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Protection of spruce from colonization by the bark beetle, *Ips perturbatus*, in Alaska

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ABSTRACT

Field bioassays were conducted in south-central Alaska in a stand of Lutz spruce, *Picea* × *lutzii*, to determine whether a semiochemical interruptant (verbenone and *trans*-conophthorin) and/or a defense-inducing plant hormone (methyl jasmonate, MJ) could be used to protect individual standing trees from bark beetle attack. During two experiments (initiated in May 2004 and 2005, respectively), attacks by *Ips perturbatus* on standing trees were induced by using a three-component aggregation pheromone (ipsenol, *cis*-verbenol, and ipsdienol) and prevented by using the interruptant. In 2005, treatments from 2004 were repeated and additional treatments were evaluated by using MJ spray or injection with and without the interruptant. Aggregation began before 3 or 7 June, and attack density was monitored through 3 or 16 August. During both years, tree mortality caused by *I. perturbatus* was recorded twice (in August, and in May of the following year). In both experiments, attack density was greatest on trees baited with the three-component attractive pheromone, but was significantly reduced by addition of the semiochemical interruptant to trees baited with the attractant. There were no significant differences in attack density between attractant + interruptant-treated trees and unbaited trees. In 2004, mortality was highest among attractant-baited trees, whereas addition of the interruptant significantly reduced the level of initial (10 week post-treatment) and final (54 week post-treatment) mortality. In 2005, no significant reduction in attack density occurred on trees baited with the attractant when MJ was sprayed or injected. The highest initial (10.6 week post-treatment) and final (49.4 week post-treatment) mortality was observed among trees that had been injected with MJ and baited with the attractant. Mortality at the final assessment was significantly lower in all other treatment groups. As in 2004, addition of the interruptant to attractant-baited trees significantly reduced the level of final mortality compared to attractant-baited trees. MJ was not attractive or interruptive to *I. perturbatus* or associated bark beetles in a flight trapping study. However, MJ-treated trees (sprayed or injected) exuded copious amounts of resin on the bark surface. Anatomical analyses of felled trees from four treatment groups [Tween (solvent)-sprayed, MJ-sprayed, Tween-injected, and MJ-injected + attractant baited] showed that treatment with MJ increased the number and size of resin ducts produced following treatment. These analyses also revealed a reduction in radial growth in MJ-treated trees. Our results show that during both years, treatment with a simple, two-component interruptant system of verbenone and *trans*-conophthorin significantly reduced *I. perturbatus* attack density and tree mortality on attractant-baited trees and provided a full year of protection from bark beetle attack.

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1. Introduction

Lutz spruce, *Picea* × *lutzii* Little, a natural hybrid of white spruce, *Picea glauca* (Moench) Voss, and Sitka spruce, *Picea sitchensis* (Bong.) Carr., occurs primarily where their distributions overlap on the Kenai Peninsula of Alaska (Viereck and Little, 1972). A major pest of young *P.* × *lutzii* is the northern spruce engraver, *Ips perturbatus* (Eichhoff) (Coleoptera: Scolytidae), which is distrib-

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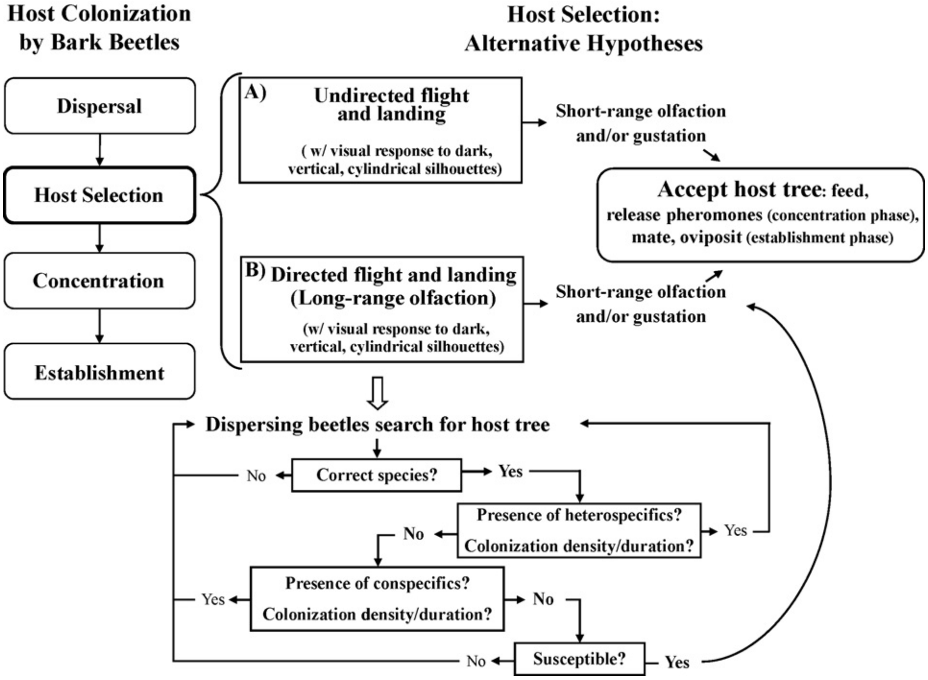

Fig. 1. Host colonization by bark beetles can subdivided into four phases (dispersal, host selection, concentration, and establishment) according to D.L. Wood (1982a). Two major hypotheses to describe host selection are based on (A) undirected flight or (B) directed flight in response to long-range olfactory signals (Wood, 1982a; Borden, 1997). Prior to (long-range) or after landing, a bark beetle may undergo a series of binary decisions to evaluate the species status, followed by the level of occupancy, suitability, and susceptibility of a host (Borden, 1997). Figure adapted from Graves (2008).

uted transcontinentally in the boreal region of North America (Gobeil, 1936; Bright, 1976; Wood, 1982b; Robertson, 2000). Normally, endemic populations infest individual standing spruce trees under environmental stress, but during warm, dry summers following mild winters, engraver beetle populations can increase significantly and kill groups of standing spruce trees (Holsten and Werner, 1987; Holsten, 1996, 1997, 1998). Økland et al. (2005) considered two species of *Ips* [the spruce bark beetle, *I. typographus* (L.) in Eurasia and *I. perturbatus* in North America] "tree-killing bark beetles." Damage caused by *I. perturbatus* and other *Ips* spp. in northern regions may assume greater economic importance as more habitat is provided through climate change and human activities (Robertson, 2000). For example, in 1996 more than 47% of the residual spruce trees in a thinned area near Granite Creek on the Kenai Peninsula became infested with *I. perturbatus* and *I. tridens* (Mannerheim). Spring drought conditions as well as the recent overall warming of the Kenai Peninsula apparently led to this rapid increase in *Ips* activity in 1996 (Holsten, 1996, 1997, 1998; Anon., 1999).

In the spring, male *I. perturbatus* disperse from overwintering sites in the forest litter or downed trees to seek out new host material. After finding a host (see below), males bore through the outer bark and begin excavating a nuptial chamber in the phloem. They release aggregation pheromones and are joined by one to four females, which oviposit in the phloem following mating. Larvae feed on the phloem and develop throughout the summer. Following pupation and eclosion in the late summer and fall, the adults either remain in or leave their brood host to overwinter in the forest litter. *I. perturbatus* has one generation per year in Alberta, Canada (Robertson, 2000), and this is likely the case in Alaska as well.

