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High Release Rate 3-Methylcyclohex-2-en-1-one Dispensers Prevent Douglas-Fir Beetle (Coleoptera: Curculionidae) Infestation of Live Douglas-Fir

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ABSTRACT The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Curculionidae), antiaggregation pheromone, 3-methylcyclohex-2-en-1-one (MCH), has been used by natural resource managers and landowners to protect high-value, high-risk trees from Douglas-fir beetle infestation throughout the western United States since 2000. Labor is a major portion of the cost of MCH treatments. MCH is applied by walking through treatment areas and stapling the formulated pheromone in bubble capsules to trees and other objects on a regular grid pattern. Reducing the number of MCH release points and increasing the distance between them could significantly reduce labor costs, particularly in areas with steep terrain or large volumes of woody debris that could impede the movement of applicators. This study compared the standard MCH application method with a method releasing MCH at a 3 times higher rate and placed at three times fewer release points per unit area. Treatments were applied to 2-ha plots simulating an operational application. Aggregation pheromone-baited traps were placed at plot centers to ensure that dispersing adult beetles would be present on all plots. Both MCH treatments were equally effective at preventing the infestation of live Douglas-fir, *Pseudotsugae menziesii* (Mirbel) Franco, trees (≥ 30 cm diameter at breast height). These results confirm that MCH formulated to release at three times the current standard rate and placed at 3 times fewer points per unit area can effectively prevent the infestation of live Douglas-fir. The new treatment will significantly reduce the labor cost of MCH applications making them feasible for areas that may have previously been marginal economically.

KEY WORDS *Dendroctonus pseudotsugae*, 3-methylcyclohex-2-en-1-one, antiaggregation pheromones

The antiaggregation pheromone, 3-methylcyclohex-2-en-1-one (MCH), is highly effective in preventing the infestation of high-risk trees by Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Curculionidae) (Ross and Daterman 1995, Ross et al. 1996). MCH was first registered by the U.S. Environmental Protection Agency in 1999 and has been used operationally in the Pacific Northwest and Rocky Mountains since 2000 to protect high-valued trees and stands from Douglas-fir beetle infestation (Ross et al. 2006). Each year since 2000, >1,000 ha of high-value stands such as campgrounds and other recreational areas, old-growth reserves, and residential sites have been successfully treated with this pheromone.

MCH is applied in the spring before Douglas-fir beetle flight, which usually begins sometime between late April and mid-May in the interior Pacific North-

west and Rocky Mountains depending upon weather and local site conditions. The pheromone is formulated in plastic bubble capsule dispensers that are stapled to trees or other objects in a grid pattern throughout the area to be treated (Ross et al. 2006). The pheromone elutes from the bubble capsules throughout the main flight period which occurs between mid-May and early July. Because Douglas-fir beetle outbreaks may last several years in a given location, stands need to be treated each spring as long as Douglas-fir beetle populations remain high to be most effective in preventing tree mortality.

A large portion of the cost of an MCH treatment is attributable to the time applicators spend walking through an area dispersing the plastic bubble capsules. Application of fewer MCH dispensers eluting at a higher rate than those currently registered for operational use could potentially reduce treatment costs significantly. Applicators would walk a shorter distance and stop less often because the dispensers would be more widely spaced to achieve the same application rate per unit area. A previous study compared a dose of MCH dispensers (i.e., 72 bubble capsules per

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ha) very close to the recommended standard dose of 75 bubble capsules per ha to those releasing and spaced at 3 and 9 times the standard. Only the 3 times higher release rate and spacing provided results equal to the standard release rate and spacing based on the percentages of host trees mass attacked by Douglas-fir beetle (Ross et al. 2002). This earlier study used small 1-ha circular plots with MCH deployed around the perimeter to test the feasibility of higher MCH release rates.

The current study was conducted to test the three times higher release rate in a manner more representative of an operational treatment. In contrast to the earlier study (Ross et al. 2002), this study used larger 2-ha plots, and the MCH was applied on a grid pattern throughout the treated area. Conditions in this study were more typical of treatments that would be applied by resource managers both in scale and application method. Furthermore, because of the variability of forest environments, it is important to verify results in different years and locations before widespread implementation.

