spruce beetle attacks

Site	Latitude; longitude (WGS84)	Treatments	Replicates	Distance (m) (SD)
2017				
Mill Park, UT	40° 36' N; 110° 26' W	2	10	47.1 (22.1)
Guanella Pass, CO	39° 36' N; 105° 43' W	2	5	39.0 (11.8)
The Craggs, CO	38° 54' N; 105° 08' W	2	5	25.3 (6.8)
2018				
Denali State Park, AK	62° 41' N; 150° 14' W	5	9 ¹	49.7 (22.8)
Togwotee Pass, WY	43° 49' N; 110° 12' W	4	12	30.9 (17.4)
Mill Park, UT	40° 36' N; 110° 26' W	4	12	58.0 (132.7)
Hidden Valley, CO	40° 24' N; 105° 39' W	4	6 ²	28.3 (11.1)
Guanella Pass, CO	39° 36' N; 105° 43' W	4	18	32.3 (8.5)
Hopewell Lake, NM	36° 43' N; 106° 15' W	4	12	45.0 (22.8)

Minimum spacing between treatment trees was 20 m.

¹Three additional replicates were installed near Houston, Alaska, but data were not used due to lack of spruce beetle attacks.

²Twelve replicates were planned, but six of these were instead installed at Guanella Pass due to lack of live spruce >30 cm dbh at Hidden Valley. The extra replicates at Guanella Pass were not baited (i.e., 12 baited and 6 unbaited replicates at Guanella Pass).

3. Second year attack—fine twigs attached, most or all needles fallen, no live brood present; or
4. Older attack—no needles, some or many fine twigs missing.

Note that treatment trees were uninfested when trials began, thus, classes 2–4 apply only to spruce in the surrounding 10 m radius.

Area Protection Treatments

For area protection trials, we deployed MCH-AKB (see load and release information above; Synergy Semiochemicals Corp.) at 30 sets per hectare (~18.2 m spacing). This density was extrapolated from MCH-only treatments tested at 20, 40, and 80 g/ha (Hansen et al. 2017). Repellents were stapled to north aspects of boles about 2 m above ground level. Spacing of MCH-AKB was determined using compass and hip chain with a repellent pair attached to the nearest tree with preference given to large diameter spruce when available within arm's reach. Treatment blocks were ~1.25 ha with a nested 0.64 ha survey block to minimize edge effects. Three replicates each of treated and control blocks were installed during June, 2017 at Mill Park, Utah plus a single replicate near Guanella Pass, Colorado. Additional replicates were planned for Rocky Mountain National Park, Colorado, but our permit was not approved until well after beetle flight had begun. To ensure spruce beetle pressure within the plots, funnel traps baited with frontalinal, MCOL, and a host terpene blend (Hansen et al. 2006b; Synergy Semiochemicals Corp.) were deployed ~50 m within two opposing treatment block corners. Control plots included the two baited funnel traps but no other treatment.

After beetle flight in September, 2017, we conducted ground surveys within 80 × 80 m squares centered within the treatment blocks to quantify posttreatment spruce beetle attacks (Gillette et al. 2012, Hansen et al. 2017). We surveyed all live trees >10 cm dbh and all spruce estimated to have been infested within the previous 5 yr. Previously attacked spruce were used as a surrogate for local beetle population size in analyses. Data collected included species, dbh (measured with Biltmore sticks), status (live, spruce beetle mass-attacked, strip-attacked, unsuccessfully-attacked, or other mortality), and year of attack. Year of attack was determined using characters described above.

Analyses

We used generalized linear mixed models to analyze the trapping bioassay data (PROC GLIMMIX, SAS Institute, Inc., Cary, NC;

Littell et al. 2006). Collection day-of-year was used as a covariate and replicate within trap location by area was specified as a random effect. The response distribution was specified as log-normal to satisfy assumptions regarding residuals. Degrees of freedom were calculated using the Kenward–Roger method (Kenward and Roger 1997). Pairwise comparisons were made using the Tukey adjustment for *P*-values and confidence limits.

