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A Biodegradable Formulation of MCH (3-Methylcyclohex-2-en-1-one) for Protecting *Pseudotsuga menziesii* from *Dendroctonus pseudotsugae* (Coleoptera: Curculionidae) Colonization

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Abstract

Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, trees and stands can be protected from Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins (DFB)-caused mortality by application of synthetic formulations of the beetle's antiaggregation pheromone, 3-methylcyclohex-2-en-1-one (MCH). A biodegradable formulation of MCH, SPLAT MCH, was developed and evaluated for protecting individual Douglas-fir trees and small stands from colonization and mortality by DFB. In an individual-tree experiment in Idaho, both MCH bubble capsules and SPLAT MCH significantly reduced the proportion of treated trees colonized and killed by DFB compared to untreated controls. SPLAT MCH was as effective as MCH bubble capsules for protecting individual trees. Both MCH bubble capsules and SPLAT MCH significantly reduced the proportion of trees colonized and killed by DFB within 0.04-ha circular plots surrounding each treated tree compared to untreated controls. In 0.41 ha stands in New Mexico, both MCH bubble capsules and SPLAT MCH significantly reduced the proportion of trees colonized and killed by DFB compared to untreated controls, again with no differences observed between MCH treatments. In a similar stand level trial in Idaho, neither MCH treatment significantly reduced the proportion of trees colonized by DFB, and only MCH bubble capsules significantly reduced levels of tree mortality compared to untreated controls, but no significant difference was observed between SPLAT MCH and MCH bubble capsules. Overall, the results indicate that SPLAT MCH is as effective as MCH bubble capsules for protecting individual trees and small stands of Douglas-fir from DFB-caused mortality.

Key words: Douglas-fir beetle, Douglas-fir, SPLAT MCH, Scolytinae, forest health

Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins (DFB), is the most damaging insect pest of Douglas-firs, *Pseudotsuga menziesii* (Mirb.) Franco (Pinales: Pinaceae), in North America (Furniss and Carolin 1977; Furniss 2014a,b). Populations typically occur at low densities due to limited availability of optimal hosts (recently dead or stressed Douglas-firs), but often increase following wildfires, wind storms or defoliation events that create large amounts of susceptible trees (Furniss and Carolin 1977; Furniss 2014a,b). Under these conditions, DFB populations may reach high enough densities to successfully colonize and kill large numbers of healthy trees for several years.

The antiaggregation pheromone 3-methylcyclohex-2-en-1-one (MCH) (Kinzer et al. 1971) signals to late arriving beetles that a tree is fully occupied, causing them to search for uncolonized or less

densely populated host trees (McMullen and Atkins 1961, Pitman and Vité 1974, Hedden and Gara 1976). Several products containing MCH can reduce undesirable levels of tree mortality attributed to DFB (Seybold et al. 2018). MCH bubble capsules were registered with the United States Environmental Protection Agency (USEPA) in 1999 and first used operationally in 2000 (Ross et al. 2015). Recent efforts have focused on increasing the release rate and reducing the number of release points per unit area with the goal of reducing the labor required to deploy and retrieve bubble capsules (Ross et al. 2002, Ross and Wallin 2008, Brookes et al. 2016).

Fettig et al. (2015) developed a biodegradable formulation of the antiaggregation pheromone, verbenone (SPLAT Verb, ISCA Technologies Inc., Riverside, CA), for protecting lodgepole pines, *Pinus contorta* Dougl. ex Loud. (Pinales: Pinaceae), from mortality caused by mountain pine beetles, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae). SPLAT Verb was registered with the USEPA in 2013, and first used operationally in 2014 (Fettig et al. 2016). SPLAT is a monolithic 'matrix-type' diffusion controlled-release device designed to release semiochemicals over a sustained period at relatively low doses (Mafra-Neto et al. 2013) and has been used in both agriculture and forestry to manage coleopteran pests (Mafra-Neto et al. 2014). Because SPLAT is a flowable emulsion, the user can adjust the size, and, therefore, release rate, of each release point (dollop) according to desired treatment application parameters. Semiochemicals are completely released within at most months after application, and dollops of inert ingredients biodegrade within 1–2 yr (Mafra-Neto et al. 2014, Fettig et al. 2016). Consequently, there is no need to retrieve them. In campgrounds or other high use recreational areas retrieval of MCH bubbles capsules is often required. Labor costs for retrieval could be eliminated if a biodegradable formulation of MCH were available. The objective of this study was to assess the efficacy of SPLAT MCH for protecting individual Douglas-firs and small stands of Douglas-fir from mortality attributed to colonization by DFBs.