Materials and Methods

The study sites were located on the Council Ranger District of the Payette National Forest at $\approx 45^{\circ} 05' N$ and $116^{\circ} 35' W$ (Zone 11 at 4988710.275 [W], 448814.634 [N] UTM coordinates) ≈ 160 km NNW of Boise, ID. Elevations across the plots ranged from 1,460 to 1,740 m. The Bear Creek drainage where the plots were located had been experiencing a low to moderate level of Douglas-fir beetle-caused tree mortality in the years just before the study. In addition, a tornado on 4 June 2006 in the Bear Creek drainage damaged many Douglas-fir, *Pseudotsugae menziesii* (Mirbel) Franco, trees in an area ≈ 20 km long and 1–2 km wide, providing highly suitable breeding sites for the Douglas-fir beetle during the second half of the 2006 flight period. A survey on 19 June 2006 found Douglas-fir beetle brood in trees blown over during the tornado at two different locations (C. Jorgensen, personal communication). It was expected that the population of dispersing Douglas-fir beetles would be moderate to high in 2007.

This study included four replications of three treatments in a randomized complete block design. Square, 2-ha plots were located in mixed conifer stands with a large component of mature Douglas-fir. Plots were established on one and 2 May 2007 before initiation of the annual Douglas-fir beetle flight. Plots were located in undamaged stands within 2 km of the stands impacted by the tornado. No currently infested trees were located on any of the plots. All plots were at least 150 m from any other plots. Treatments included two different MCH release rates and spacings and an untreated control. The formulation of MCH used in this study was a bubble capsule containing 400 mg of MCH and releasing 3.8 mg/d at 20°C (Chemtica Internacional, San Jose, Costa Rica). The low release rate MCH treatment followed current standard recommendations of placing individual bubble capsules on an

11.5-m grid throughout the plot (Ross et al. 2006). The high release rate MCH treatment was achieved by placing three bubble capsules together at each release point on a 20-m grid throughout the plot. Both treatments resulted in an application rate of 75 bubble capsules per ha.

In the earlier study testing elution rate and spacing of MCH dispensers, it was reported that the individual bubble capsules produced by Pherotech International Inc. (Delta, BC, Canada) released ≈ 2 mg/d (Ross et al. 2002). That approximate release rate was based on weight loss of bubble capsules over a 20-wk period at a field site in northeastern Oregon (Ross and Daterman 1995). The actual release rate of any passive release pheromone formulation will depend upon environmental conditions, primarily temperature and air movement, and the age of the dispenser. Release rates determined under field conditions will differ from those determined under controlled laboratory conditions. The bubble capsules used in the current study were designed by Chemtica Internacional to have release properties similar to the original MCH bubble capsule produced by Pherotech International Inc. Although we did not compare the release rates of MCH bubble capsules produced by the two companies under the same conditions, the product labels for bubble capsules from both companies indicate that they should have very similar release rates. Based on label information, both bubble capsules contain ≈ 400 mg of MCH, both companies recommend applying 100 bubble capsules per ha, and both state that the bubble capsules are effective in the field for 60 d.

A pheromone-baited, 16-unit multiple-funnel trap (Lindgren 1983) (Pherotech International Inc.) was placed at the center of each plot to provide a standard source of attraction and to monitor beetle activity. The primary purpose of these traps was to confirm the presence of beetles on all plots and to ensure some level of beetle pressure for evaluating the effectiveness of the MCH treatments. Each trap was baited with a lure containing ≈ 10 mg of frontalinal and 5 mg of seudenol impregnated in polyvinyl chloride cord formulations produced in our laboratory (Daterman 1974). Release rates at 24°C and chemical purities for frontalinal and seudenol were 0.5 and 0.25 mg/d and 95.0 and 99.3%, respectively. A small piece of dichlorvos-impregnated plastic (3 by 3 cm) was placed in each collection cup to kill trapped insects. Trap catches were collected on 23 May and 6 June 2007. Traps were removed after the second sample date. Numbers of Douglas-fir beetles and associated predators in each sample were counted in the laboratory. The gender of 100 Douglas-fir beetles in each sample was determined (Jantz and Johnsey 1964). Six samples contained <100 beetles, so the gender of all beetles in those samples was determined.

Basal area of all live trees with diameter at breast height (dbh) ≥ 30 cm was measured and recorded by species on variable radius plots located at the treatment plot center and 40 m from the plot center in each cardinal direction. The Douglas-fir component was

expressed as a percentage of total basal area of all species.