When selecting a coniferous host to colonize (Wood, 1982a), bark beetles like *I. perturbatus* encounter a series of alternatives. Under the hypothesis of directed flight and landing guided by long-range olfaction, a searching beetle must determine if a tree is (1) the correct species; (2) not occupied at a high colonization density or for a long duration by other bark beetle species or woodborers; (3) not occupied at a high colonization density or for a long duration by bark beetles of the same species; and (4) susceptible to attack (Fig. 1, Borden, 1997). If these criteria are met, a beetle may be able to accept and successfully colonize its host. If not, the beetle may reject the host and continue searching. Each of these decision nodes, save perhaps the final one, is marked by an olfactory behavioral chemical that is either attractive or interruptive to the flying or walking insect. Because of the reliance by the beetle on this chemical communication-based decision process, there is potential to exploit this process to reduce the impact of bark beetles on forest trees (Borden, 1997).

Increased tree mortality in Alaska caused by *Ips* spp. has stimulated research on new management tactics based on semiochemicals from these decision nodes. Field tests of various bark beetle semiochemicals (Fig. 2) showed that racemic ipsenol (2-methyl-6-methylene-7-octen-4-ol) (**1A, B**), 83%-(−)-*cis*-verbenol (*cis*-4,6,6-trimethyl-bicyclo[3.1.1]hept-3-en-2-ol) (**2A, B**), and racemic ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) (**3A, B**) were highly attractive to *I. perturbatus* (Holsten et al., 2000). Addition of a high release rate (~7 mg/day) of 84%-(−)-verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) (**4A, B**) to the three-component attractant significantly reduced trap catches (Holsten et al., 2001). Verbenone is known to be produced by bark beetles, symbiotic fungi, and by auto-oxidation of α-pinene (reviewed in Seybold et al., 2000). Verbenone has also been found among volatiles trapped above both sexes of *I. perturbatus* feeding in cut logs of *P.* × *lutzii* (Graves, 2008). *trans*-Conophthorin [(*E*)-7-methyl-1,6-dioxaspiro[4.5]decane] (**5A, B**), another bark beetle semiochemical, is found in the bark of at least seven angiosperm trees (*Populus tremuloides* Michx., *P. trichocarpa* Torr. and A. Gray, *Acer macrophyllum* Pursh., and *Betula papyrifera* Marsh. in North America), and (*B. papyrifera*, *B. pubescens* Ehrh., *P. tremula* L., and *Quercus suber* L. in Europe) (Huber et al., 1999, 2000; Zhang et al.,

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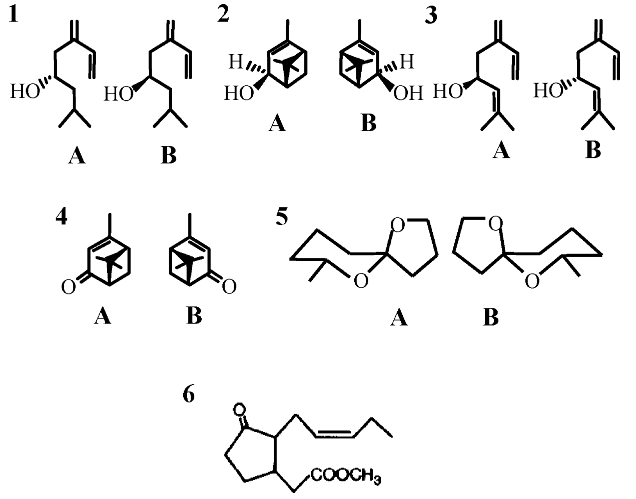

Fig. 2. Semiochemicals associated with *Ips perturbatus* and the plant hormone methyl jasmonate. (**1-A**) (4*S*)-(−)-ipsenol (2-methyl-6-methylene-7-octen-4-ol); (**1-B**) (4*R*)-(+)-ipsenol; (**2-A**) (1*S*,2*S*,5*S*)-(−)-*cis*-verbenol (*cis*-4,6,6-trimethylbicyclo [3.1.1]hept-3-en-2-ol); (**2-B**) (1*R*,2*R*,5*R*)-(+)-*cis*-verbenol; (**3-A**) (4*S*)-(+)-ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol); (**3-B**) (4*R*)-(−)-ipsdienol; (**4-A**) (1*S*)-(−)-verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one); (**4-B**) (1*R*)-(+)-verbenone; (**5-A**) *trans*-conophthorin [(5*R*,7*R*)-(*E*)-7-methyl-1,6-dioxaspiro[4.5]decane]; (**5-B**) (5*S*, 7*S*)- *trans*-conophthorin; and (**6**) methyl jasmonate [3-oxo-2-(2-pentenyl)-cyclopentaneacetic acid, methyl ester].

2000, 2002). This compound, originally isolated from two wasps, *Vespula vulgaris* (L.) and *V. germanica* (F.), was later identified as a repellent of the ash bark beetle, *Leperisinus varius* (F.) (reviewed in Francke and Kitching, 2001). Although the compound was named subsequently because of its behavioral activity with cone beetles in the genus *Conophthorus* (Pierce et al., 1995), conophthorin also functions as an interruptant for several other species of bark beetles, including the pine engraver, *I. pini* (Say) (Huber et al., 1999, 2000), *I. typographus* (Zhang and Schlyter, 2003, 2004), and the primary focus of this study, *I. perturbatus* (Graves, 2008).

A promising technology for enhancing the resistance of conifers to bark beetles and fungi is the application of the naturally occurring plant hormone, jasmonic acid, or its analog, methyl jasmonate [3-oxo-2-(2-pentenyl)-cyclopentaneacetic acid, methyl ester] (MJ-**6**, Fig. 2) (Creelman and Mullet, 1997). Topical application of these compounds to a variety of conifers has caused anatomical changes related to oleoresin and phenolic synthesis and storage (Francheschi et al., 2002; Martin et al., 2002; Hudgins et al., 2003, 2004; Hudgins and Franceschi, 2004; Huber et al., 2004; Erbilgin et al., 2006; Krokene et al., 2008). Increased titers of terpenoid and phenolic chemical defense compounds can present a toxic or physical (sap flow) barrier to bark beetles attempting to enter a tree (Hudgins et al., 2003; Franceschi et al., 2005; Seybold et al., 2006). In spruce and Douglas-fir, MJ induces accumulation of resin chemicals, gene expression, and enzyme activities of metabolic pathways related to terpene synthesis (Martin et al., 2002, 2003; Fäldt et al., 2003; Huber et al., 2004). Specifically, it increases the activity of enzymes called monoterpene synthases, which are directly responsible for the accumulation of monoterpenes in the oleoresin. MJ has been widely studied in relation to agricultural crop plant responses to insect pests (e.g., Thaler et al., 2001), but has only recently been investigated in forest tree-insect pest systems (Erbilgin et al., 2006; Zeneli et al., 2006).

In a novel approach to reducing damage by bark beetles, we used the *I. perturbatus*/*P.* × *lutzii* system as a model to integrate and manipulate two aspects of the natural processes that occur when bark beetles interact with conifers. Specifically, we coupled the phenomena of interruptive insect behavioral chemistry (verbenone and conophthorin) with induced host resistance (by MJ) to develop and test a management strategy for *I. perturbatus* with low environmental impact. This study investigated whether the combination of a beetle-produced compound (verbenone), a non-host volatile (conophthorin), and the induction of a host defense mechanism by MJ, is effective in reducing the colonization density and, ultimately, mortality of *P.* × *lutzii* associated with *I. perturbatus*. The long-term goal is to assess whether behavioral chemicals, induction of host resistance, or the dual application of behavioral chemicals and induced resistance could reduce tree mortality caused by bark beetles to levels equivalent to those of successful insecticide trials (Shea et al., 1984; Fettig et al., 2006).