Attack severity was the response variable for the individual tree protection trials. This variable is ordinal in that the responses can be ranked but with unknown distances between classes (0 = unattacked; 1 = unsuccessful-attacked or “pitchout”; 2 = strip-attacked; 3 = mass-attacked) and these data were analyzed with an ordinal logistic regression model (Hosmer et al. 2013). We used a generalized linear mixed model with a multinomial response distribution and the cumulative logit link function to accommodate “replicate within area” as a random effect (PROC GLIMMIX, SAS Institute, Inc., Cary, NC; Littell et al. 2006). Degrees of freedom were calculated using the Kenward–Roger method (Kenward and Roger 1997). Covariates explored included dbh as well as summaries of conditions within 10 m of each treated tree: basal area and stems counts for spruce, non-host, recently infested spruce (1–2 yr before surveys), older infested spruce (3–5 yr prior); mean dbh of spruce >25 cm; and the percent of spruce component. Pairwise comparisons were made using the Tukey–Kramer adjustment for *P*-values and confidence limits (Kramer 1956).

To test for a repellent halo effect, we conducted a similar analysis for all spruce within 10 m of the central, treated spruce. In this case, each spruce within the 10 m radius was considered an experimental unit with distance to the central spruce used as a covariate. Replicate within area was used as a random variable to account for subsample correlation. Data from the two years, 2017 and 2018, were analyzed separately because of the differences in treatments and baiting schemes.

Model residuals, for the central treated trees only, were tested for spatial dependence using Moran's *I* test (spdep package, R statistical software; r-project.org; Bivand 2002). We did this as a check for inter-plot influences with some treated trees as close as 20 m. Although bait “spillover” is concentrated within 10 m (Hansen et al. 2006a, Klutsch et al. 2017), *Dendroctonus* have been observed to respond to baits at least as far as 400 m (Dodds and Ross 2002). Thus, inter-plot beetle attraction was likely influenced by our spacing but with unknown effects on tree mortality. Likewise, inter-plot

influence of MCH-*AKB* is unknown although MCH-only data suggest that repellent effects might occur to at least 10 m (Hansen et al. 2017). PROC GLIMMIX cannot output model residuals for multinomial responses, so we first re-coded the response into binomial classes: unsuccessful attack (unattacked or pitchout) or successful attack (strip- or mass-attack). We were not able to use Moran's *I* test for the surrounding 10 m spruce because those trees were not georeferenced.

For the area protection trials, each spruce tree was considered an experimental unit with attack severity as the response variable. We used an ordinal logistic regression model similar to that described above. Replicate within Area was specified as a random effect to account for subsample correlation. Tested covariates included the dbh of each individual spruce and plot-level measures such as average dbh of spruce >25 cm, spruce basal area, non-host basal area, the percent of spruce component, and basal area or stem counts of previously infested spruce within each plot. Pairwise comparisons were made using the Tukey-Kramer adjustment for *P*-values and confidence limits (Kramer 1956).

Results

Trapping Bioassays

Most semiochemicals tested did not significantly reduce trap captures relative to the reference lure alone. For example, none of the eight tested in 2014 had any measureable effect (Table 3). A few compounds or aggregated compounds, however, significantly reduced attraction to baited traps. We tested isophorone plus sulcatone twice; it reduced captures ~64% in 2013 and ~73% in 2015. High-dose, high-release rate MCH consistently reduced captures >90% compared with reference traps. Adding isophorone plus sulcatone or leaf alcohols to MCH further reduced mean trap captures but the differences were not significant relative to MCH-alone. *AKB* also significantly reduced trap captures, ~80% relative to lure only traps.

Focusing on the *AKB* components, β -caryophyllene and leaf alcohol alone did not significantly reduce trap captures compared with lure-only traps at Colorado and Utah sites. Linalool significantly repelled spruce beetles in Colorado but not in Utah. Adding two of the three *AKB* components to MCH appears superior to MCH alone (Table 3; compare reductions for MCH alone in 2017 Utah "A" to that for MCH plus two *AKB* components in 2017 Utah "B"), but MCH with all three *AKB* components resulted in maximal repellency.

