Forest Entomology

Journal of Economic Entomology, XX(X), 2017, 1–9
doi: 10.1093/jee/tox208
Research Article

# 3-Methylcyclohex-2-en-1-one for area and individual tree protection against spruce beetle (Coleoptera: Curculionidae: Scolytinae) attack in the southern Rocky Mountains

**E. Matthew Hansen,[1,5] A. Steven Munson,[2] Darren C. Blackford,[2] Andrew D. Graves,[3] Tom W. Coleman,[3] and L. Scott Baggett[4]**

[1]US-Forest Service, Rocky Mountain Research Station, 860 North 1200 East, Logan, UT 84321, [2]US-Forest Service, Forest Health Protection, 4746 South 1900 East, Ogden, UT 84403, [3]US-Forest Service, Forest Health Protection, 333 Broadway Boulevard SE, Albuquerque, NM 87102, [4]US-Forest Service, Rocky Mountain Research Station, 240 West Prospect Street, Fort Collins, CO 80526, and [5]Corresponding author, e-mail: matthansen@fs.fed.us

Subject Editor: John Trumble

Received 14 April 2017; Editorial decision 4 July 2017

## Abstract

We tested 3-methylcyclohex-2-en-1-one (MCH) and an *Acer* kairomone blend (AKB) as repellent semiochemicals for area and single tree protection to prevent spruce beetle (*Dendroctonus rufipennis* Kirby) attacks at locations in Utah and New Mexico. In the area protection study, we compared host infestation rates of MCH applications at three densities (20, 40, and 80 g MCH ha$^{-1}$) against a control treatment over 0.64 ha plots centered within ~1.25 ha treatment blocks. All treatments included two baited funnel traps within the plot to assure spruce beetle pressure. Following beetle attack, plots were surveyed for new spruce beetle attacks and to quantify stand characteristics. The probability of more severe spruce beetle attacks was significantly reduced, by ~50%, in each of the MCH area treatments compared with the control treatment but there was no significant treatment difference among the MCH deployment densities. For the single tree protection study, we compared attack rates of MCH, *Acer* kairomone blend (AKB), and MCH plus AKB on spruce beetle-baited trees against bait-only trees. Each treatment was applied over a range of host diameters to test for host size effects. Seventy-five percent of control trees were mass-attacked, about one-third of MCH- and AKB-alone spruce was mass-attacked, and no MCH plus AKB spruce were mass-attacked. These results suggest that MCH alone is a marginal area and single tree protectant against spruce beetle but that deployment with other repellents can significantly increase treatment efficacy.

**Key words:** pheromone, bark beetle management, *Dendroctonus rufipennis*, semiochemicals, Engelmann spruce

The spruce beetle, *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae, Scolytinae), is the most important disturbance agent of North American spruce species. For example, an ongoing outbreak in Colorado has infested about 0.7 million hectares of Engelmann spruce, *Picea engelmannii* Parry ex Engelm (Colorado State Forest Service 2016). The life cycle ranges from 1 to 3 yr depending on local temperatures, with most attacks on new hosts occurring during early summer (Holsten et al. 1999). Spruce beetle prefers downed host trees at endemic population levels. Outbreaks affecting live standing trees often result following population growth in widespread windthrow, exacerbated by factors such as high stand susceptibility and favorable weather, the latter decreasing life cycle duration and winter-related beetle mortality (Reynolds and Holsten 1994). Larger diameter spruce (e.g., > 40 cm diameter at breast height [dbh]) are preferentially attacked during outbreaks with females laying upwards of 100 eggs in vertical galleries within host phloem. The larvae feed horizontally, consuming the host's phloem. Occasionally, only one bole face is colonized, creating a "strip-attack" wherein the tree will remain alive unless mass attacks occur in a subsequent year (Holsten et al. 1999). As with other *Dendroctonus* species, chemoreception is critical for identifying suitable hosts, finding mates, and attracting conspecifics to overwhelm host defenses via mass attack (Jenkins et al. 2014).

Management options include indirect control through vegetation management (i.e., tree thinning to increase tree vigor and size-age class diversity) and direct suppression strategies such as preventive insecticide treatments, removal or burning of infested trees, trapping using funnel traps or baited host trees, and semiochemical repellents

Published by Oxford University Press on behalf of Entomological Society of America 2017.
This work is written by (a) US Government employee(s) and is in the public domain in the US.



Downloaded from https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox208/4107613/3-Methylcyclohex-2-en-1-one-for-area-and
by DigiTop USDA's Digital Desktop Library user
on 08 September 2017

(Bentz and Munson 2000, Fettig et al. 2007, Hansen et al. 2010, Munson 2010, Windmuller-Campione and Long 2015, Fettig and Hilszczański 2015). Semiochemical strategies exploit spruce beetle's olfactory communications by attracting beetles to traps or repelling them from potential hosts. The rationale for choosing among these strategies will depend on factors such as budgetary constraints, resource value, location and size of outbreak, management goals, and environmental concerns.

Semiochemical repellents can be used to reduce or arrest bark beetle attraction to host trees and can be an effective management tool (Samman and Logan 2000). Moreover, this strategy may be the only option for areas with logging or pesticide application restrictions. Additionally, passive semiochemical release devices are highly portable, enabling deployment in areas lacking road access, have no known non-target effects compared with insecticides, and are often less expensive than alternative control strategies.

3-Methylcyclohex-2-en-1-one (MCH) is a semiochemical repellent found in the spruce beetle (Rudinsky et al. 1974) and previous studies have shown that MCH reduced spruce beetle attraction to female-infested logs (Kline et al. 1974; Furniss et al. 1976), traps baited with synthetic semiochemical lures (Furniss et al. 1976; Lindgren et al. 1989), stumps, windthrow, and felled boles (Rudinsky et al. 1974, Furniss et al. 1976, Dyer and Hall 1977, Lindgren et al. 1989). MCH is frequently deployed to reduce Douglas-fir beetle (*D. pseudotsugae* Hopkins) attacks on Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), completely arresting infestations in some trials despite the presence of baited traps (Ross and Wallin 2008, Brookes et al. 2016). Results have been mixed, however, in trials using MCH to protect live, standing spruce from spruce beetle attack. Testing proved unsuccessful using passive release devices (2–10 mg MCH $day^{-1}$ at 22–25°C) for single tree and area protection in Alaska (Werner and Holsten 1995) and area protection in Utah (Ross et al. 2004).

The first successful study used microinfusion pumps as the release device (2.6–5.0 mg MCH $day^{-1}$ regardless of temperature) resulting in an 80–87% reduction in area-wide host infestation rates compared with control plots in Alaska (Holsten et al. 2003). A recent test of MCH to reduce spruce beetle attacks used a high-release passive device (1,000 mg bubble, eluting 12 mg MCH $day^{-1}$ at 25°C, Synergy Semiochemicals Corp., Burnaby, BC, Canada), combined with the semiochemicals isophorone (1,800 mg bubble, eluting 6.5 mg $day^{-1}$ at 25°C) and sulcatone (1,700 mg bubble, eluting 35 mg $day^{-1}$ at 25°C) (Hansen et al. 2016). Although the study was focused on the effects of pesticide treatment to suppress the spruce beetle population and attacks on live hosts, results suggested that the repellents might be effective as an area protection treatment. Engelmann spruce in plots that included these repellents had a significantly reduced probability of beetle attack compared with similarly treated plots without repellents (Hansen et al. 2016).