2. Materials and methods

2.1. Locations and characteristics of field sites

Field studies with *I. perturbatus* and *P.* × *lutzii* were conducted in 2004 (N 60°43.859′,W 149°19.404′) and 2005 (N 60°44.090′,W 149°21.084′) at two adjacent sites in the vicinity of Granite and Silvertip Creeks on the Kenai Peninsula in Alaska (approx. 60 km southeast of Anchorage). This location (approx. 250 m elevation) is characterized by a montane/maritime climate, and the forest stand mainly contained spruce trees that were 84 ± 3 years old (mean ± S.E.M., *N* = 30) with a diameter at breast height (d.b.h.) of 20.43 ± 0.53 cm (mean ± S.E.M., *N* = 151) measured at 1.3 m height; a mean height of approx. 9 m; and a stand density of about 376 stems/ha. There were also a few paper birch, *B. papyrifera*, and mountain hemlock, *Tsuga mertensiana* (Bong.) Carr., in the stand. Shrub cover consisted primarily of various species of willow (*Salix*), alder (*Alnus*), and dwarf birch (*Betula*), which may contribute non-host volatile compounds like conophthorin that could influence the behavior of *I. perturbatus*.

2.2. Trap placement, semiochemicals, flight bioassay experimental design, and statistical analysis

A flight bioassay of the potential behavioral activity of MJ with *I. perturbatus* was conducted from 16 May to 17 August 2005. For this study, 12-unit, Lindgren funnel traps (Lindgren, 1983) were hung individually on 1.5 m aluminum poles, positioning the bottom of each collection cup between 0.1 and 0.3 m above the forest floor. Poles and traps were placed at least 10 m apart (Bakke et al., 1983) and six treatments were replicated 10 times (for a total of 60 traps) in a completely randomized design because of the homogeneity of the cover type. Traps were baited with semiochemicals (Table 1) dispensed from polyvinyl chloride bubble caps, polyethylene centrifuge tubes, or urethane pouches as release devices (Pherotech International Inc., Delta, BC, Canada and ChemTica Internacionale S.A., Heredia, Costa Rica). The treatments were selected to test whether MJ at two release rates was attractive or interruptive relative to the three-component pheromone attractant (Table 2). Treatments included (1) an unbaited trap; (2) a trap baited with the three-component pheromone attractant of ipsenol, *cis*-verbenol, and ipsdienol (Holsten et al., 2000); (3) a trap baited with a low release rate of MJ; (4) a trap baited with a high release rate of MJ; (5) a trap baited with the three-component attractant and the low release rate of MJ; and (6) a trap baited with the three-component attractant and the high release rate of MJ (Table 2). Each component of the attractant and each formulation of MJ were released from a separate release device. MJ for the lowest release rate elution device and for application to trees (see below) was purchased from Sigma–Aldrich (St. Louis, MO, product #392707, 95% chemical purity). Traps were emptied weekly throughout the study to record the seasonal responses of various forest insects to

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Table 1
Synthetic semiochemicals used in *Ips perturbatus* trapping and standing tree studies near Silvertip Creek, Kenai Peninsula, AK, 2004–2005

Semiochemical	Enantiomeric composition	Load (mg)[a]	Release rate (mg/day)[b]	Chemical purity (%)	Cost/device[c]
Ipsenol	Racemic	20	0.2	>98	$4.48
cis-Verbenol	83%-(−)	75	0.3–0.6	>98	$3.16
Ipsdienol	Racemic	40	0.1	>98	$2.96
Verbenone (pouch)	84%-(−)	4.7[d]	40.0	>97	$6.28
trans-conophthorin	Racemic	70	3.0	~90	$13.68
Methyl jasmonate (tube)	n.a.	103	~0.06[e]	95	$0.25
Methyl jasmonate (pouch)	n.a.	1.0[d]	50–60[f]	95	$3.00

[a] Polyethylene centrifuge tube release device (400 μl) for conophthorin and methyl jasmonate (low release rate), urethane pouch for verbenone and methyl jasmonate (high release rate); all other semiochemicals used a polyvinyl chloride bubble cap release device.
[b] Release rates determined at 22 °C by manufacturer.
[c] 2005 cost estimates.
[d] Release device load in grams.
[e] Release rates measured at 22 °C in our laboratory.
[f] Release rate determined by manufacturer; release devices were deployed from 16 May to 17 August 2005 and stored at −32 °C until March 2007. A release rate of ~3.8 mg/day was measured on the used devices at 22 °C in our laboratory (20 March–1 May 2007).

the semiochemicals. Trapped insects were placed in labeled plastic bags and frozen for later identification and counting. All Scolytidae were identified to species and tallied.

For presentation, data were normalized by determining the mean number of insects caught per trap per 7-day trapping interval. For analysis, full season data were transformed by using the $\log_{10}(x+1)$ (Zar, 1984) function for stabilizing variances (Snedecor and Cochran, 1980) and to meet the assumptions of homoscedasticity and normality. Transformed data were then analyzed by ANOVA followed by a comparison of means by using the Ryan–Einot–Gabriel–Welsch Multiple *Q*-test (REGWQ procedure) (Day and Quinn, 1989; SAS Institute Inc., 2001) and SAS software (GLM procedure) (SAS Institute Inc., 2001). For all analyses $\alpha = 0.05$. Only non-transformed means are reported in the results.

2.3. Formulation and application of methyl jasmonate

Methyl jasmonate was diluted in a 0.1% aqueous solution of Tween® 20 (polyethylene glycol sorbitan monolaurate = Tween, Sigma–Aldrich product #274348-4L, CAS 9005-64-5). Two liters of methyl jasmonate solution (22.4 g/l, 100 mM, 200 mmoles/tree) were applied evenly from the base to a height of 3.7 m of each relevant experimental tree by using a NorthStar 16 Gallon (60.6 liter) ATV Tree Sprayer (Northern Tool + Equipment, Burnsville, Minnesota, USA, product #268180). The quantity of methyl jasmonate applied was adjusted for the chemical purity of the material. The first application was on 18 May 2005; the second was on 1 June. At both times, two liters of Tween (0.1%) were applied in identical fashion to a second set of control trees.

Methyl jasmonate was also formulated for injection in another set of experimental trees by mixing 476 ml of methyl jasmonate with 24 ml of Tween (934.6 g/l, 4166.7 mM in Tween). Next, 48 ml of this solution (200 mmoles/tree) was applied through short pieces of polyethylene tubing to 12 holes/tree (4 ml/hole) by hand injection with a 10 ml plastic Luer tip syringe. The quantity of methyl jasmonate applied was adjusted for the chemical purity of the material. Holes were drilled from the four cardinal directions toward the stem axis and at a 30° angle from above by using a 1.25 cm bit. The holes were drilled to a total depth of 3.2 cm, which reflected a penetration of about 2.2 cm into the stem xylem. The first set of four holes was located immediately above the root collar, the next set was approx. 0.9 m higher, and the third set was approx. 0.9 m above the second set. A 7.62-cm long piece of polyethylene tubing (0.95 cm i.d., 1.25 cm o.d.) was inserted into each hole. The end of the tube inserted into the tree had four 1.25 cm long, wide-kerfed slits cut into it at 90° angles from each other to facilitate movement of the liquid into the xylem sapwood. The first set of injections was performed on 19 May 2005; the second was on 2 and 3 June 2005. Concurrently with the methyl jasmonate injections, a solution of Tween was prepared for injection (25 ml Tween in 500 ml of water, 4.8% Tween in water) and injected as above into another set of control trees.