Individual Tree Protection

Results from 2017 testing in Utah and Colorado showed a significant treatment effect ($F_{1,36} = 16.21, P = 0.0003$). Control trees were estimated to be 73.3 times more likely to be in a higher severity attack class (e.g., mass-attack over strip-attack, strip-attack over unsuccessful attack, or unsuccessful attack over unattacked) compared with MCH-*AKB*-treated trees (odds ratio 95% confidence limits: 8.4–638.2). No covariates were significant including dbh. The Moran's *I* test for spatial dependence of model residuals found no evidence of autocorrelation (Moran's *I* = -0.0675; $P = 0.6783$).

Broadening the analysis to include all spruce within 10 m of the central treated tree, but exclusive of the central tree (i.e., halo effect), spruce in control plots were estimated to be 15.5 times more likely to be in a higher severity attack class compared with spruce within 10 m of an MCH-*AKB*-treated tree (odds ratio 95% confidence limits: 8.9–26.7; $F_{1,656} = 54.43, P < 0.0001$). Diameter was a significant covariate with a positive relationship between dbh and the probability of a higher severity attack. There was a significant interaction

Table 3. Generalized linear mixed model (GLIMMIX) predicted mean weekly spruce beetle trap captures for the reference treatment (three-component lure in a 12-unit funnel trap) and potential semiochemical repellents added to an otherwise similar trap

Compound	Log-scale	Back-transformed
	Mean (SE)	Mean
2013		
(-) <i>cis</i> -Carveol	6.25(0.12)a	518.0
Reference	6.17(0.13)a	478.2
Verbenene	6.15(0.13)a	468.7
(-) Carvone	6.03(0.12)a	415.7
Piperitone	5.98(0.12)a	395.4
Isophorone plus sulcatone	5.15(0.13)b	172.4
2014 "A"		
Eucarvone	5.19(0.26)a	179.5
Pentyl furan	5.14(0.26)a	170.7
Carene oxide	5.12(0.26)a	167.3
(+) <i>cis</i> -Carveol	5.11(0.26)a	165.7
3-Pinene-2-ol	5.09(0.26)a	162.4
Reference	5.07(0.26)a	159.2
(+) Limonene epoxide	5.01(0.26)a	149.9
2014 "B"		
Celery ketone	5.27(0.10)a	194.4
Reference	5.22(0.10)a	184.9
(+) Carvone	5.15(0.10)a	172.4
2015 "A"		
Reference	5.50(0.18)a	244.7
Nopol	5.38(0.18)a	217.0
Terpene-4-ol	5.24(0.18)a	188.7
Isophorone plus sulcatone	4.18(0.18)b	65.4
MCH (1,000 mg)	2.26(0.18)c	9.6
MCH plus isophorone plus sulcatone	1.94(0.19)c	7.0
2015 "B"		
Reference	5.00(0.11)a	148.6
Methylbutenol	5.00(0.11)a	147.9
Sulcatol	4.97(0.11)a	143.5
Cymene-8-ol	4.87(0.11)a	129.8
<i>AKB</i>	3.37(0.11)b	29.2
2015 ChemTica		
Reference	5.08(0.18)a	160.5
MCH (400 mg × 10)	2.29(0.21)b	9.8
MCH with leaf alcohols	1.88(0.21)b	6.6
2017 Colorado		
Reference	5.22(0.15)a	184.9
β -Caryophyllene	5.02(0.15)ab	150.9
Linalool + β -caryophyllene	4.79(0.15)b	120.7
Linalool	4.77(0.15)b	118.3
MCH (reduced)	3.38(0.15)c	29.2
2017 Utah "A"		
β -Caryophyllene	5.93(0.10)a	376.9
Leaf alcohol	5.91(0.10)a	370.1
Reference	5.69(0.10)a	296.5
Linalool	5.65(0.10)a	283.5
MCH (reduced)	4.01(0.10)b	55.0
2017 Utah "B"		
Reference	6.26(0.12)a	524.8
MCH + linalool + β -caryophyllene	3.96(0.12)b	52.7
MCH + linalool + leaf alcohol	3.90(0.12)b	49.6
MCH + β -caryophyllene + alcohol	3.89(0.12)b	48.9
MCH + <i>AKB</i>	3.50(0.12)c	33.0

2013–2015 trials were conducted in the western Uinta Mountains, UT. 2017 trials were conducted at Rocky Mountain National Park and Guanella Pass, CO and in the western Uinta Mountains, UT. Except for "2015 ChemTica," all semiochemicals were provided by Synergy Semiochemicals Corp.