Our objective was to determine the efficacy of MCH and another semiochemical repellent as area and individual tree treatments to reduce attacks by spruce beetles. Field studies were conducted at three southern Rocky Mountain locations with a range of spruce beetle population pressure. We used the same high-release MCH passive device as Hansen et al. (2016) to test for protection of individual trees and areas. MCH was deployed at three densities as area protection treatments. We also deployed an *Acer* (maple genus) kairomone blend (AKB) on individual trees, alone and paired with MCH. AKB was previously tested in a Utah trapping bioassay study and was found to reduce spruce beetle captures by ~80% compared with a reference treatment (E. M. Hansen, A. S. Munson, and D. Wakarchuk unpublished data).

## Materials and Methods

### Study area descriptions

Study sites were identified on the Duchesne/Roosevelt Ranger District, Ashley National Forest, Utah ("Mill Park"), Beaver Ranger District, Fishlake National Forest, Utah ("Big Flat"), and Tres Piedras Ranger District, Carson National Forest, New Mexico ("Hopewell Lake") (Fig. 1). Our search criteria included sites with active spruce beetle populations (assessed using aerial detection survey (ADS) maps and confirmed with ground reconnaissance) and susceptible Engelmann spruce stands that included live, large diameter trees (≥40 cm dbh) (Table 1). The Mill Park area had epidemic beetle populations (two clumps of five or more infested trees every 2 ha; Bentz and Munson 2000, Hansen et al. 2006) and the Big Flat and Hopewell Lake areas had building populations (>2 infested trees for every 2 ha). ADS conducted during 2015 by the US Forest Service, Forest Health Protection staff estimated that there were 5,400 fading spruce (with yellow-green needles) within 5 km of the center of the Mill Park area compared with 200 fading spruce within 5 km of the center of the Big Flat area (ADS records spruce needle fade the year following infestation). ADS did not detect spruce beetle-caused mortality within 5 km of Hopewell Lake, although there was a mortality polygon about 5 km from the northern-most plots. Spruce trees near Hopewell Lake were heavily defoliated by western spruce budworm, *Choristoneura occidentalis* Freeman, confounding identification of beetle-killed trees from the air. Stands at Mill Park were partially cut ca. 1960 contributing to relatively smaller average diameter trees and lower basal area (BA) (Table 1).

### Area protection

For area protection trials, we deployed MCH (1,000 mg bubble, eluting 12 mg $day^{-1}$ at 25 °C; Synergy Semiochemicals Corp) at 20 (22.4 m spacing), 40 (15.8 m spacing), and 80 (11.2 m spacing) g $ha^{-1}$. This release device has at least twice the MCH load and release rate compared to devices that have been successfully used against Douglas-fir beetle for many years (400–500 mg bubbles, eluting at 3.8–5 mg $d^{-1}$ at 20–25°C; Ross and Wallin 2008, Brookes et al. 2016). MCH was stapled to north aspects of boles about 2 m above ground level (Fig. 2). To attain a uniform buffer distance from the treatment edge to the surveyed plot (0.64 ha) edge among all spacings, treatment blocks were ~1.25 ha (Fig. 2). Plots were spaced >30 m apart and treatments randomly assigned with replicates blocked within stands where possible (Fig. 1). The minimum spacing was closer than we preferred but was necessitated by the lack of accessible stands meeting our criteria combined with the need for homogenous conditions within each replicate. We recognize that wind transport of semiochemicals may have influenced nearby plots at this spacing. This downside was ameliorated by the buffer between the treatment and measure block edges (Fig. 2) which resulted in ~60 m minimum spacing between measure blocks. For reference, MCH in Douglas-fir beetle systems has an effective repellent range up to ~30 m depending on the release rate at a single point. For example, seven co-located MCH devices (500 mg releaser, each eluting 5 mg $d^{-1}$ at 25°C for a total of 35 mg $d^{-1}$) spaced on a 31 m grid was as effective at inhibiting Douglas-fir beetle attacks as single devices (5 mg $d^{-1}$) spaced on a 12 m grid (Brookes et al. 2016). In another study, Ross et al. (2002) found that three 2 mg $d^{-1}$ MCH releasers spaced at 15 m significantly reduced Douglas-fir beetle infestation rates compared

Downloaded from https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox208/4107613/3-Methylcyclohex-2-en-1-one-for-area-and
by DigiTop USDA's Digital Desktop Library user
on 08 September 2017