Table 2
Treatments used in *Ips perturbatus* funnel trapping studies and the corresponding figure legend abbreviations near Silvertip Creek, Kenai Peninsula, AK, 2005

Semiochemicals	Figure abbreviation
Unbaited	(A)
Racemic ipsenol, 83%-(−)-*cis*-Verbenol, racemic ipsdienol (attractant)	(B)
Methyl jasmonate vial (MJ low release rate)	(C)
Methyl jasmonate pouch (MJ high release rate)	(D)
Attractant + MJ low release rate	(E)
Attractant + MJ high release rate	(F)

2.4. Standing tree treatments, experimental design, and data collection

2.4.1. 2004 study

The objectives of this experiment were to assess the attack behavior of *I. perturbatus* in response to attractant-baited trees with and without verbenone and conophthorin, and to evaluate the effects of sodium *N*-methyl-dithiocarbamate (MS) (a soil fumigant) as an agent of tree stress. Treatments were applied to trees on 25 May 2004. There were five treatments in the study (Table 3); each treatment was replicated 10 times; and the study was arranged in a completely randomized design. Fifty trees were selected [d.b.h., 15.63 ± 0.35 cm (mean ± S.E.M.); height, 8.95 ± 0.24 m (mean ± S.E.M.), $N = 50$] with a distance of at least 30 m between selected trees. Following selection, treatments were assigned randomly and all semiochemical release devices were stapled to the south-facing sides of trees at a height of ~1.5 m.

In previous pilot studies, treatment of standing trees with a partial attractant (ipsenol + ipsdienol) did not elicit colonization by *I. perturbatus*. We hypothesized that tree stress may be an important precursor to colonization by *I. perturbatus*, so in 2004 one treatment examined the effect of MS on tree vigor. Sodium *N*-methyldithiocarbamate (anhydrous, 32.7% chemical purity, 67.3% inert ingredients, WOODFUME, Osmose Wood Preserving, Inc., Buffalo, NY, EPA Registration No. 3008-33) was dissolved in dimethyl sulfoxide (DMSO) (Riedel-deHaën Product No. 60153, marketed by Sigma–Aldrich Laborchemikalien GmbH, Seelze, Germany, 99.5% chemical purity, Lot 33530). It was applied to

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Table 3
Treatments used in *Ips perturbatus* standing tree studies near Silvertip Creek, Kenai Peninsula, AK, 2004 and 2005

Semiochemicals	Figure abbreviation
2004	
Unbaited	(A)
Verbenone pouch, *trans*-conophthorin	(B)
Racemic ipsenol, 83%- (−)-*cis*-verbenol, racemic ipsdienol (Attractant)	(C)
Attractant-baited, verbenone pouch, *trans*-conophthorin	(D)
Artificially stressed tree (MS dissolved in DMSO)	(E)
2005	
Unbaited	(A)
Tween-sprayed	(B)
Attractant-baited	(C)
Attractant-baited, verbenone, *trans*-conophthorin	(D)
Attractant-baited, MJ-sprayed	(E)
Attractant-baited, MJ-sprayed, verbenone, *trans*-conophthorin	(F)
MJ-sprayed	(G)
Verbenone, *trans*-conophthorin	(H)
MJ-sprayed, verbenone, *trans*-conophthorin	(I)
Attractant-baited, MJ-injected	(J)
Tween-injected	(K)

unbaited standing trees to mimic natural tree stress. MS has been shown to act as a stressing agent in conifers by reducing resin yields to almost zero less than 2 week following treatment and increasing the susceptibility of these trees to bark beetle attack (Hayes et al., 1994; Miller et al., 1995; Strom et al., 2004). Application followed the method of Strom et al. (2004) where a 4:1 mixture of MS was prepared in DMSO and a cotton ball soaked with ~10 ml of the solution was inserted into one 2 cm-deep wound in each cardinal direction created with a hatchet in the sapwood of the stem. The wounds were located 5–10 cm above the soil level.

For all treated trees, attack progression by *I. perturbatus* in 2004 was monitored from the day when attacks were initially observed (3 June) and every 3 days thereafter to determine if attractant-baited trees came under a mass attack by *I. perturbatus* (as described in Wood and Vité, 1961). Entrance holes of *I. perturbatus* were relatively unique in their diameter, external presentation, seasonal occurrence, and location on the bole when compared to other main stem-infesting scolytids associated with *P.* × *lutzii* at this site [e.g., *Dendroctonus rufipennis* (Kirby), *Dryocoetes affaber* (Mannerheim), *Dr. autographus* (Ratzeburg), *Dr. caryi* Hopkins, *Hylurgops rugipennis* (Mannerheim), *Ips concinnus* (Mannerheim), *I. tridens tridens* (Mannerheim), *Polygraphus rufipennis* (Kirby), and *Trypodendron lineatum* (Oliv.)] (Graves, 2008). The number of *I. perturbatus* entrance holes was recorded from the complete bark surface area of the bole from 1 m to approx. 1.75 m height. This number was converted to the number of attacks/dm^2 of bark surface area on attractant-baited trees. Final attack density was assessed on each tree on 3 August 2004 by counting the number of *I. perturbatus* entrance holes in three randomly selected 1 dm^2 bark surface sections per tree (at approx. 1.75 m height) and calculating a mean density per tree. From this estimate, mean densities were calculated for each treatment group ($N = 10$).

Tree mortality resulting from this experiment was assessed on 3 August 2004, and again on 7 June 2005. Trees were considered dead when the foliage was absent or dry, discolored (but not necessarily brown), easy to remove from the branches and/or if the phloem was completely necrotic and the bark was easily dislodged from the stem. Tree mortality was expressed as the percentage of dead trees in each treatment group ($N = 10$). Attractive and interruptive semiochemical release devices were left on treated trees until the date of initial mortality assessment (3 August).

2.4.2. 2005 study

The objectives of this experiment were to assess the attack behavior of *I. perturbatus* in response to attractant-baited trees with and without verbenone, conophthorin, MJ spray, or MJ injection. Treatments in this study were initiated on 18 May 2005 and applications continued until 3 June 2005. There were 11 treatments in the study (Table 3); each treatment was replicated 10 times in a completely randomized design. One hundred and ten trees were selected [d.b.h., 16.27 ± 0.28 cm (mean ± S.E.M.); height, 8.75 ± 0.22 m (mean ± S.E.M.), $N = 110$] with spacing as in 2004. MJ was formulated and applied as described in Section 2.3. The first spray application was on 18 May; the second on 1 June 2005. The first set of injections was performed on 19 May 2005; the second set on 2 and 3 June 2005. Concurrently with the MJ spray application and injection, an aqueous solution of Tween was sprayed on or injected into respective sets of control trees. Following the second set of spray and injection treatments, semiochemical treatments were applied on 3 June 2005 in the same manner as in 2004.