Materials and Methods

Individual-Tree Study

This study was conducted on the Boise National Forest (43.708° N, –116.092° W; 1,700–1,900 m elevation) in southwestern Idaho. During June 2017 (before DFB adult flight began), 30 trees were selected >50 m apart along a forest road >100 m from any trees that were colonized and killed by DFB the previous year, based on crown fade and DFB galleries in the phloem (Furniss and Carolin 1977). In a completely randomized design, each tree received one of three treatments (n = 10): 1) MCH bubble capsules (500 mg released at ~5 mg/d at 25°C, Synergy Shield MCH, Product #3311, Synergy Semiochemicals Corp., Burnaby, BC, Canada), [two capsules per tree ≤61 cm DBH (diameter at breast height, 1.37 m), four capsules per tree >61 cm DBH]; 2) SPLAT MCH (10.0% MCH by weight, ISCA Technologies Inc.) (two 10-g dollops per tree ≤61 cm DBH, four 10-g dollops per tree >61 cm DBH); and 3) untreated controls. Gas chromatographic analysis revealed that the average release rate of a 10 g dollop of SPLAT MCH over 21 d at an average temperature of 26°C (range from 21 to 34°C) was 42 mg/d, eight times higher than from MCH bubble caps. Each experimental tree was baited with a low dose, of aggregation pheromone (Pitman and Vité 1970, Libbey et al. 1983) [10 mg of frontalin (0.5 mg/d at 24°C) and 5 mg of seudenol (0.25 mg/d at 24°C) in polyvinyl chloride formulations (Daterman 1974)] to enhance DFB attraction. MCH release devices and aggregation pheromone lures were attached to the tree bole at approximately 1.4 m height. Pheromone lures were attached to the north side and MCH release devices were spaced equidistant around the circumference.

Experimental trees were assessed for colonization by DFB, based on presence of large amounts of boring dust at the base of the bole, in July 2017 and for mortality, based on crown fade, in July 2018. All trees (≥20 cm DBH) within a 0.04-ha circular plot (11.3 m radius) centered on each experimental tree were also assessed for colonization by DFB in July 2017 and mortality in July 2018. The DBH and azimuth from plot center of each dead tree was recorded. Differences in tree DBH among treatments were tested using a one-way analysis of variance (ANOVA). Differences in the proportions of baited experimental trees colonized and killed were tested using logistic regression. Proportions of colonized and killed trees within the 0.04-ha circular plots centered on each experimental tree were examined using logistic regression. For both individual trees and plots, means were compared and separated by Tukey's HSD test. Raleigh's Z test (Watson and Batschelet 1982) was used to determine whether tree protection was radially uniform. In all cases $\alpha = 0.05$. All statistical analyses were performed using JMP 13 software (JMP, Version 13. SAS Institute Inc., Cary, NC, 1989–2019).

Forest Stand Studies

Studies were conducted at the Cibola National Forest in west-central New Mexico (33.991°N, –107.182°W; 2,500–3,000 m elevation) and the Boise National Forest (43.708°N, –116.092°W; 1,700–1,900 m elevation) in southwestern Idaho. Aerial and ground surveys from the previous year indicated that DFB was causing noticeable mortality within surrounding stands (>10 trees or >4.6 m^2 of basal area killed by DFB per ha within the last 2 yr). For each site, a completely randomized design was used with three treatments and six replications (0.41-ha square plots separated by >100 m, n = 18). Treatments applied in May 2016 were: 1) MCH bubble capsules applied at 30 per plot containing ~500 mg of MCH (released at ~5 mg/d at 20°C), spaced on approximately a 12 × 12 m grid; 2) SPLAT MCH applied at 15 g (10.0% MCH by weight) per plot using 15 dollops (release rate 63 mg/d at 26°C based on the laboratory release rate data described above) spaced on a 13 × 21 m grid; and 3) untreated controls. The bubble capsule treatment was based on the established operational recommendations (Ross et al. 2015) and the SPLAT MCH treatment was based on manufacturer's recommendations. MCH release devices were attached to the north side of the tree bole at approximately 1.4 m height. One 16-unit multiple-funnel trap (Lindgren 1983) baited with the Douglas-fir Beetle Lure (Product # 3187, Synergy Semiochemicals Corp., Burnaby, BC, Canada) [frontalin (released at ~2.5 mg/d at 20°C), seudenol (released at 1.5 mg/d at 20°C), reconstituted Douglas-fir turpentine (released at ~150 mg/d at 20°C), and ethanol (released at ~10 mg/d at 20°C)] was placed near the plot center to provide a similar level of DFB attraction on all plots. We chose to use this relatively strong attractant in our initial evaluations of SPLAT MCH in order to provide a very conservative evaluation of efficacy before considering further commercialization of this product. Trap contents were collected every 2 wk until beetle flight ended in 2016. This general study design was based on several previous studies testing MCH for protection of Douglas-fir stands from DFB (Ross and Daterman 1994, 1995; Ross et al. 1996, 2002; Ross and Wallin 2008; Brookes et al. 2016).