Within each grouping, means followed by the same letter are not significantly different at $P > 0.05$ using tests of pairwise differences (Tukey-Kramer). *AKB* includes linalool, β -caryophyllene, and leaf alcohol.

between treatment and distance to the treated tree ($F_{1,656} = 17.17$, $P < 0.0001$). Spruce farther from a bait only-treated tree were less likely to have a higher severity attack rating, whereas spruce farther from a bait plus MCH-AKB-treated tree were more likely to have a higher severity attack rating (Fig. 1).

Data from 2018 were analyzed separately for Alaska and the southern Rocky Mountains because the responses clearly differed by region. In the Rocky Mountains, there was a significant treatment effect ($F_{3,232} = 12.01$, $P < 0.0001$). Control trees were estimated to be 20.8–69.1 times more likely, depending on the pairwise comparison, to be in a higher severity attack class compared with repellent-treated trees (odds ratio 95% confidence limits: 4.9–583.8). This was the third consecutive year of testing MCH-AKB for single tree protection and this semiochemical combination showed significant tree protection, relative to controls, for each year (Fig. 2). There were no significant differences, however, among the three repellent treatments (MCH-AKB, MCH-AKB plus sulcatone, and double-dose MCH-AKB) and no covariates were significant. The Moran's I test found no evidence of spatial dependence among model residuals (Moran's $I = -0.0054$; $P = 0.5123$). For Alaska data, there was no significant treatment effect ($F_{4,39} = 0.98$, $P = 0.4286$), with repellent-treated spruce incurring beetle attacks at levels indistinguishable from controls. Again, spatial dependence was not detected (Moran's $I = -0.0345$; $P = 0.5546$).

For trees at Rocky Mountain locations within 10 m of the treated tree but excluding that tree, spruce in control plots were 1.8–3.6 times more likely to be in a higher severity attack class compared with spruce in repellent-treated plots, depending on the pairwise comparison (odds ratio 95% confidence limits: 1.2–6.0; $F_{3,2295} = 13.07$, $P < 0.0001$). Diameter and spruce basal area were significant covariates, each with a positive relationship to the probability of a higher severity attack. As with the 2017 results, there was a significant interaction between treatment and distance to the treated tree ($F_{3,2295} = 5.53$, $P = 0.0009$). Increasing distance from controls trees reduced the probability of higher severity attack, whereas the opposite trend was observed among repellent-treated trees (Fig. 1).

In Alaska, contrary to results for the treated tree, there was a significant halo effect. Spruce within 10 m of a control spruce were

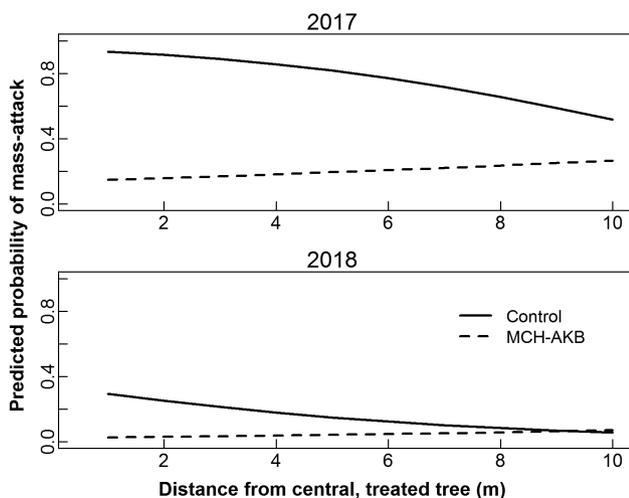


Fig. 1. Modeled probability of mass-attack by distance from central, treated tree for a 50.8-cm dbh spruce (individual tree protection). The probability of attack is positively correlated to dbh (see Results). Spruce basal area within 10 m of the treated tree was a significant covariate for 2018 data and its mean value was held constant to produce this figure. The 2018 data are exclusive of Alaska results.