For all treated trees, attack progression by *I. perturbatus* in 2005 was monitored from the day of initial attack (7 June) and every 3 days thereafter until attack density saturated. In a slightly different approach from 2004, numbers of *I. perturbatus* entrance holes were recorded from four randomly selected 1 dm^2 bark surface sections per tree (at approx. 1.75 m height) and used to calculate a mean density per dm^2 bark surface per tree. From this estimate, mean densities were calculated for each treatment group ($N = 10$). Final attack density was assessed on each tree on 16 August 2005 in the same fashion as the initial 2005 estimates.

Tree mortality resulting from this experiment was assessed on 18 August 2005 and again on 16 and 17 May 2006. Foliar and stem criteria for classifying a tree as dead were generally the same as in 2004 except that we verified that phloem was necrotic up to ~2 m around the entire circumference of the tree. In August, we assumed that trees with partially dead phloem at the base would die during the remainder of the growing season, and this was confirmed in every case during the May assessment. Attractive and interruptive semiochemical release devices were left on treated trees until the date of initial mortality assessment (18 August). Because limited data are available in the literature on the specific impact of MJ treatment on commercial trees in the field, at the time of initial assessment, we also made observations on the condition of each study tree, including whether the tree was infested by subcortical insects other than *I. perturbatus* (i.e., *D. rufipennis*, *Dryocoetes* spp., or *Polygraphus* spp.), and visible external effects of treatments to the bole such as resin exudation, and crown condition (i.e., wilting, flagging branches, or discolored foliage).

2.5. Standing tree studies—statistical analyses

When necessary, measurements of colonization density were transformed by using the $\log_{10}(x + 1)$ (Zar, 1984) function for stabilizing variances (Snedecor and Cochran, 1980) and to meet the assumptions of homoscedasticity and normality. Transformed data were then analyzed by ANOVA followed by a comparison of means by using the REGWQ procedure (Day and Quinn, 1989; SAS Institute Inc., 2001) and SAS software (GLM procedure) (SAS Institute Inc., 2001). Tree mortality data (expressed as a percentage of 10 trees) were analyzed by using a modified Student–Newman–Keuls procedure with arcsine-transformed proportional data (Steel et al., 1997). The arcsine square roots of each proportion were ranked, and the largest and smallest values were used for the calculation of the test statistic, which was compared to a 'Studentized' range distribution with ∞ d.f. (Steel et al., 1997; Table A.8; pp. 622–623). Comparisons continued in this manner until significance was no longer achieved. The key modification to

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the procedure is that the variance for the sample is derived by using the variance formula for the binomial distribution. For all analyses $\alpha = 0.05$. Only non-transformed means or percentages were reported in results.

2.6. Stem analyses

In May 2006 prior to the growth period, 40 trees from four treatment groups (Tween-sprayed, MJ-sprayed, Tween-injected, and MJ-injected + attractant baited) were felled, limbed, and sectioned (1 m) to provide material for an analysis of resin duct density, resin duct size, and radial growth (2004–2005). Disks (5–10 cm wide) were removed from the end of each section and labeled, placed in plastic bags, frozen, and shipped to the University of Minnesota for analysis. Disks from four heights (2, 3, 6, and 7 m) were chosen for further analysis based on their proximity to either the spray or injection treatment (i.e., both within and above the treatment zone). Four subsamples, ~2.5 cm^2 and containing outer bark, inner bark, cambium, and the final two growth rings (2004 and 2005), were randomly removed from each disk by using a chisel and hammer.

These samples were further sectioned in the lab by using razor blades to create a piece that was ~1 cm × 1 cm × 2 cm containing the anatomical features described above. Thin (~1 μm) tangential sections were made and rinsed in 95% ethanol to remove excess particulate matter and resin. Next, the sections were rinsed several times in deionized water and stained by using first, a 1% aqueous Toluidine Blue O (Sigma–Aldrich, #198161) solution and second, a 2% aqueous Safranin O (Sigma–Aldrich, #S2255) solution. Staining was used to better delineate physical features of the sections, not for histological purposes. Samples were thoroughly rinsed in water to remove excess stain and placed on a glass slide for detailed analysis. Measurements were taken with a microscope fitted with a 10× objective lens (overall 100× magnification). Measurements included the number of resin ducts in a randomly chosen, single mm wide radial incremental section of newly formed xylem; the size of each resin duct in the xylem produced in 2005; and radial growth increment of the xylem during the 2004 and 2005 growing seasons. Resin duct size (cross-sectional area in μm^2) was determined by calculating the estimated area of a circle with the mean of the length (radial direction) and the width (tangential direction) measurements for the radius. Tree radial growth was normalized by dividing the radial growth increment in 2005 (post-treatment) by the radial growth increment in 2004 (pre-treatment). In addition, pictures of samples were taken (100× magnification) with a Nikon eclipse E600 (Nikon Instruments Inc., Melville, NY) microscope with a Nikon DXM1200F digital camera (Nikon Instruments Inc., Melville, NY) mount and Nikon ACT-1 imaging software.

2.7. Stem analysis—statistical analyses

Statistical analyses were performed to analyze the effect of MJ on (1) the number of resin ducts per 1 mm linear section of newly formed xylem (2005); (2) the size of resin ducts; and (3) the radial growth. Tree growth was subjected to factorial treatment design analyses to better interpret the interactions among factors affecting growth. Initially, the model included all factors including year, height, and treatment and their interactions with mean growth increment as the response variable. There was no significant treatment by height interaction, but there was a significant treatment by year interaction. Therefore, the final analysis of growth included the pooled means of the growth increment analyzed separately by year. Orthogonal contrasts were used to determine the effects of application technique (sprayed vs. injected) and the plant hormone (Tween vs. Tween + MJ); treatment means were compared with the REGWQ procedure (Day and Quinn, 1989; SAS Institute Inc., 2001) and SAS software (GLM procedure) (SAS Institute Inc., 2001). In all cases, analyses were performed to determine if there was an interaction between treatment and height of observation. If there was a significant interaction ($P < 0.05$), heights were analyzed separately. Data were transformed by using the $\log_{10}(x + 1)$ transformation and analyzed by ANOVA followed by a comparison of means with the REGWQ procedure (Day and Quinn, 1989; SAS Institute Inc., 2001) and SAS software (GLM procedure) (SAS Institute Inc., 2001). For all analyses $\alpha = 0.05$. Only non-transformed means are reported in the results.

2.8. Preparation of stem samples for electron microscopy

Wood samples were also prepared for scanning electron microscopy (SEM) by using techniques described previously by Blanchette and Simpson (1992). Specifically, 0.5–1.5 cm^3 pieces of *P.* × *lutzii* encompassing outer bark, inner bark, cambium, and xylem were infiltrated under vacuum with Tissue Freezing Medium^{TM} (Triangle Biomedical Sciences, Durham, NC), frozen to −20 °C, and cut transversely with a Tissue-Tek II cryostat (Elkhart, IN). Samples were then rinsed in deionized water to remove freezing medium, air dried, mounted on aluminum stubs, sputter-coated with gold, and observed by using a Hitachi S-3500N scanning electron microscope (SEM).