Basal area for all trees ≥20 cm DBH was measured at plot center and ~25 m from plot center in each cardinal direction using variable radius sampling (n = 5 points per plot, basal area factor = 10). Basal area was recorded by species and the percentage of total basal area

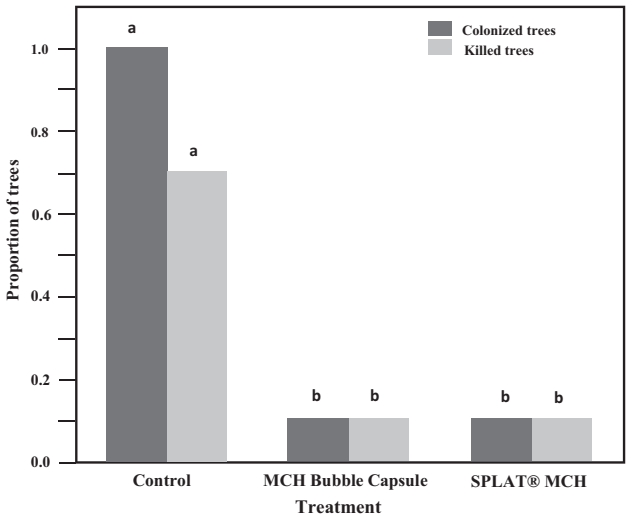

Fig. 1. Proportions of treated and control *Pseudotsuga menziesii* (n = 10) colonized and killed by *Dendroctonus pseudotsugae* in Boise National Forest, Idaho on untreated control plots and plots treated with MCH bubble capsules or SPLAT MCH. Within columns of same shade of gray, different letters indicate significant differences using Tukey's HSD test ($P < 0.001$).

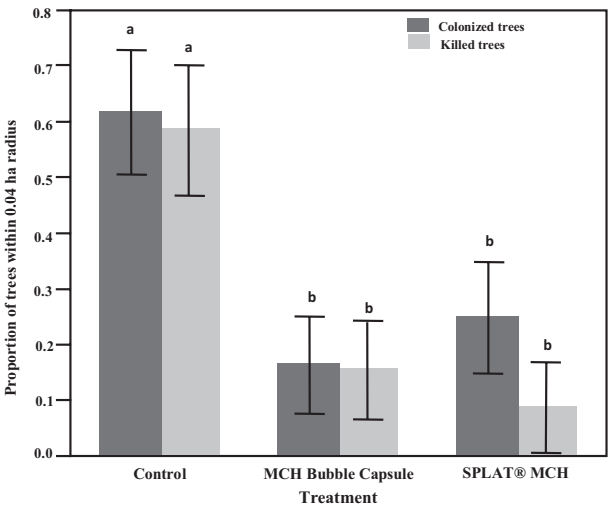

Fig. 2. Mean proportions (± SE) of *Pseudotsuga menziesii* >20 cm DBH infested and killed by *Dendroctonus pseudotsugae* within a 0.04-ha circular plot (11.3 m radius) centered around experimental trees (untreated controls, MCH bubble capsule, and SPLAT MCH), Boise National Forest, Idaho. Within columns of same shade of gray, different letters indicate significant differences using Tukey's HSD test ($P < 0.001$).