A 1-ha circular area at the center of each plot was surveyed on 13 and 14 August 2007 after beetle flight had ended to determine the infestation status of all large Douglas-fir trees (≥ 30 cm dbh). Only the central portion of each plot was surveyed because in previous studies with similar experimental designs almost all infested trees were located near the pheromone-baited traps at the plot centers (Ross and Daterman 1994, Ross et al. 1996). Also, this avoided any potential edge effect at the plot perimeters. The dbh and infestation status of each tree were recorded. Trees were classified as mass attacked or unattacked based on the presence or absence of large amounts of frass on the lower several meters of the bole (Knopf and Pitman 1972, Ringold et al. 1975). There were no intermediate attack levels; all trees either had a high density of attacks evidenced by frass piles covering the visible portions of the bole or they had no apparent attacks. The percentage of large Douglas-fir trees that were mass attacked was calculated for each plot.

Data Analyses. Total cumulative numbers of Douglas-fir beetles and predators caught in multiple-funnel traps over the entire collection period (1 or 2 May through 6 June) and percentage of male Douglas-fir beetles in trap catches during each collection period were subjected to analysis of variance (ANOVA) for a randomized complete block design (Steel and Torrie 1980). Specifically, the model included a fixed factor, MCH treatment, crossed with a random factor, replication. Tree and stand data also were subjected to ANOVA. Before analysis, each variable was tested for homogeneity of treatment variances by Levene's method (Milliken and Johnson 1984). All treatment variances were found to be homogeneous, so no data were transformed. Means were compared and separated by Fisher protected least significant difference (LSD) when $P < 0.05$ (Steel and Torrie 1980). All statistical analyses were performed with SAS computer programs (SAS Institute 2004).

Results and Discussion

Several pheromone-baited traps were damaged and the corresponding samples were lost from plots in one replication. Therefore, numbers of beetles captured from only three replications were analyzed. Significantly more Douglas-fir beetles were caught in traps on control plots than in traps on either the low release or high release MCH plots ($F = 10.78$; $df = 2, 4$; $P = 0.0245$) (Table 1). There are several potential reasons for the smaller numbers of beetles captured on the MCH-treated plots. The presence of MCH on those plots may have reduced the response of flying beetles to the aggregation pheromones in the traps (Ross and Daterman 1995, Ross et al. 1996). In addition, it is possible that the aggregation pheromones released by beetles attacking trees near the traps on the control plots may have increased the number of beetles on those plots and subsequently the numbers captured in the traps (Ross et al. 2002). In any case, the trap catch

Table 1. Mean number \pm SEM of Douglas-fir beetles and clerid predators, *T. undatulus*, caught in traps baited with aggregation pheromones on MCH-treated and control plots in southwestern Idaho, between 2 May and 6 June 2007 ($n = 3$)

Treatment	Douglas-fir beetles (mean no./trap)	<i>T. undatulus</i> (mean no./trap)
Control	1,343.7 \pm 262.6a	127.0 \pm 26.2
Low release MCH	156.7 \pm 69.5b	181.3 \pm 50.9
High release MCH	499.3 \pm 217.5b	262.7 \pm 10.5

Means in each column followed by the same letter or no letter are not significantly different at $\alpha = 0.05$.

data indicate that dispersing adult beetles were present on all of the plots.

Data from all four replications were used to compare the mean percentage of male Douglas-fir beetles among treatments for each collection period. However, there was one missing value for each period, so the error degrees of freedom were reduced by one in the analyses. The mean percentage of male Douglas-fir beetles in trap catches ranged from 68 to 78% on the first collection date and from 74 to 84% on the second collection date. A high relative abundance of males is typical for pheromone-baited trap catches early in the flight season (Ross and Daterman 1997, Ross et al. 2002). There were no significant differences among treatments on either date ($F = 1.20$; $df = 2, 5$; $P = 0.3760$ and $F = 0.66$; $df = 2, 5$; $P = 0.5548$, respectively). These data suggest that both male and female Douglas-fir beetles responded similarly to the MCH treatments.

Two predators were captured in the pheromone-baited traps, *Thanasimus undatulus* (Say) and *Temnochila chlorodia* (Mannerheim). *T. undatulus* accounted for >99% of the 2,711 predators collected in all of the traps. Consequently, only the *T. undatulus* data were analyzed. There were no significant differences in mean number of *T. undatulus* captured among treatments ($F = 4.53$; $df = 2, 4$; $P = 0.0938$) (Table 1). These results are consistent with earlier studies and support the conclusion that *T. undatulus* is not repelled by MCH (Ross and Daterman 1995; Ross et al. 1996, 2002).