3. Results

3.1. Flight bioassays

The flight response of *I. perturbatus* to all funnel trap treatments began prior to 24 May 2005 and ended in early to mid-August 2005 (total catch = 13,470) (Fig. 3A). The maximum flight response of *I. perturbatus* was recorded during the week of 31 May –7 June 2005 (catch = 5843). The maximum flight response of *I. concinnus*, an associated subcortical scolytid, was recorded between 26 July–17 August 2005 (catch = 1195; total catch = 1841) (Fig. 3A). There was an overall treatment effect on the response of *I. perturbatus* in the experiment ($F_{5,54} = 76.14$; $P < 0.001$). The response of *I. perturbatus* to attractant-baited traps was significantly higher than the response to unbaited traps (Fig. 3B). Neither release rate of MJ was attractive alone. The addition of either release rate of MJ to the attractant slightly enhanced the trap catch of *I. perturbatus*, but this increase was not significant. There were also significant treatment effects for male *I. concinnus* ($F_{5,54} = 30.77$; $P < 0.001$), female *I. concinnus* ($F_{5,54} = 81.28$; $P < 0.001$), and *D. rufipennis* ($F_{5,54} = 2.99$; $P = 0.019$). The responses of male and female *I. concinnus* to attractant-baited traps alone or with MJ at either of the two release rates were significantly higher than the responses to the MJ-baited traps or the unbaited traps (data not shown). Thus, there were no sex-specific effects of MJ on the flight behavior of this species, whose sexes are relatively easy to separate by external morphological characters. Though there was an overall significant effect of treatment on the flight response *D. rufipennis*, mean separation tests did not detect specific treatment differences. Flight responses of eight other species of Scolytidae [*Cryphalus ruficollis* Hopk., *Dryocoetes affaber*, *D. autographus*, *H. rugipennis*, *Ips tridens tridens*, *Pityophthorus* spp. (primarily *nitidulus* (Mannerheim)), *Polygraphus* spp. (primarily *rufipennis*), and *Trypodendron* spp. (primarily *lineatum*)] were also not affected significantly by either release rate of MJ. Subsequent gravimetric analysis of the two MJ release devices in the laboratory at 22 °C revealed that the used devices continued to release 5.7 μg/d and 3.8 mg/d, respectively, over a 40 d period (Table 1).

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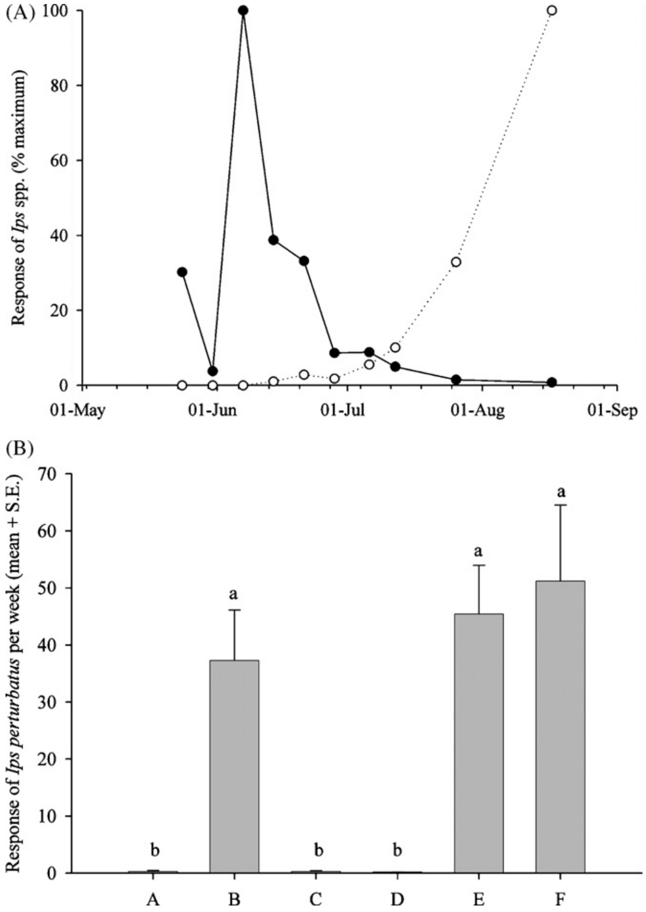

Fig. 3. (**A**) Seasonal flight period of *Ips perturbatus* (solid line) and *Ips concinnus* (dashed line) to treatments near Silvertip Creek, Kenai Peninsula, AK (16 May–17 August, 2005) (total catch = 13,470 and 1841, respectively). Points correspond to the dates when the 60 funnel traps were emptied. The maximum flight response of *I. perturbatus* was recorded during the period of 31 May–7 June 2005 (catch = 5843). The maximum flight response of *I. concinnus*, an associated subcortical scolytid, was recorded during the period of 26 July–17 August 2005 (catch = 1195). (**B**) Flight response of *I. perturbatus* to funnel traps baited with its attractant and two release rates of methyl jasmonate (16 May–17 August 2005) near Silvertip Creek, Kenai Peninsula, AK ($N = 10$). Treatments included: (A) blank; (B) *I. perturbatus* attractant (synthetic ipsenol, *cis*-verbenol, and ipsdienol); (C) MJ-low (low release rate); (D) MJ-high (high release rate); (E) attractant + MJ-low; and (F) attractant + MJ-high. Bars with the same letters are not significantly different, REGWQ test, $F_{5,54} = 76.14$, $P < 0.001$.

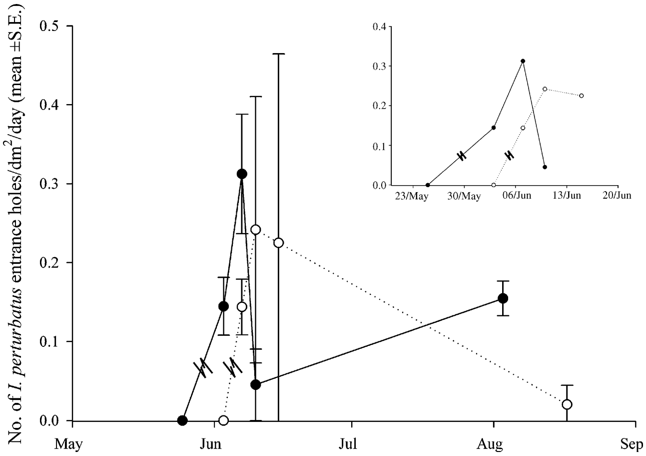

Fig. 4. Attack progression by *Ips perturbatus* in 2004 (solid line) and 2005 (dashed line) on attractant-baited standing *Picea* × *lutzii* near Silvertip Creek, Kenai Peninsula, AK ($N = 10$). Broken lines between the first two data points in each line indicate uncertainty as to when the first attacks actually occurred between the time when the baits were placed and the first observations were recorded. In 2004, the total number of *I. perturbatus* entrance holes from 1 m to approx. 1.75 m the trunk of the tree was calculated for the first three data points. This number was converted to the number of attacks/dm^2. The final data point for 2004 was an average of three random samples/tree (1 dm^2 each) at 1.75 m. All data points for 2005 refer to an average of four random samples/tree (1 dm^2 each) at 1.75 m.