4.2–6.9 times more likely to be in a higher severity attack class than spruce near a repellent-treated spruce (odds ratio 95% confidence limits: 1.2–24.6, depending on pairwise comparison; $F_{4,194} = 3.46$, $P = 0.0094$). Pairwise comparisons among the four repellent treatments found no significant differences at $\alpha = 0.05$. Spruce basal area and dbh were significant covariates positively related with probability of higher attack severity and distance to the central tree was significant with a negative relationship. The interaction between treatment and distance was not significant.

Area Protection

Beetle pressure was extremely high at the three Utah replicates where control plots lost 75–80% of total spruce basal area during 2017 (total includes spruce beetle-killed up to 5 yr previous). The control plot in Colorado lost about 50% of total basal area that year, also indicating high beetle pressure. Treatment was a significant effect in the ordinal logistic model ($F_{1,3785} = 14.33$, $P = 0.0002$). Spruce in control plots were 2.4 times more likely to be in a higher severity attack class compared with those in MCH-AKB-treated plots (odds ratio 95% confidence limits: 1.5–3.8; Fig. 3). Tree diameter, the interaction between dbh and treatment, and counts of recently infested stems (i.e., 2015 and 2016 mass-attacks) were significant covariates, all positively related to the probability of a more severe attack (Fig. 4).

Discussion

Our trapping bioassays confirmed MCH as a spruce beetle repellent in Utah despite the failure of MCH as an area protectant elsewhere in the state (Ross et al. 2004). This discrepancy, however, might

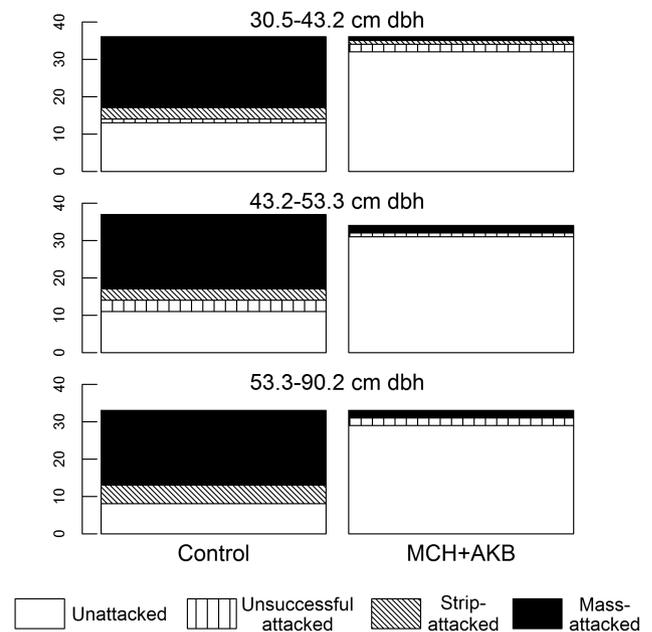


Fig. 2. Numbers of spruce in each spruce beetle attack class for combined individual tree protection trials in 2016 (Hansen et al. 2017) and 2017 and 2018 (data presented herein), by diameter class. These data are from the central, treated tree and not any of the surrounding trees within 10 m. Data are not included from Alaska and Rocky Mountain National Park nor the two other 2018 repellent treatments (MCH-AKB plus sulcatone and double-dose MCH-AKB). The diameter classes depicted do not reflect the diameter classes used in field trials (see Methods); the breakpoints used here are intended to aid interpretation.