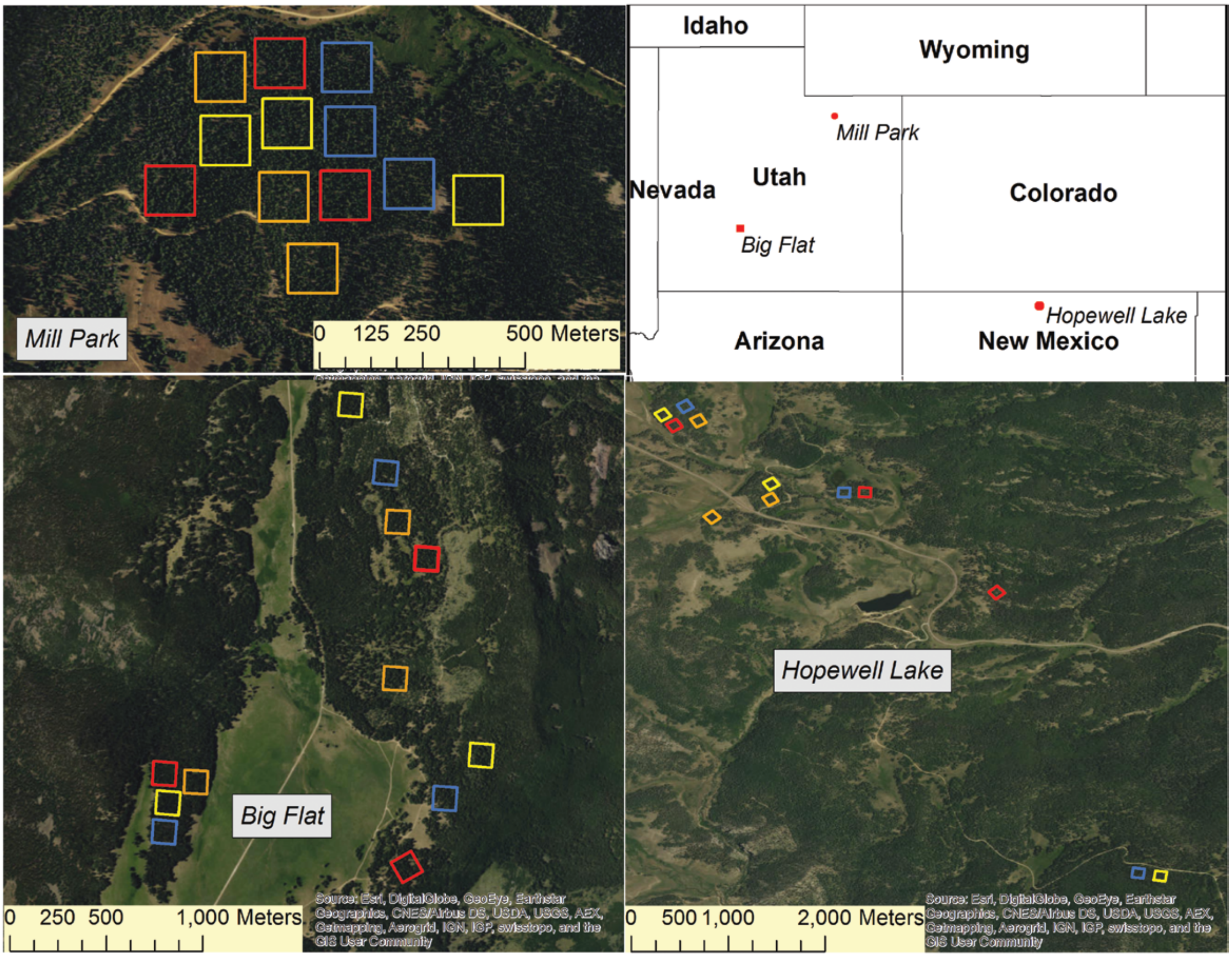


**Fig. 1.** Plot locations for area treatments installed at "Big Flat" (Beaver Ranger District, Fishlake National Forest, Utah), "Mill Park" (Duchesne/Roosevelt Ranger District, Ashley National Forest, Utah), and "Hopewell Lake" (Tres Piedras Ranger District, Carson National Forest, New Mexico). Treated areas were ~1.25 ha squares with a 0.64 ha measure block centered within. Control plots are outlined in blue, MCH-treated plots deployed at 20 g ha$^{-1}$ (22.4 m spacing) are outlined in yellow, MCH-treated plots deployed at 40 g ha$^{-1}$ (15.8 m spacing) are outlined in orange, and MCH-treated plots deployed at 80 g ha$^{-1}$ (11.2 m spacing) are outlined in red. Trees used in the individual tree protection trials were in the vicinity of each of the three locations.

to control plots but nine releasers (18 mg MCH d$^{-1}$) spaced at 44 m incurred infestations rates indistinguishable from those in controls. Therefore, the data from Douglas-fir beetle systems suggest that beetle-caused tree mortality was minimally modified in our study, if at all, especially given that recently infested BA was generally similar within treatments at each of the three study locations (i.e., treatments had similar within-plot beetle pressure; Table 1).

To ensure spruce beetle pressure within the plots, funnel traps baited with frontalin, 1-methyl-2-cyclohexen-l-ol, and a host terpene blend (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. Trap captures were collected every 2 wk during peak beetle flight. Hopewell Lake plots were installed late May, 2016 and Big Flat and Mill Park plots were installed mid-June, 2016. At Mill Park, we observed fresh attacks on a few trees in the area, indicating that local beetle flight had already begun at the time of plot establishment.

Plot measurements occurred within 80 × 80 m squares (0.64 ha) centered within the treatment blocks (Fig. 2) (Gillette et al. 2012). In September, 2016, after beetle flight, we conducted ground surveys of the measured blocks to quantify post-treatment spruce beetle attacks. We surveyed all live trees >10 cm dbh and all spruce estimated to have been infested within the previous 10 yr (trees attacked before 2016 were a surrogate for local beetle population size, a covariate in analyses). Data collected included species, dbh (measured with Biltmore sticks), 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. (2006):

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

## Individual tree protection

For individual tree protection trials, we selected live and unattacked Engelmann spruce >50 m from stands used for the area protection

Downloaded from https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox208/4107613/3-Methylcyclohex-2-en-1-one-for-area-and
by DigiTop USDA's Digital Desktop Library user
on 08 September 2017

**Table 1.** Pre-treatment stand characteristics, by area, for each treatment within 80 × 80 m square plots (0.64 ha)

| | Live BA ($m^2$, SD)[a] | ES BA ($m^2$, SD)[b] | ES dbh (cm, SD)[c] | Percent ES (%, SD)[d] | Recently infested BA ($m^2$, SD)[e] |
|---|---|---|---|---|---|
| *Big Flat* | | | | | |
| Control | 33.3 (3.0)a | 30.2 (3.4)a | 33.1 (1.3)a | 74.9 (5.3)a | 0.22 (0.22)a |
| 20 g MCH $ha^{-1}$ | 35.2 (3.0)a | 31.9 (3.4)a | 34.8 (1.3)a | 73.5 (5.3)a | 0.29 (0.22)a |
| 40 g MCH $ha^{-1}$ | 39.5 (3.0)a | 34.4 (3.4)a | 34.3 (1.3)a | 69.5 (5.3)a | 0.13 (0.22)a |
| 80 g MCH $ha^{-1}$ | 30.0 (3.0)a | 26.9 (3.4)a | 33.6 (1.3)a | 76.0 (5.3)a | 0.36 (0.22)a |
| *Hopewell Lake* | | | | | |
| Control | 18.0 (1.6)a | 11.9 (1.6)a | 28.2 (2.2)a | 48.0 (12.1)a | 0.27 (0.15)a |
| 20 g MCH $ha^{-1}$ | 22.2 (1.6)a | 15.1 (1.6)a | 30.8 (2.2)a | 53.9 (12.1)a | 0.27 (0.15)a |
| 40 g MCH $ha^{-1}$ | 21.3 (1.6)a | 16.2 (1.6)a | 29.9 (2.2)a | 57.0 (12.1)a | 0.39 (0.15)a |
| 80 g MCH $ha^{-1}$ | 18.1 (1.6)a | 13.1 (1.6)a | 28.2 (2.2)a | 67.5 (12.1)a | 0.27 (0.15)a |
| *Mill Park* | | | | | |
| Control | 17.7 (1.2)a | 15.7 (1.1)a | 23.7 (1.5)a | 80.1 (4.5)a | 0.15 (0.19)b |
| 20 g MCH $ha^{-1}$ | 14.3 (1.2)a | 12.5 (1.1)a | 22.0 (1.5)a | 81.5 (4.5)a | 0.24 (0.19)b |
| 40 g MCH $ha^{-1}$ | 17.1 (1.2)a | 14.6 (1.1)a | 20.2 (1.5)a | 85.1 (4.5)a | 0.78 (0.19)a |
| 80 g MCH $ha^{-1}$ | 17.9 (1.2)a | 16.2 (1.1)a | 21.7 (1.5)a | 90.4 (4.5)a | 0.31 (0.19)ab |

For each area, within the same column means followed by the same letter are not significantly different at $P > 0.05$ using tests of pairwise differences (Tukey–Kramer).

[a]Total live BA for all species. Big Flat species were Engelmann spruce (ES), subalpine fir, and quaking aspen. Mill Park species were Engelmann spruce, subalpine fir, and lodgepole pine. Hopewell Lake species were Engelmann spruce, corkbark fir, white fir, Douglas-fir, and quaking aspen. Spruce that were beetle-infested during 2016 was recoded as live to estimate pre-test conditions.

[b]Engelmann spruce BA.

[c]Average dbh of Engelmann spruce ≥25 cm.

[d]Percent of all live stems that were Engelmann spruce. Percent by BA can be derived by dividing ES BA by Live BA.

[e]Spruce BA infested during 2014 and 2015.

trials. Selected trees were spaced at >20 m intervals. All trees were baited with frontalin (Synergy Semiochemicals Corp. ) to ensure treatments were challenge by spruce beetle. Treatments were: (1) control (bait only), (2) MCH (single 1,000 mg bubble), (3) AKB (~6.8 g active ingredient), and (4) MCH plus AKB. AKB consists of three active ingredients, linalool (47.5% of the active ingredient, by weight), β-caryophyllene (42% of the active ingredient), and leaf alcohols, Z3-hexenol (10.5% of the active ingredient). Elution rates are unknown for each compound in the AKB pouch. Repellents and baits were stapled to north aspects of boles about 2 m above the ground. Each treatment was replicated 10 times at each of the three areas, although extra sets of baits and repellents allowed us to include four additional trees, one control and one MCH plus AKB at Mill Park and Big Flat.