3.2. 2004 standing tree study

3.2.1. Colonization density

In 2004, attacks by *I. perturbatus* were first observed on 3 June (approx 9 d after placement of semiochemical baits). Attacks continued to accumulate on trees for 7 more days (until 10 June) when the rate of new attacks reached approx 0.3 attacks/(dm^2 day) (Fig. 4). There was a significant treatment effect on the ultimate density of attacks on trees in the experiment ($F_{4,45} = 104.70$; $P < 0.001$) (Fig. 5). Following the end of the flight period, the *I. perturbatus* attack density (observed 3 August) was highest on attractant-baited trees (11.03 ± 1.3 attacks/dm^2 tree) and was significantly lower in all other treatments (Fig. 5). Trees treated with the combination of the attractant + verbenone and conophthorin (interruptant), and with the injection of MS in DMSO sustained low levels of *I. perturbatus* attacks (0.4 ± 0.22 and 0.5 ± 0.4 attacks/dm^2, respectively) (Fig. 5). *I. perturbatus* attacks were not observed on unbaited control trees, or on those that had been treated with the combination verbenone and conophthorin in absence of the attractant. There was no significant difference between untreated trees and those that had been treated with the attractant and the interruptant.

3.2.2. Tree mortality

During our initial assessment of the experimental trees (3 August 2004, 10 week post-treatment), mortality was significantly higher in attractant-baited trees (40%) than in most other treatment groups (Fig. 6A). In addition to the trees killed in the attractant-baited treatment group, only one other tree died during this initial assessment (MS + DMSO treatment group) (Fig. 6A). During the final assessment (7 June 2005, 54 week post-treatment), all trees that had been treated with the attractant had died (Fig. 6B). Significantly fewer trees died in all other treatment groups including trees treated with the combination of MS + DMSO (60% mortality) followed by trees treated with the attractant and the interruptant (30% mortality). Mortality was

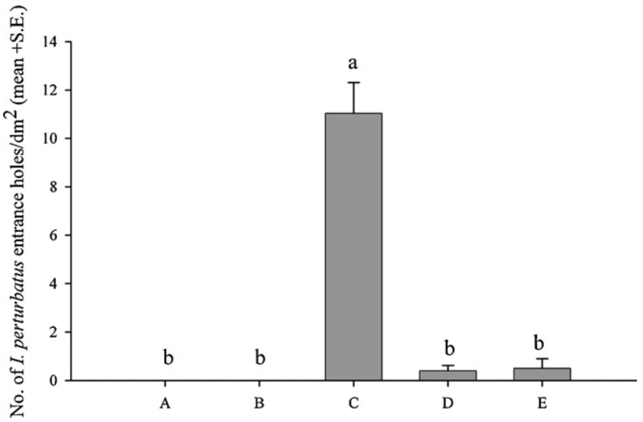

Fig. 5. *Ips perturbatus* attack density on standing *Picea* × *lutzii* near Silvertip Creek, Kenai Peninsula, AK (3 August 2004) ($N = 10$ trees/treatment). Treatments included: (A) untreated; (B) verbenone and *trans*-conophthorin; (C) *I. perturbatus* attractant (synthetic ipsenol, *cis*-verbenol, and ipsdienol); (D) attractant + verbenone + *trans*-conophthorin; and (E) injected MS in DMSO.

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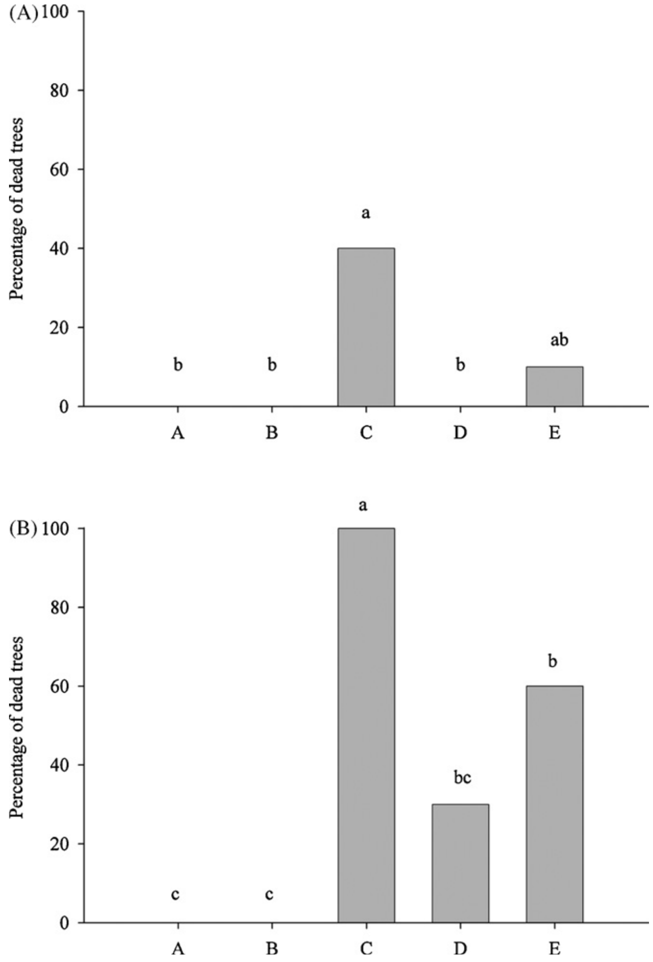

Fig. 6. Mortality of standing *Picea* × *lutzii* attributed to *Ips perturbatus* near Silvertip Creek, Kenai Peninsula, AK, (**A**) 3 August 2004 and (**B**) 7 June 2005 ($N = 10$ trees/treatment). Treatments included: (A) untreated; (B) verbenone and *trans*-conophthorin; (C) *I. perturbatus* attractant (synthetic ipsenol, *cis*-verbenol, and ipsdienol); (D) attractant + verbenone + *trans*-conophthorin; and (E) injected MS in DMSO. Histogram bars labeled with different letters indicate significantly different percentages of tree mortality (modified Student–Newman–Keuls procedure for multiple comparisons with arcsine-transformed proportional data, $P < 0.05$, $N = 10$).

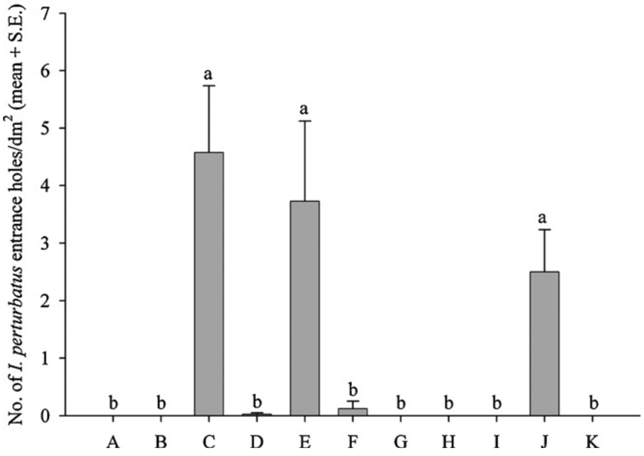

Fig. 7. *Ips perturbatus* attack density on standing *Picea* × *lutzii* near Silvertip Creek, Kenai Peninsula, AK (16 August 2005) ($N = 10$ trees/treatment). Treatments included: (A) untreated; (B) Tween-sprayed; (C) *I. perturbatus* attractant (synthetic ipsenol, *cis*-verbenol, and ipsdienol); (D) attractant + verbenone + *trans*-conophthorin; (E) attractant + methyl jasmonate (MJ)-sprayed; (F) attractant + MJ-sprayed + verbenone + *trans*-conophthorin; (G) MJ-sprayed; (H) verbenone + *trans*-conophthorin; (I) MJ-sprayed + verbenone + *trans*-conophthorin; (J) attractant + MJ-injected; and (K) Tween-injected.

not observed in the treatment groups that contained unbaited trees or trees that had been baited solely with the interruptant. There was no significant difference in mortality between untreated trees and those that had been treated with the attractant and the interruptant (Fig. 6B).