The different responses of Douglas-fir beetles and their predator, *T. undatulus*, to the presence of MCH can be discussed from an evolutionary perspective. Because high concentrations of MCH are typically found around habitat with a high density of mated Douglas-fir beetles (Rudinsky et al. 1973, Pitman and Vité 1974), adult Douglas-fir beetles that avoid MCH when searching for a mate and reproductive habitat would have a selective advantage over those that do not. Those that do not respond to MCH would be unlikely to find sufficient food for their offspring to develop to maturity in the habitat already exploited by conspecifics; and their offspring would be unlikely to survive. Conversely, those that avoid MCH would be more likely to locate habitat with sufficient food for their offspring by searching in habitat unexploited by conspecifics regardless of the abundance of resources. In contrast, because immature *T. undatulus* larvae en-

Table 2. Mean \pm SEM stand and tree characteristics for Douglas-fir ≥ 30 cm dbh on MCH-treated and control plots in southwestern Idaho, 2007 ($n = 4$)

Treatment	Basal area (m ² /ha)	% basal area (% of total)	Tree density (stems/ha)	dbh (cm)	% mass attacked
Control	6.9 \pm 1.0	41.2 \pm 6.9	27.2 \pm 4.0	50.3 \pm 1.9	12.6 \pm 2.8a
Low release MCH	13.1 \pm 1.7	56.0 \pm 6.1	34.2 \pm 10.3	49.6 \pm 3.8	0 \pm 0b
High release MCH	9.3 \pm 1.4	46.4 \pm 5.4	41.0 \pm 10.5	45.5 \pm 1.8	0 \pm 0b

Means in each column followed by the same letter or no letter are not significantly different at $\alpha = 0.05$.

ter Douglas-fir beetle galleries to feed on the developing brood, *T. undatulus* gain no advantage by avoiding MCH. *T. undatulus* arriving at trees with a high density of mated Douglas-fir beetles could potentially locate mates and abundant food for their offspring provided that other predators had not already exploited that habitat. Therefore, *T. undatulus* might be expected to show a positive response to MCH in searching for mates and food for their offspring. In this study, more *T. undatulus* were caught in traps on MCH-treated plots than in traps on control plots, although the difference was not statistically significant (Table 1).

There were no significant differences among treatments for basal area ($F = 4.25$; $df = 2, 6$; $P = 0.0708$), percentage of total basal area ($F = 0.58$; $df = 2, 6$; $P = 0.5877$), tree density ($F = 1.15$; $df = 2, 6$; $P = 0.3769$), or dbh ($F = 2.64$; $df = 2, 6$; $P = 0.1506$) for large Douglas-firs (Table 2). This was anticipated because the plots were selected to be as similar as possible in stand structure and composition. However, there was a significant difference among treatments in the mean percentage of large Douglas-fir trees that were mass attacked by Douglas-fir beetle ($F = 20.43$; $df = 2, 6$; $P = 0.0021$). More than 12% of the large Douglas-fir trees on the control plots were mass attacked, but not a single tree was mass attacked on either the low release or high release MCH treatment plots (Table 2). For comparison, the percentage of host trees mass attacked on control plots in five earlier studies ranged from 5.6 to 17.8% (Ross and Daterman 1994, 1995; Ross et al. 1996, 2002). The actual numbers of mass attacked trees on the 1-ha areas surveyed on the control plots ranged from 1 to 7.

These results confirm earlier findings that placing MCH dispensers releasing at three times the current recommended rate at three times fewer release points per unit area can effectively prevent Douglas-fir beetle infestation of high-risk trees. The current study also demonstrates that this new treatment is effective when the MCH is applied on a grid pattern to areas up to 2 ha. Using the new recommendations for release rate and spacing may make MCH treatments economically feasible on areas that were otherwise marginal by reducing labor costs associated with MCH application. Because the new treatment requires only one third as many release points as the current treatment, labor costs associated with the time that applicators spend placing pheromone dispensers in the field should be reduced by a similar amount. Although an earlier study found that a release rate and spacing that

was nine times the current standard was not effective (Ross et al. 2002), it is possible that release rates and spacing between three and nine might be effective. Further studies will be needed to determine the feasibility of even higher release rates and wider spacing of MCH to optimize application strategies.

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