that was Douglas-fir was determined for each plot. Trees within variable radius plots were assessed for signs of colonization by DFB in July 2016 and for mortality in July 2017. Significant ($\alpha = 0.05$) differences in the proportion of trees colonized and killed by DFB among treatments were tested using ANOVA, after transforming proportions using arcsine $\sqrt{x + 0.01}$. Mean proportions of both colonized and killed trees were compared and separated by Tukey's HSD test. Total cumulative numbers of DFB and predators [*Thanasimus undatulus* (Say), *Enoclerus sphegeus* F. (Coleoptera: Cleridae), and *Temnochila* spp. (Coleoptera: Trogossitidae)] collected in baited multiple-funnel traps throughout the duration of the DFB flight period (June–August) were determined for each plot. Significant ($\alpha = 0.05$) differences in DFB and predator abundances among treatments were examined using a one-way ANOVA. Means of both DFB and predator abundance were compared and separated by Tukey's HSD test. All statistical analyses were performed using JMP 13 software (JMP, Version 13. SAS Institute Inc., Cary, NC, 1989–2019).

Results

Individual-Tree Study

There was no difference in DBH ($F_{2,27} = 0.1$; $P = 0.891$) among treatments, MCH bubble capsule = 52.0 cm, SPLAT MCH = 52.1 cm, Control = 52.6 cm. Both MCH bubble capsules and SPLAT MCH reduced the proportion of baited trees colonized ($F_{3,26} = 26.3$; $P < 0.001$) and killed ($F_{3,26} = 8.0$; $P < 0.001$) by DFB compared to the untreated control (Fig. 1). No differences were observed between MCH treatments in the proportion of baited trees colonized or killed. Both MCH bubble capsules and SPLAT MCH significantly reduced the proportion of trees colonized ($F_{3,26} = 5.8$; $P = 0.004$) and killed by DFB ($F_{3,26} = 6.7$; $P = 0.002$) within 0.04-ha circular plots surrounding each treated tree compared to the untreated control (Fig. 2). No differences were observed between MCH treatments within the circular plots (Fig. 2), and protection of neighboring trees was radially uniform for both MCH treatments [Raleigh's Z-test ($Z_{observed} < Z_{critical}$; $P > 0.05$ for all treatments)] (Table 1).

Forest Stand Studies

Boise National Forest, Idaho

The stand composition was >88% Douglas-fir in the experimental area. The mean numbers of DFB ($F_{2,15} = 1.9$, $P = 0.18$), *Temnochila* spp. ($F_{2,15} = 0.9$ $P = 0.44$), *T. undatulus* and *E. sphegeus* ($F_{2,15} = 0.6$, $P = 0.48$) collected in baited multiple-funnel traps did not differ among treatments (Table 2). There were no differences in basal area ($F_{2,15} = 0.5$; $P = 0.60$), DBH ($F_{2,15} = 0.05$; $P = 0.95$), and percent Douglas-fir ($F_{2,15} = 1.8$; $P = 0.20$) among treatments (Table 3). Neither MCH bubble capsules nor SPLAT MCH significantly reduced the proportion of trees colonized by DFB compared to the untreated control ($F_{2,15} = 2.2$, $P = 0.07$) (Table 3). MCH bubble capsules, but not SPLAT MCH reduced levels of tree mortality compared to the untreated control ($F_{2,15} = 3.7$, $P = 0.03$) (Table 3). However, no difference in tree mortality was observed between SPLAT MCH and MCH bubble capsules (Table 3).

Cibola National Forest, New Mexico

The stand composition was >66% Douglas-fir in the experimental area. The mean number of DFB collected in baited multiple-funnel traps was significantly lower in plots treated with MCH bubble capsules compared to both SPLAT MCH and untreated control plots ($F_{2,15} = 5.3$, $P = 0.02$) (Table 2). The mean number of *T. undatulus* and *E. sphegeus* collected in baited multiple-funnel traps was lower for both MCH treatments compared to the untreated control ($F_{2,15} = 4.3$, $P = 0.03$) (Table 2). No *Temnochila* spp. were collected in New Mexico (Table 2). There were no differences in basal area ($F_{2,15} = 0.2$; $P = 0.82$), DBH ($F_{2,15} = 0.2$; $P = 0.86$) and percent Douglas-fir ($F_{2,15} = 0.1$; $P = 0.94$) among treatments (Table 3). Both MCH treatments significantly reduced the proportion of trees colonized ($F_{2,15} = 10.1$, $P = 0.002$) and killed by DFB ($F_{2,15} = 13.2$, $P < 0.001$) compared to untreated controls (Table 3). No differences were observed between MCH treatments (Table 3).