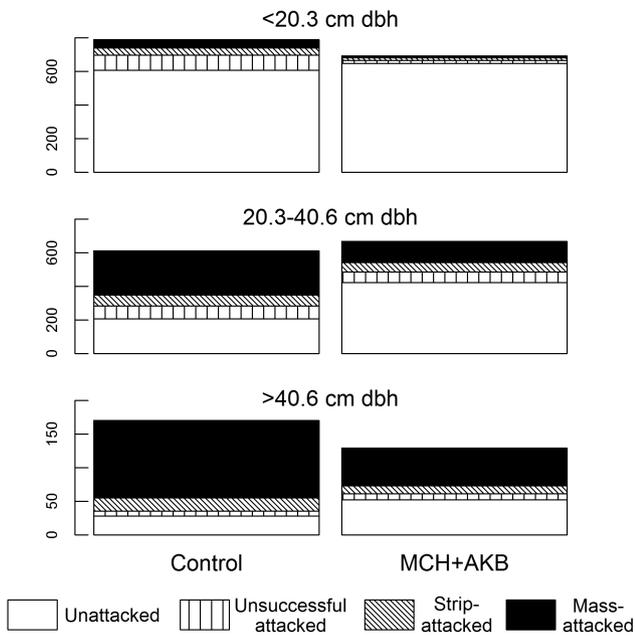


Fig. 3. Numbers of spruce in each spruce beetle attack class for area protection trials in 2017, by diameter class. These data are combined from three replicates in Utah and one in Colorado. The diameter breakpoints are arbitrary and intended to aid visualization of the effect of diameter.

be explained by differences in dose and release rates. Our testing also discovered novel spruce beetle repellents, the most promising being AKB. Although we did not directly compare MCH alone to MCH+AKB, 2017 results suggest the latter would capture significantly fewer spruce beetles (Table 3). Also, individual tree protection trials had intermediate results with MCH or AKB alone but revealed maximal protection using AKB as an MCH adjuvant (Hansen et al. 2017). Isophorone plus sulcatone also significantly reduced beetle captures but at rates less likely to prove useful as a tree protectant. Adding isophorone plus sulcatone or leaf alcohol to MCH did not significantly reduce captures relative to MCH alone. Verbenene, a structural analog of verbenone, was found to be *attractive* to spruce beetles in British Columbia (Gries et al. 1992). We tested it in Utah as a potential aggregant to enhance baits and lures but beetle captures were not significantly different compared with the lure-only reference. Finally, testing each of the three AKB components alone found little, if any, repellency but adding the full blend to MCH offers maximal repellency of any combination we tested, a result confirmed in individual tree protection trials (Hansen et al. 2017).

In individual tree protection trials in the Rocky Mountains, MCH+AKB-treated trees were significantly less likely to be in a higher severity attack category than control trees. This confirms the results of Hansen et al. (2017) albeit that earlier trial, with a smaller sample size, had no attacks whatsoever on MCH+AKB-treated trees. Combining the Hansen et al. results with the Rocky Mountains data herein, 70/106 control trees were strip- or mass-attacked compared with 6/103 MCH+AKB-treated trees (Fig. 2). We consider this an excellent result with efficacy nearly as good as topical insecticides such as carbaryl (Fettig et al. 2013) but without the environmental and human health risks. Additionally, semiochemical applications are typically a fraction of the cost compared with topical pesticides. For example, carbaryl treatment of a mature spruce can cost US\$25–300 per tree depending on size, location, and number of trees, among other factors. In comparison, the MCH+AKB bulk purchase price is expected ~\$5 per pair. Assuming a technician at \$20 per hour

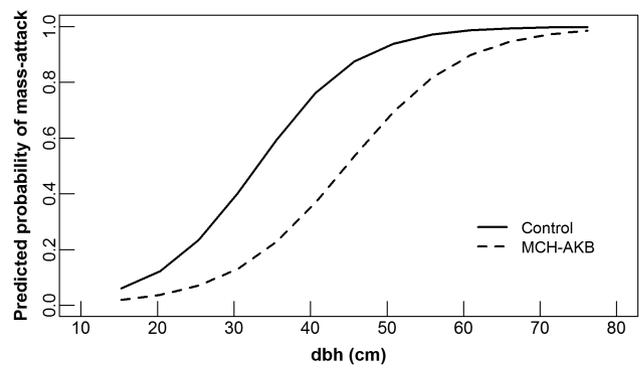


Fig. 4. Modeled probability of mass-attack by dbh for area protection trials, 2017. Recently infested stem count (2015 and 2016 infestation) was a significant covariate, and its mean value (6.9) was held constant to produce this figure.