To test tree diameter as a covariate, we selected a range of trees with diameters starting at 30.5 cm. At Mill Park and Hopewell Lake, we grouped trees in diameter classes every 7.6 cm up to 68.6 cm dbh and randomly applied one of the four treatments to two trees in each diameter class per treatment. Tree diameters were generally larger at Big Flat where we used diameter classes every 10.2 cm up to 81.4 cm dbh. The additional trees at Mill Park and Big Flat were of larger dbh classes, up to 90.2 cm at Big Flat. Individual tree treatments were examined for attack status in September, 2016.

## Analyses

The response variable "status" (2016 attacks only) describes attack severity and is ordinal, meaning that the categorical responses can be ranked but with unknown distances between classes (0 = unattacked; 1= unsuccessful-attacked or "pitchout"; 2 = strip-attacked; 3 = mass-attacked). We analyzed the area data with an ordinal logistic regression model (Hosmer et al. 2013). Each spruce tree was considered an experimental unit and the model results give the probability or likelihood of attack severity as a function of the treatment and any covariates. 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). Tested covariates included the dbh of each individual spruce and plot-level measures such as (1) stem counts by infestation year for previously infested spruce, (2) average dbh of spruce >25 cm (Schmid and Frye 1976), (3) spruce BA, (4) total BA, and (5) the percent of spruce component. To calculate stem counts of previously infested spruce, each tree mass-attacked before 2016 was counted as 1.0 killed stem, and each strip-attacked tree was counted as 0.5 killed stem. Pairwise comparisons were made using the Tukey–Kramer adjustment for $P$-values and confidence limits (Kramer 1956).

We attempted a similar analysis for the individual tree data but one treatment had all observations in a single status class and the complete separation prevented estimation of model parameters. Instead, we combined status classes resulting in two classes: survived (unattacked, unsuccessfully attacked, and strip attacked) and mass-attacked. This enabled use of a binomial response model (Allison 2008) and application of rules developed by Shea et al. (1984). Shea et al. used a similar binomial response (i.e., killed versus survived) and assumed that at least 60% of control trees needed to be killed to demonstrate "sufficient" beetle pressure. Further, they established a null hypothesis test that tree survival was ≥90% in the tree protection treatment, a standard met with no more than 20% mortality. We used a generalized linear mixed model to accommodate "area" as a random effect (PROC GLIMMIX, SAS Institute, Inc.). Because quasi-separation could still

Downloaded from https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox208/4107613/3-Methylcyclohex-2-en-1-one-for-area-and
by DigiTop USDA's Digital Desktop Library user
on 08 September 2017

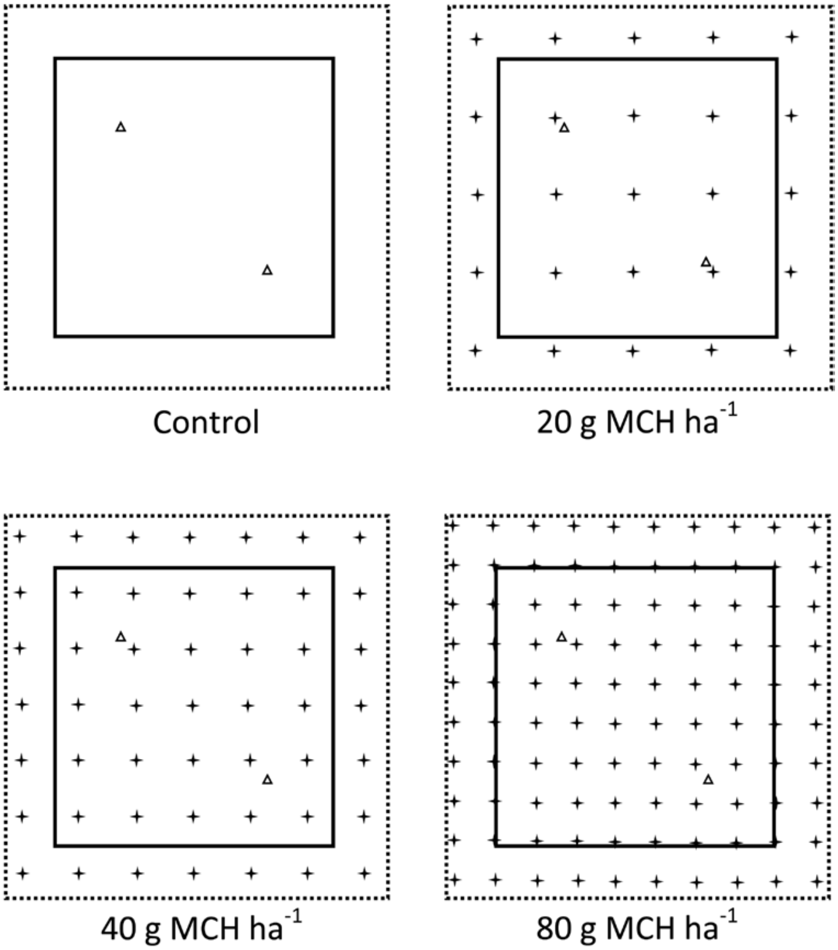


**Fig. 2.** Diagrams of area treatments using MCH to reduce spruce beetle attacks on live spruce. Dotted lines represent the treatment block ~1.25 ha (~110 × ~110 m), the solid lines represent the 100% survey plot 0.64 ha (80 × 80 m), the triangles represent baited funnel traps, and the stars represent 1,000 mg MCH bubbles. MCH spacing was 22.4, 15.8, and 11.2 m for the 20, 40, and 80 g MCH ha$^{-1}$ treatments, respectively.

be an issue, we confirmed conclusions using Firth's penalized likelihood method (Firth 1993) (PROC LOGISTIC, SAS Institute, Inc.). Results were identical with both model types.

## Results

For the area protection tests, pre-treatment stand conditions were mostly uniform among the treatments at each of the three locations, the exception being recently infested spruce BA at Mill Park (Table 1). The raw data (Table 2) suggested a significant treatment effect, confirmed by statistical analyses. Spruce in the control plots were significantly more likely, by a factor of 1.9–2.2, to be classified as more severely attacked (e.g., mass-attacked rather than strip-attacked, strip-attacked rather than unsuccessfully attacked, or unsuccessfully attacked rather than unattacked) than spruce in any of the MCH-treated treatments ($F_{3,\,10,024} = 11.62$, $P < 0.0001$) (Table 3). Spruce in the three densities of MCH deployment did not significantly differ from each other with respect to likelihood of a more severe attack. Significant covariates were the dbh of each spruce ($F_{1,\,10,024} = 128.04$, $P < 0.0001$; positive correlation) and plot-level average spruce dbh ($F_{1,\,252.5} = 15.45$, $P = 0.0001$; negative correlation), spruce density ($F_{1,\,1743} = 7.49$, $P = 0.0063$; negative correlation), and counts of previously infested spruce, all years combined ($F_{1,\,10,024} = 4.93$, $P = 0.0265$; negative correlation).