Table 1. Mean angular dispersion (*R*) of trees killed by *Dendroctonus pseudotsugae* relative to experimental trees within 0.04-ha circular plots, Boise National Forest, Idaho. $R = 0.0$ indicates completely uniform dispersion; $R = 1.0$ indicates nonuniform dispersion (i.e., directionality). Rayleigh's Z test was used to reject H_A (that mean angular direction of trees killed by *D. pseudotsugae* is nonuniform) in support of H_0 ($Z_{observed} < Z_{critical}$; $P > 0.05$ for all treatment groups)

Treatment	Number of *D. pseudotsugae*-killed trees	Mean angular dispersion of *D. pseudotsugae*-killed trees (*R*)	Z (NR^2)	*P*-value
Control	35	0.18	1.09	>0.05
MCH bubble capsule	15	0.45	3.21	>0.05
SPLAT MCH	16	0.60	3.55	>0.05

Table 2. Mean numbers of *Dendroctonus pseudotsugae* and associated predators collected in baited multiple-funnel traps placed at the center of each plot. For each location, means (± SE) followed by the same letter or no letter within columns are not significantly different ($P > 0.05$)

	Mean numbers captured (±SE)		
Location and Treatment	*D. pseudotsugae*	Trogossitidae spp.	Cleridae spp.
Boise National Forest			
Control	1043.3 ± 121.4	3.0 ± 0.4	34.1 ± 5.5
MCH bubble capsule	1399.6 ± 204.3	3.5 ± 0.3	20.1 ± 11.7
SPLAT MCH	1191.9 ± 180.2	4.2 ± 1.0	44.6 ± 22.8
Cibola National Forest			
Control	418.6 ± 107.4 a	0	19.5 ± 4.0 a
MCH bubble capsule	189.0 ± 50.0 b	0	5.7 ± 1.6 b
SPLAT MCH	540.7 ± 141.3 a	0	8.2 ± 1.3 b

Table 3. Stand and infestation characteristics (means ± SE) within 0.41-ha square plots for *Pseudotsuga menziesii* ≥20 cm DBH[a]. For each location, means followed by the same letter or no letter within columns are not significantly different ($P > 0.05$)

Location and Treatment	Basal area (m^2/ha)	% *P. menziesii*	DBH (cm)	% Colonized[b]	% Mortality[c]
Boise National Forest					
Control	25.3 ± 1.9	89.1 ± 8.0	50.3 ± 17.0	52.7 ± 5.2	45.6 ± 7.8 a
MCH bubble capsule	18.8 ± 1.9	98.6 ± 1.4	51.8 ± 16.2	26.1 ± 4.9	18.6 ± 9.3 b
SPLAT MCH	21.1 ± 1.9	96.6 ± 2.2	51.6 ± 15.3	47.1 ± 11.6	29.3 ± 7.1 ab
Cibola National Forest					
Control	28.0 ± 2.0	66.4 ± 8.6	45.7 ± 3.2	63.9 ± 11.3 a	43.3 ± 6.7 a
MCH bubble capsule	27.1 ± 7.3	71.3 ± 5.1	48.0 ± 1.2	13.2 ± 4.6 b	6.5 ± 3.2 b
SPLAT MCH	29.4 ± 3.1	66.0 ± 10.5	44.5 ± 6.5	29.5 ± 4.1 b	21.3 ± 5.2 b

[a]DBH, diameter at breast height (1.37 m).
[b]Based on presence of large amounts of boring dust at the base of the tree bole.
[c]Based on presence of crown fade.

Discussion

Several studies have reported higher abundances of DFB collected in baited multiple-funnel traps placed at the center of control plots than in MCH-treated plots (Ross and Daterman 1994, 1995; Ross et al. 1996; Ross and Wallin 2008), apparently due to inhibition of DFB attraction to baited traps by MCH-treated trees surrounding them (McMullen and Atkins 1961, Hedden and Gara 1976, Pitman and Vité 1974). In Idaho, where MCH treatments did not protect stands (Table 2), we observed no differences in DFB or predator trap catches between MCH treatments and the untreated control (Table 3). Nonetheless, these data indicate that DFBs were present on all plots and thus represented a challenge for MCH to provide protection of trees therein. In contrast to the Idaho site, the mean number of clerid predators was reduced in both MCH treatment groups compared to the control at the New Mexico site (Table 3), apparently because the clerids were repelled by MCH. MCH has not previously been reported to have a repellent effect on predators of DFB (Ross and Daterman 1995; Ross et al. 1996, 2002; Zhou et al. 2001; Ross and Wallin 2008).