labor for four hours, \$50 mileage costs, and treatment of 40 spruce, MCH+AKB application would cost ~\$8.25 per tree. A second trip to remove the release devices would increase the cost to ~\$11.50 per tree (although note that carbaryl typically provides 2 yr of protection, whereas MCH+AKB should be assumed efficacious for a single year; as with carbaryl application, actual cost of MCH+AKB deployment will widely vary due to multiple factors). This result is even more compelling considering that proper insecticide treatment of large diameter spruce (e.g., >60 cm dbh) requires a lift or bucket truck to spray high enough to prevent top-kill.

Surprisingly, there was no significant effect of tree diameter on 2017 or 2018 results. This is contrary to the results of Hansen et al. (2017) wherein increasing diameter was positively correlated with infestation probability. Those results, however, included MCH- and AKB-alone, whereas MCH+AKB-treated spruce were unattacked regardless of diameter. Adding sulcatone or doubling MCH+AKB pouches had no significant effect compared with single pouches of MCH+AKB. In Alaska, however, none of the repellent treatments had significantly different attack rates compared with the control treatment, and most treated trees were mass-attacked regardless of treatment (8/9 control trees and 26/36 repellent trees with another four strip-attacked).

Conceivably, our decision to apply baits on nearby posts or forego them altogether during 2018 testing hampered our ability to detect differences among MCH+AKB, MCH+AKB plus sulcatone, and double-dose MCH+AKB. This decision contributed to fewer attacks on control trees relative to 2017 trials wherein baits were attached directly to experimental trees, and may have masked differences among the repellent treatments. Still, we think this approach yielded results more representative of applications wherein baits would never be used alongside semiochemical repellents. Multiple previous studies used rules developed by Shea et al. (1984) to determine efficacy of pesticides and semiochemicals for protection against bark beetles. Shea et al. used a binomial response (survived or killed) and assumed treatment success only if results met thresholds of mortality among controls ($\geq 60\%$) and survival among treated trees ($\geq 90\%$). On one hand, treatment efficacy cannot be demonstrated without losses among the control group. But on the other hand, differences among treatments is more easily demonstrated with modern statistical models, and relatively low rates of attacks on control trees can be countered by larger sample sizes. In 2018, we installed 72 total replicates of each treatment spread among six geographically distinct areas, enhancing the statistical power to detect differences among treatments even in the event that a minority of control trees were attacked. Our multinomial

approach with a larger sample size offers future researchers an alternative for analyzing tree protection data.

Analyzing beetle attacks on spruce up to 10 m distant from the individual tree treatments revealed a halo effect for the bait-only and bait plus repellent-treated trees (Fig. 1). This was most pronounced near control trees where increasing distance from the baited tree resulted in diminishing probability of a higher severity attack, consistent with observations that spillover is concentrated within 10 m of a bait (Hansen et al. 2006a, Klutsch et al. 2017). An opposite but weaker trend was found for repellent-treated trees. While MCH-AKB offers a degree of protection to nearby spruce, we are unable to infer a threshold distance beyond which the probability of infestation is indistinguishable from background levels. It is worth noting, however, that the modeled probability of mass-attack for a spruce 10 m distant from a tree treated with bait plus MCH-AKB was about one-half that of a similar tree 10 m distant from a bait only tree (Fig. 1, 2017 panel). Still, these results should consider the influence of the tree baits which might have established secondary attraction surrounding bait-only trees as well as confounded results for the repellent-treated trees. Interestingly, spruce around the repellent-treated tree at Alaska plots *did* benefit from the nearby repellents. Hypothetically, the presence of the bait overwhelmed repellent efficacy for the treated tree yet the repellents offered a measure of protection to spruce away from the bait. Additionally, the Alaskan site had more treatments and fewer replicates than other sites, potentially reducing the power to detect a treatment difference. Regardless, our Alaskan testing joins several other MCH experiments from that region with inconclusive results (Werner and Holsten 1995, but see Holsten et al. 2003).