Area (i.e., Mill Park, Big Flat, and Hopewell Lake) was a significant covariate when included as a main rather than random effect ($F_{2,\,9.8} = 9.44$, $P = 0.0052$). The probabilities of more severe infestations were significantly higher at Mill Park and Hopewell Lake compared to Big Flat, whereas the probabilities of more severe infestations did not significantly differ among spruce at Mill Park and Hopewell Lake. Results for the 80 g MCH ha$^{-1}$ treatment at Big Flat were potentially biased after a trap at one replicate was knocked to the ground during the peak flight period.

For the individual tree tests, control trees were significantly more likely to be mass-attacked than trees treated with either MCH or AKB ($F_{3,\,119} = 18.04$, $P < 0.0001$) but the latter treatments did not significantly differ (Fig. 3). Trees treated with either MCH or AKB alone were significantly more likely to be mass-attacked than trees treated with MCH plus AKB. Applying the rules of Shea et al. (1984), 75% of control trees were mass-attacked, indicating a rigorous challenge from spruce beetles. Although MCH- or AKB-alone-treated trees incurred fewer mass attacks than control trees, mortality was too high (33–37%) for these treatments to be considered effective using Shea et al.'s rules (threshold of 20%). The MCH plus AKB treatment, however, incurred no mass-attacks and can be considered effective using the rules of Shea et al. (1984). In fact, no MCH plus AKB-treated tree was even unsuccessfully attacked (pitch-out). Tree

Downloaded from https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox208/4107613/3-Methylcyclohex-2-en-1-one-for-area-and
by DigiTop USDA's Digital Desktop Library user
on 08 September 2017

**Table 2.** Frequency tables of spruce beetle attack severity among treatments, by area, in area protection tests

| Severity | Attack severity | | | | Total |
|---|---|---|---|---|---|
| | Unattacked | Unsuccessful | Strip | Mass | |
| *Big Flat, Utah* | | | | | |
| 20 g MCH ha$^{-1}$ | 545 | 0 | 0 | 1 | 546 |
| 40 g MCH ha$^{-1}$ | 605 | 2 | 0 | 1 | 608 |
| 80 g MCH ha$^{-1}$ | 528 | 3 | 0 | 0 | 531 |
| Control | 557 | 3 | 1 | 5 | 566 |
| *Mill Park, Utah* | | | | | |
| 20 g MCH ha$^{-1}$ | 370 | 7 | 10 | 26 | 413 |
| 40 g MCH ha$^{-1}$ | 435 | 6 | 9 | 8 | 458 |
| 80 g MCH ha$^{-1}$ | 481 | 5 | 8 | 19 | 513 |
| Control | 371 | 12 | 28 | 57 | 468 |
| *Hopewell Lake, New Mexico* | | | | | |
| 20 g MCH ha$^{-1}$ | 363 | 0 | 18 | 11 | 392 |
| 40 g MCH ha$^{-1}$ | 430 | 5 | 13 | 12 | 460 |
| 80 g MCH ha$^{-1}$ | 326 | 8 | 13 | 9 | 356 |
| Control | 329 | 4 | 13 | 27 | 373 |

These data are summations of three replicates per treatment at each area in 80 × 80 m measurement plots. Data for Engelmann spruce ≥ 20.3 cm dbh shown.

**Table 3.** Odds ratios from an ordinal logistic regression model comparing the likelihood of an Engelmann spruce stem being classified with a higher severity spruce beetle attack rating (0 = unattacked; 1 = unsuccessful-attacked or "pitchout"; 2 = strip-attacked; 3 = mass-attacked) among treatment pairs within 80 × 80 m measure plots (0.64 ha)

| Treatment pair | Odds ratio | Lower | Upper | Adj. *P* |
|---|---|---|---|---|
| Control > MCH at 20 g ha$^{-1}$ | 2.2 | 1.4 | 3.3 | <0.0001 |
| Control > MCH at 40 g ha$^{-1}$ | 2.2 | 1.3 | 3.6 | 0.0002 |
| Control > MCH at 80 g ha$^{-1}$ | 1.9 | 1.2 | 3.1 | 0.0015 |
| MCH at 20 g ha$^{-1}$ > MCH at 40 g ha$^{-1}$ | 1.0 | 0.6 | 1.5 | 1.0 |
| MCH at 80 g ha$^{-1}$ > MCH at 20 g ha$^{-1}$ | 1.1 | 0.7 | 1.8 | 0.9839 |
| MCH at 80 g ha$^{-1}$ > MCH at 40 g ha$^{-1}$ | 1.1 | 0.7 | 1.9 | 0.9839 |

The odds ratio is the probability that a spruce tree in the first treatment of the pair was classified as more severely beetle-attacked.

diameter was a significant covariate, with a positive correlation to probability of mass-attack ($F_{1, 119} = 5.61$, $P = 0.0195$) (Fig. 4).

## Discussion

High-release MCH (1,000 mg bubbles eluting 12 mg MCH d$^{-1}$ at 25°C), deployed at all three densities in our area protection study, significantly reduced the probability of host infestation by spruce beetle compared with the control treatment. Spruce in the control plots was about twice as likely to have more severe attack levels compared with MCH-treated spruce (Table 3). The probabilities of more severe infestations did not significantly differ among the three MCH densities (20, 40, and 80 g MCH ha$^{-1}$). MCH efficacy appears to be comparable in areas with low spruce beetle population levels (Big Flat) and areas with high spruce beetle population levels (Mill Park) (Table 2).

In contrast, Hansen et al. (2016) reported an ~15-fold decrease in the probability of mass-attacks among Engelmann spruce in plots treated with a grid of MCH (1,000 mg bubble caps) at 16 m (~39 g MCH ha$^{-1}$) surrounding carbaryl-treated trap trees compared with plots with carbaryl-treated trap trees but without a grid of MCH. The Hansen et al. (2016) study conducted near our Mill Park and Big Flat plots, included the semiochemicals isophorone and sulcatone deployed with each MCH bubble, and a comparison of both results suggests that MCH alone has limited efficacy as an area protectant against spruce beetle attack. Holsten et al. (2003) reduced the attack rates in MCH-treated plots by 80–87% compared with control plots. In that study, conducted with Lutz spruce, *Picea* × *lutzii* Little, and Sitka spruce, *P. sitchensis* (Bong.) Carr., in southcentral Alaska, the release device was a microinfusion pump emitting a constant dose of MCH regardless of temperature or time since deployment. Use of the microinfusion pump confounds direct comparison to the release rates of MCH emitted from a passive releaser (e.g., 40 g MCH ha$^{-1}$). Nevertheless, Holsten et al. (2003) deployed the micro-infusion pumps on a 9 m grid (~123 ha$^{-1}$ over 0.2 ha plots) which is a considerably higher density than even our 80 g MCH ha$^{-1}$ treatment. Direct comparisons to our results should consider differences in host species and geographic variation in spruce beetle populations.

Although the three MCH densities we tested did not significantly differ in efficacy, we recommend that any operational deployments use a higher density of MCH, e.g., 40 g MCH ha$^{-1}$. This opinion was informed by examination of the raw data (Table 2), without consideration of covariates such as tree diameter or stand characteristics, that suggest the 20 g MCH ha$^{-1}$ treatment had intermediate efficacy relative to the control and higher density MCH treatments. MCH deployed at rates as low as 20 g MCH ha$^{-1}$ can effectively reduce infestation rates of Douglas-fir by Douglas-fir beetle, a spruce beetle congener (Ross et al. 1996, 2015) but operational deployments should allow for a margin of error to maximize treatment efficacy (Brookes et al. 2016).