The equal protection of individual pheromone-baited trees and trees surrounding them achieved with SPLAT MCH and MCH bubble capsules in Idaho (Figs.1 and 2) indicates that either treatment could be used with equal effect. In New Mexico, the statistically similar reductions in colonization rates and mortality in 0.41 ha plots achieved with both SPLAT MCH and MCH bubble capsules suggests that either treatment could be used interchangeably on an area basis. The lack of a significant treatment effect in Idaho disagrees with numerous studies evaluating the efficacy of MCH bubble capsules (Ross and Daterman 1994, 1995; Ross et al. 1996, 2002; Ross and Wallin 2008; Brookes et al. 2016). Forest health surveys indicate that DFB populations were declining in New Mexico and increasing in Idaho when this study was initiated (USDA Forest Service 2018a,b). The high numbers of DFBs captured in traps in the Boise National Forest plots (up to 7.4× the numbers in the Cibola National Forest) (Table 3) suggest that the population was so high that treatments with antiaggregation pheromone were overwhelmed. However, previous MCH studies have resulted in reduced attack incidence in areas with populations as high as or higher than those at the Idaho sites (e.g., Ross and Daterman 1994, 1995). Therefore, the

lack of efficacy at the Idaho site may have been due to a combination of an increasing population and the very strong three-component attractant used in our study. In previous studies very weak attractant lures were used to simply ensure that beetles were drawn equally to the plots (Ross and Daterman 1994; Ross and Daterman 1995; Ross et al. 1996, 2002; Ross and Wallin 2008; Brookes et al. 2016). Moreover, in operational treatments, no attractant would be placed anywhere near the target stand. The high levels of efficacy observed in the individual tree study in Idaho for both MCH treatments, where a weaker attractant was used (Figs. 1 and 2), supports this hypothesis.

In the individual-tree study, each bubble capsule was replaced with a 10g dollop of SPLAT MCH containing 1g of MCH releasing at 42 mg/d. This is more than eight times the release rate of a bubble capsule (5 mg) and would result in depletion of the formulated MCH in 24 d. In comparison, a bubble capsule containing 500 mg and releasing 5 mg/d would not be depleted of MCH until 100 d. In the forest stand studies, two bubble capsules were replaced by a 15 g dollop of SPLAT MCH containing 1.5 g of MCH releasing at 63 mg/d. Based on laboratory release rates, 30 bubble capsules would release 150 mg/d/0.41 ha plot while fifteen 15 g dollops of SPLAT MCH would release 945 mg/d/0.41 ha plot over six times the release rate of bubble capsules on an area basis. As with the individual-tree study, MCH would be depleted in SPLAT MCH in 24 d, but not for 100 d in the bubble capsule treatment. The laboratory release rates of MCH were determined at slightly different temperatures for bubble capsules (20°C) and SPLAT MCH (26°C), but that small difference is unlikely to account for the large differences in release rate of the two formulations. Although actual MCH release rates would vary with fluctuating temperatures in the field from those determined at a constant temperature in the laboratory, from an operational perspective, the high release rate of SPLAT MCH might require more than one application per year to protect trees throughout the DFB flight period which can extend over as much as 2 mo depending upon weather conditions. Additionally, the cost of a second application of SPLAT MCH might offset any cost savings over a bubble capsule treatment where retrieval of the bubble capsules is required. Since the SPLAT MCH treatments tested here released MCH at much higher rates than the MCH bubble capsule treatments, future studies should test lower doses of SPLAT MCH to determine the optimal application rates.

Because of these promising results, ISCA Technologies Inc. is pursing USEPA registration of SPLAT MCH. The inert ingredients of SPLAT MCH have already been certified as food safe by the USEPA (Mafra-Neto et al. 2013), and it is likely that SPLAT MCH, like SPLAT Verb (Fettig et al. 2015), will be granted organic production status by the United States Department of Agriculture. Due to its biodegradable qualities (dollops degrade within about a year post-application), SPLAT MCH may be a preferable treatment method in areas where retrieval of MCH bubble capsules is required to satisfy management objectives.

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