Although the nearest neighboring test tree averaged 25–58 m, some experimental units were as close as 20 m in the individual tree protection trials. This spacing was likely insufficient to ensure zero inter-plot influence of beetle activity. For example, Douglas-fir beetles have been captured in baited traps from as far away as 400 m (Dodds and Ross 2002). But experimental units spaced at, say, >500 m would introduce other confounding factors such as differences in stand conditions and beetle population levels. MCH-AKB might also influence spruce beetle activity in excess of 10 m, enough to result in inter-plot influence. But we think these influences are small, affecting beetle flight activity more so than tree mortality. These confounding factors are likely overwhelmed when trees baits are deployed, i.e., baited trees are likely to attract sufficient beetle pressure to result in successful attack regardless of the influence of other nearby baits or repellents. This speculation is supported by the lack of spatial dependence among model residuals. To the degree that this conclusion may be wrong, our test results are conservative rather than incorrect. That is, if the MCH-AKB halo protected control trees >20 m away, then the reduced attack rate on controls masked the true efficacy of MCH-AKB. Similarly, a possible confounding effect of baited control trees “too close” to repellent-treated trees would be to expose the latter to even greater beetle pressure.

For area protection, MCH-AKB deployed at 30 sets per hectare resulted in significantly fewer spruce beetle attacks compared with controls (Fig. 3). Anecdotally, a disproportionate amount of mortality in repellent-treated plots occurred near the baited traps suggesting better performance in applications that would preclude baits (albeit unbaited controls would also have fewer attacks). Successful beetle attacks in repellent-treated plots were about one-half that in control plots. Note that this result was under extreme beetle population pressure (among control plots, 50–80% of total spruce basal area was killed the year of the trials). The probability of attack was also influenced by tree diameter and the interaction between

diameter and treatment (Fig. 4). The largest diameter spruce were likely to have successful attacks regardless of treatment (i.e., >50 cm dbh). Our area repellent deployments focused on spacing over host diameter but our combined individual tree and area protection results suggest that larger trees should receive MCH-AKB regardless of spacing if the intent is to protect those size classes. That is, the individual tree protection trials found good efficacy of MCH-AKB regardless of host diameter. One possible operational approach for area protection is to hybridize our individual tree and area protection methods by applying repellents to all hosts >50 cm dbh and with no spacing gap >18 m.

Our results are generally applicable to spruce type in the southern Rocky Mountains (Table 2). MCH-AKB in pouch release devices did not work for individual tree protection in Alaska although results for the surrounding 10 m suggest the possibility of positive results in the absence of baits (also see Holsten et al. 2003). Additional testing is needed to determine efficacy elsewhere such as western Canada. MCH is commercially available and is EPA-registered for use against spruce beetles (EPA Registration No. 27586-5). Although linalool is EPA-registered it is not yet labeled for use against bark beetles, and the other AKB components may require registration before they can be sold commercially. For area protection, Hansen et al. (2017) found high-dose, high-release MCH alone to reduce the probability of a higher severity spruce beetle attack by 1.9–2.2 times. This is nearly as good as our MCH-AKB result of 2.4 times reduction in probability of a higher severity attack. Enhanced efficacy could result from tighter grid spacing with emphasis on applying repellents to the largest spruce. This strategy requires further testing to confirm improved efficacy. For individual tree protection, MCH-AKB is clearly superior to MCH alone (Hansen et al. 2017; results herein). If AKB becomes commercially available and labeled for bark beetle applications, MCH-AKB should be an effective, economical, and environmentally benign tool for protecting Engelmann spruce from spruce beetle attacks. We envision its deployment in areas with high-value spruce such as in campgrounds, around cabins/homes, and ski areas.

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