Downloaded from https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox208/4107613/3-Methylcyclohex-2-en-1-one-for-area-and
by DigiTop USDA's Digital Desktop Library user
on 08 September 2017

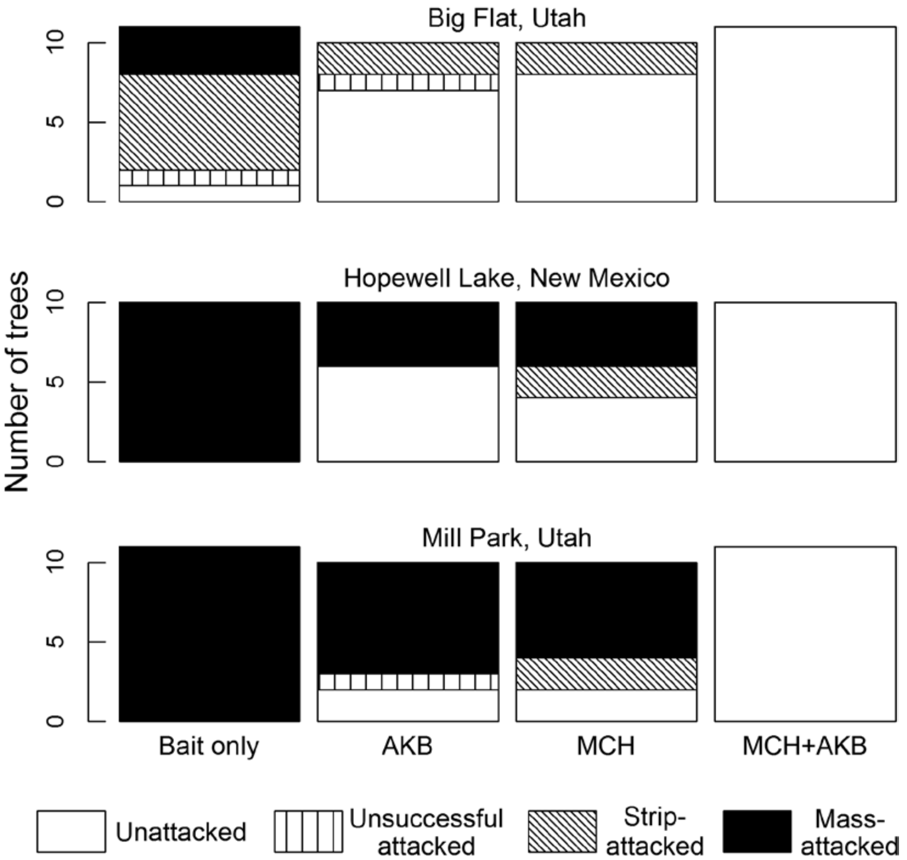


**Fig. 3.** Counts of attack status by treatment for the individual tree protection tests at three southern Rocky Mountain locations. Beetle pressure was greatest at Mill Park and lightest at Big Flat (see Table 2, counts of mass-attacks by area). For the analysis, unattacked, unsuccessfully attacked, and strip-attacked trees were combined into a single category (i.e., "survived") to enable use of a binomial logistic model.

Managers need to consider whether an ~50% reduction in probability of successful spruce beetle attack justifies the expense of labor and materials. Although the cost of deploying MCH at 40 g per ha is ~10% the cost of insecticide application, efficacy is also considerably less than insecticide applications. It should be noted that our test protocol included baited funnel traps which would not be deployed within an MCH-treated area and could have affected efficacy. This is important because we observed a disproportionate amount of currently infested trees near the baited traps regardless of treatment. Therefore, our field trials may not represent the true efficacy of MCH as an area protectant compared with a scenario of no management. Nevertheless, the combined results of previous studies (Werner and Holsten 1995, Holsten et al. 2003, Ross et al. 2004, Hansen et al. 2016) and those herein suggest that MCH alone is marginally effective as an area protection treatment for spruce beetle and that efficacy might be substantially improved by combining MCH with other repellent semiochemicals.

A final caveat is that attack rates were relatively low in our control plots even if considerably higher than epidemic threshold values defined by Bentz and Munson (2000) and Hansen et al. (2006). Control plots at Mill Park had 12% of spruce >20 cm dbh mass-attacked compared with 68% in the control plots of Ross et al. (2004) wherein MCH did not significantly reduce infestation rates. In the other successful tests of MCH against spruce beetle, Holsten et al.'s (2003) study was conducted in "an area of low spruce beetle population density" while Hansen et al.'s (2016) control plots in the Mill Park area had about 10% mass-attacks on spruce >20 cm dbh. Because beetle population density is thought to have contributed to previous MCH failures against spruce beetle (Holsten et al. 2003), the positive results we observed may not be repeatable when exposed to greater beetle pressure. Population pressure was extreme, however, in uncut stands just south of our Mill Park plots (recall that the Mill Park plots were partially cut ca. 1960, and the modified stand conditions possibly contributed to lower mortality rates during the current outbreak). ADS from 2015 alone estimated about 3650 infested spruce over 672 ha within 2 km to the south of our plots and most of the Mill Park individual tree trials were located in this area of very high population density. Our plots were intentionally installed in the partially cut stands because these were the only in the area that still had live, susceptible spruce in 2016 while also being nearby to stands with very high population densities.

Results were more convincing in the individual tree protection trials. The combination of MCH and AKB resulted in complete protection of treated spruce despite the presence of a spruce beetle bait. We did not observe a single spruce beetle pitch-out attack on trees treated with both repellents at all three study areas (Fig. 3). In comparison, 75% (24/32) of all bait-only control trees were mass-attacked, including all of the control trees at Mill Park and Hopewell Lake. A relatively small local beetle population at Big Flat (compare the mass-attack counts among the three locations for the area protection trials in Table 2) resulted in mass-attacks on only 3/11 of the control trees, while another six control trees were strip-attacked. We consider the overall results to represent a rigorous challenge to the treated trees (Shea et al. 1984) and expect that the MCH plus AKB treatment will perform at least as well in applications that do not include the presence of a spruce beetle

Downloaded from https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox208/4107613/3-Methylcyclohex-2-en-1-one-for-area-and
by DigiTop USDA's Digital Desktop Library user
on 08 September 2017

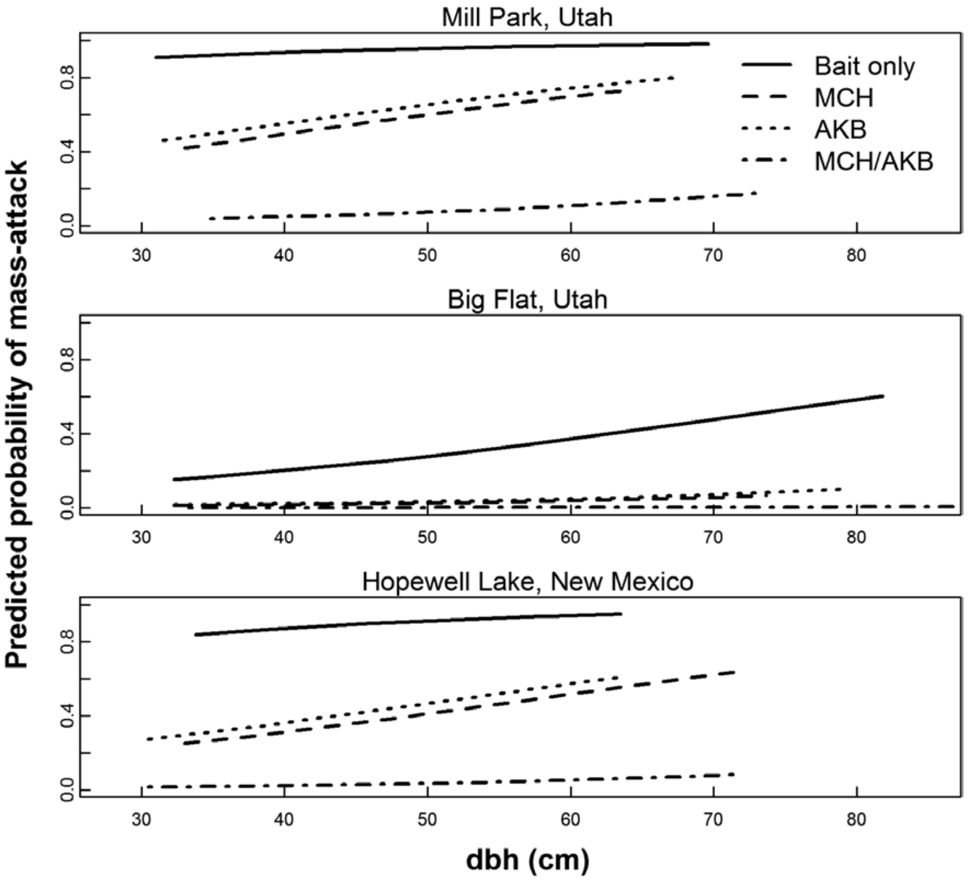


**Fig. 4.** Predicted probabilties of spruce beetle-caused mortality in individual tree protection tests using the repellents MCH and AKB as a function of spruce diameter (dbh) and treatment at two areas in Utah ("Mill Park" and "Big Flat") and one in New Mexico ("Hopewell Lake").

bait. MCH or AKB alone had significantly fewer beetle attacks than the bait-only control trees but mass-attack rates exceeded Shea et al.'s treatment efficacy threshold (<20%). Nevertheless, it is reasonable to expect lower attack rates in operational use wherein treated trees are not also baited. Efficacy of the repellents was likely dependent on the local spruce beetle population levels. Most AKB or MCH alone trees remained unattacked at Big Flat where beetle pressure was relatively low, whereas most AKB or MCH alone treated trees were mass-attacked at Mill Park where beetle pressure was high (Table 2, mass-attack counts; Fig. 3). MCH plus AKB efficacy as a single tree protectant may be reduced in areas of extreme beetle density. On the other hand, MCH plus AKB-treated trees were the only unattacked spruce we observed in portions of the Mill Park area, suggesting that the local population pressure was locally extreme.

In baited funnel trap bioassays, MCH reduced spruce beetle trap captures by ~95% using 800–1,000 mg MCH while AKB reduced captures by ~80% (E. M. Hansen, A. S. Munson, D. Wakarchuk, C. Oehlschlager unpublished data). Given the collective results of those assays and the present study, it appears that MCH synergizes with one or more of the AKB compounds to reduce spruce beetle attractiveness to potential hosts below thresholds needed for successful attack even in the presence of a spruce beetle bait. AKB includes three compounds and it is uncertain the extent to which any or all three contribute to spruce beetle repellency, alone or as an MCH synergist. Further testing is needed to determine an optimal blend of MCH and AKB components. Moreover, the EPA registration process can be simplified if not all three of the AKB components are needed in an optimized spruce beetle repellent. The terpenoid alcohol linalool is EPA registered and can be used in scented hygiene products such as soaps and lotions. Although not explicitly labeled for use against bark beetles, linalool has been used as a mosquito repellent and the EPA "has determined that there is no exposure or risk to non-target organisms, habitats or water" (EPA 2007). The other AKB compounds, β-caryophyllene and leaf alcohols (Z3-hexenol), may require EPA registration should they be found necessary as part of an optimized spruce beetle repellent. MCH is already EPA registered and labelled for use against spruce beetle and Douglas-fir beetle.

Further testing of MCH plus AKB is planned to identify optimal compound blends and to quantify efficacy as an area protection treatment. We anticipate using sites in Utah, Colorado, New Mexico, and Alaska with building-epidemic spruce beetle populations. Until those results are known, along with any necessary product registrations, resource managers can use MCH alone to reduce the probability of spruce beetle attack both on individual trees and as an area protectant. We recommend high release devices, either the single 1,000 mg device (eluting 12 mg MCH $d^{-1}$) used herein or two or more 400–500 mg devices (e.g., two devices eluting 5 mg $d^{-1}$ will effectively release 10 mg $d^{-1}$ when deployed at a single point). Because MCH alone is unlikely to provide complete protection against building-epidemic spruce beetle populations, managers should be prepared to treat trees that become infested despite the treatment (e.g., sanitation cutting). High-value spruce should be treated with an insecticide in lieu of an MCH application, particularly in sites with epidemic spruce beetle populations. Future semiochemical development will, hopefully, lead

Downloaded from https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox208/4107613/3-Methylcyclohex-2-en-1-one-for-area-and
by DigiTop USDA's Digital Desktop Library user
on 08 September 2017

to a high degree of repellent efficacy where additional treatments are not needed. This technology is most appropriate for sensitive areas where vegetation management practices or pesticide application is not allowed, is undesirable, or exceeds budgetary constraints.

## Acknowledgements

The authors thank David Wakarchuk for technical advice regarding semiochemicals. Technical help for plot installations and measurements was provided by Amanda Townsend, Jim Vandygriff, Valerie DeBlander, Ben Meyerson, and Korey Largo. They thank Kathy Johnson and Andrew Orlemann (Fishlake National Forest) and Kristy Groves and Colette Webb (Ashley National Forest) and staff on the Tres Piedras Ranger District, Carson National Forest for their cooperation. This study was funded by the Forest Service-Pesticide Impact Assessment Program and U.S. Forest Service, Forest Health Protection. Semiochemicals were donated by Synergy Semiochemicals Corp.

## References Cited

**Allison, P. D. 2008**. Convergence failures in logistic regression. SAS Global Forum 360: 1–11.

**Bentz, B. J., and A. S. Munson. 2000**. Spruce beetle population suppression in northern Utah. Western J. Appl. For. 15: 122–128.

**Brookes, H. M., D. W. Ross, T. M. Strand, H. W. Thistle, I. R. Ragenovich, and L. L. Lowrey. 2016**. Evaluating high release rate MCH (3-methylcyclohex-2-en-1-one) treatments for reducing *Dendroctonus pseudotsugae* (Coleoptera: Curculionidae) infestations. J. Econ. Entomol. 109: 2424–2427.

**(CSFS) Colorado State Forest Service. 2016**. Aerial survey highlights for Colorado, 2016. http://csfs.colostate.edu/media/sites/22/2017/01/2016COFHADSPressHighlights-CSFS.pdf (last accessed 24 February 2017).

**Dyer, E. D. A., and P. M. Hall. 1977**. Effect of anti-aggregative pheromones 3,2-MCH and trans-verbenol on *Dendroctonus rufipennis* attacks on spruce stumps. J. Entomol. Soc. B. C. 74: 32–34

**(EPA) Environmental Protection Agency. 2007**. Linalool summary document: registration review. https://archive.epa.gov/oppsrrd1/registration_review/web/pdf/linalool_summary.pdf, last accessed 24 February 2017.

**Fettig, C. J., and J. Hilszczański. 2015**. Management strategies for bark beetles in conifer forests, pp. 555–584. *In* F. E. Vega and R. W. Hofstetter (eds.), Bark beetles: biology and ecology of native and invasive species. Springer, London.

**Fettig, C. J., K. D. Klepzig, R. F. Billings, A. S. Munson, T. E. Nebeker, J. F. Negrón, and J.T. Nowak. 2007**. The effectiveness of vegetation management practices for prevention and control of bark beetle infestations in coniferous forests of the western and southern United States. Forest Ecol. Manag. 238: 24–53.

**Firth, D. 1993**. Bias reduction of maximum likelihood estimates. Biometrika 80: 27–38.

**Furniss, M. M., B. H. Baker, and B. B. Hostetler. 1976**. Aggregation of spruce beetles (Coleoptera) to seudenol and repression of attraction by methylcyclohexenone in Alaska. Can. Entomol. 108: 1297–1302.

**Gillette, N. E., C. J. Mehmel, S. R. Mori, J. N. Webster, D. L. Wood, N. Erbilgin, and D. R. Owen. 2012**. The push-pull tactic for mitigation of mountain pine beetle (Coleoptera: Curculionidae) damage in lodgepole and whitebark pines. Environ. Entomol. 41: 1575–1586.

**Hansen, E. M., B. J. Bentz, A. S. Munson, J. C. Vandygriff, and D. L. Turner. 2006**. Evaluation of funnel traps for estimating tree mortality and associated population phase of spruce beetle in Utah. Can. J. For. Res. 36: 2574–2584.

**Hansen, E. M., J. F. Negron, A. S. Munson, and J. A. Anhold. 2010**. A retrospective assessment of partial cutting to reduce spruce beetle-caused mortality in the Southern Rocky Mountains. Western J. Appl. For. 25: 81–87.

**Hansen, E. M., A. S. Munson, D. C. Blackford, D. Wakarchuk, and L. S. Baggett. 2016**. Lethal trap trees and semiochemical repellents as area host protection strategies for spruce beetle (Coleoptera: Curculionidae, Scolytinae) in Utah. J. Econ. Entomol. 109: 2137–2144.

**Holsten, E. H., R. W. Their, A. S. Munson, and K. E. Gibson. 1999**. The Spruce beetle. USDA Forest Service, Forest Insect and Disease Leaflet 127, 11 pp.

**Holsten, E. H., P. J. Shea, and R. R. Borys. 2003**. MCH released in a novel pheromone dispenser prevents spruce beetle, *Dendroctonus rufipennis* (Coleoptera: Scolytidae), attacks in south-central Alaska. J. Econ. Entomol. 96: 31–34.

**Hosmer, D., S. Lemeshow, and R. Sturdivant. 2013**. Applied logistic regression, 3rd ed. Wiley, Hoboken, NJ.

**Jenkins, M. J., E. G. Hebertson, and A. S. Munson. 2014**. Spruce beetle biology, ecology and management in the Rocky Mountains: an addendum to spruce beetle in the Rockies. Forests 5: 21–71.

**Kenward, M. G., and J. H. Roger. 1997**. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53: 983–997.

**Kline, L. N., R. F. Schmitz, J. A. Rudinsky, and M. M. Furniss. 1974**. Repression of spruce beetle (Coleoptera) attraction by methylcyclohexenone in Idaho. Can. Entomol. 106: 485–491.

**Kramer, C. Y. 1956**. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 12: 307–310.

**Lindgren, B. S., M. D. McGregor, R. D. Oakes, and H. E. Meyer. 1989**. Suppression of spruce beetle attacks by MCH released from bubble caps. Western J. Appl. For. 4: 49–52.

**Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006**. SAS system for mixed models, 2nd ed. SAS Institute, Inc, Cary, NC.

**Munson, S. 2010**. Management guide for spruce beetle. US Forest Service, Forest Health Protection. http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5187555.pdf, last accessed 24 February 2017.

**Reynolds, K. M., and E. H. Holsten. 1994**. Relative importance of risk factors for spruce beetle outbreaks. Can J. For. Res. 24: 2089–2095.

**Ross, D. W., and K. F. Wallin. 2008**. High release rate 3-methylcyclohex-2-en-1-one dispensers prevent Douglas-fir beetle (Coleoptera: Curculionidae) infestation of live Douglas-fir. J. Econ. Entomol. 101: 1826–1830.

**Ross, D. W., K. E. Gibson, R. W. Their, and A. S. Munson. 1996**. Optimal dose of an antiaggregation pheromone (3-methylcyclohex-2-en-1-one) for protecting live Douglas-fir from attack by *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae). J. Econ. Entomol. 89: 1204–1207.

**Ross, D. W., G. E. Daterman, and K. E. Gibson. 2002**. Elution rate and spacing of antiaggregation pheromone dispensers for protecting live trees from *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae). J. Econ. Entomol. 95: 778–781.

**Ross, D. W., G. E. Daterman, and A. S. Munson. 2004**. Evaluation of the antiaggregation pheromone, 3-methylcyclohex-2-en-1-one (MCH), to protect live spruce from spruce beetle (Coleoptera: Scolytidae) infestation in southern Utah. J. Entomol. Soc. B. C. 101: 145–146.

**Ross, D. W., K. E. Gibson, and G. E. Daterman. 2015**. Using MCH to protect trees and stands from Douglas-fir beetle infestation. Forest Health Technology Enterprise Team, US Department of Agriculture, Forest Service. FHTET-2001-09, revised November 2015.

**Rudinsky, J. A., C. Sartwell, T. M. Graves, and M. E. Morgan. 1974**. Granular formulation of methylcyclohexenone: an antiaggregative pheromone of the Douglas-fir and spruce bark beetles (Col., Scolytidae). Z. Angew. Entomol. 75: 254–263.

**Samman, S., and J. Logan. 2000**. Assessment and response to bark beetle outbreaks in the Rocky Mountain area: report to Congress from Forest Health Protection, Washington Office, Forest Service, USDA. The Bark Beetles, Fuels, and Fire Bibliography, p. 39.

**Schmid, J. M., and R. H. Frye. 1976**. Stand ratings for spruce beetles. USDA Forest Service Research Paper Note RM-309, Rocky Mountain. Forest and Range Experiment Station, Fort Collins, Colorado.

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

**Werner, R. A., and E. H. Holsten. 1995**. Current status of research with the spruce beetle, *Dendroctonus rufipennis*, pp. 23–29. *In* S. M. Salom and K. R. Hobson (eds.), Proceedings of the National Entomological Society meeting: application of semiochemicals for management of bark beetle infestations. US Department of Agriculture Forest Service General Technical Report INT-GTR-318.

**Windmuller-Campione, M. A., and J. N. Long. 2015**. If long-term resistance to a spruce beetle epidemic is futile, can silvicultural treatments increase resilience in spruce-fir forests in the central Rocky Mountains? Forests 6: 1157–1178.

Downloaded from https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox208/4107613/3-Methylcyclohex-2-en-1-one-for-area-and
by DigiTop USDA's Digital Desktop Library user
on 08 September 2017