3.3. 2005 standing tree study

3.3.1. Colonization density

In 2005, attacks by *I. perturbatus* were initiated prior to 7 June (6 d following placement of semiochemical baits; 21 d following initial treatment with MJ) (Fig. 5). Again, as in 2004, there was a significant difference in attack density in the experiment ($F_{10,99} = 12.81$; $P < 0.001$) (Fig. 7). As in 2004, following the end of the flight period, the *I. perturbatus* attack density was greatest on trees baited with the attractant (4.56 ± 1.16 attacks/dm^2 tree). Attack density was numerically, but not significantly, lower on trees that had been baited with the attractant and sprayed or injected with MJ (3.73 ± 1.4 and 2.5 ± 0.73 attacks/dm^2 tree, respectively). Attack density was significantly lower for all other treatments in the experiment. Regardless of treatment, all trees that had been treated with the combination of verbenone and conophthorin had attack densities not significantly different from those on the unbaited control trees (Fig. 7).

3.3.2. Tree mortality

During our initial assessment of the experimental trees (18 August 2005, 10.6 week post-treatment), mortality was significantly higher in the treatment group that had been injected with MJ and subsequently baited with the attractant (50%) than in most other treatment groups (Fig. 8A). Only two other treatment groups (attractant-baited, and MJ-sprayed + attractant-baited) contained dead trees (10% in each treatment group) (Fig. 8A). During the final assessment (16 May 2006, 49.4 week post-treatment), all trees that had been injected with MJ and baited with the attractant were dead (Fig. 8B). Two other treatment groups (attractant-baited, and MJ-sprayed + attractant-baited) sustained significantly lower levels of proportional mortality (60 and 50%, respectively). None of the trees that had been treated with the interruptant sustained mortality, even when the attractant was present. There was no significant difference in mortality between untreated trees and those in any of the treatment groups that had been treated with the interruptant (Fig. 8B).

3.4. Stem analyses

3.4.1. Resin duct density

There was an overall treatment effect on the density of resin ducts produced in the study ($F_{15,119} = 30.37$; $P < 0.001$) (Fig. 9A). In addition there was a significant height by treatment interaction ($F_{9,15} = 5.82$; $P < 0.001$) (Fig. 9A). At 2 m, the highest density of resin ducts was observed in trees that had been sprayed or injected with MJ, and there were significantly fewer resin ducts produced in Tween-injected trees ($F_{3,29} = 67.36$; $P < 0.001$). Tween-sprayed trees had a significantly lower resin duct density than all other treatments at 2 m (Fig. 9A). At 3 m, the highest density of resin ducts was observed in trees that had been sprayed or injected with MJ, and significantly fewer resin ducts were produced in trees that were either sprayed or injected with Tween ($F_{3,30} = 38.77$; $P < 0.001$) (Fig. 9A). At 6 m, the highest density of resin ducts was observed in MJ-injected trees. A significantly lower density of

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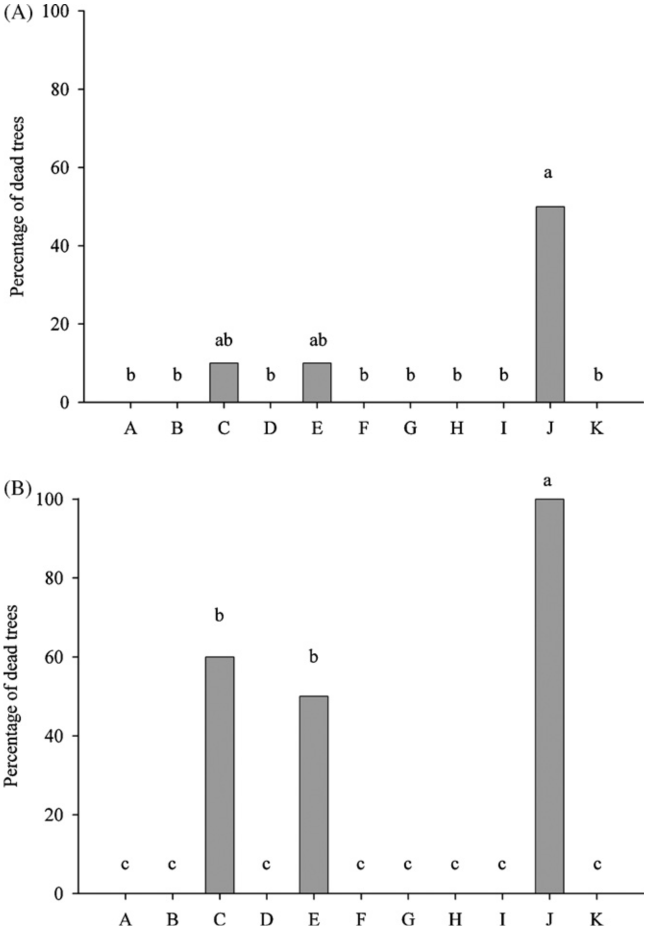

Fig. 8. Mortality of standing *Picea* × *lutzii* attributed to *Ips perturbatus* near Silvertip Creek, Kenai Peninsula, AK, (**A**) 18 August 2005 and (**B**) 16 May 2006 (N = 10 trees/treatment). Treatments included: (A) untreated; (B) Tween-sprayed; (C) *I. perturbatus* attractant (synthetic ipsenol, *cis*-verbenol, and ipsdienol); (D) attractant + verbenone + *trans*-conophthorin; (E) attractant + methyl jasmonate (MJ)-sprayed; (F) attractant + MJ-sprayed + verbenone + *trans*-conophthorin; (G) MJ-sprayed; (H) verbenone + *trans*-conophthorin; (I) MJ-sprayed + verbenone + *trans*-conophthorin; (J) attractant + MJ-injected; and (K) Tween-injected. Histogram bars labeled with different letters indicate significantly different percentages of tree mortality (modified Student–Newman–Keuls procedure for multiple comparisons with arcsine-transformed proportional data, $P < 0.05$, $N = 10$).

resin ducts was observed in MJ-sprayed trees; the density was even significantly lower in trees that had been either sprayed or injected with Tween ($F_{3,31} = 25.73$; $P < 0.001$). Finally, at the highest observation height (7 m), MJ-injected trees had the highest resin duct density, followed by MJ-sprayed trees. The lowest density was in trees that had been either sprayed or injected with Tween alone ($F_{3,29} = 25.05$; $P < 0.001$) (Fig. 9A).

3.4.2. Resin duct size

There was also an overall treatment effect on the size of resin ducts produced in the study ($F_{15,96} = 8.18$; $P < 0.001$) (Fig. 9B). In addition, there was a significant height by treatment interaction ($F_{9,15} = 2.81$; $P = 0.006$) (Fig. 9B). At 2 m, the ducts were significantly larger in trees that had been sprayed with MJ, injected with Tween alone, or injected with MJ compared with those that had been sprayed with Tween alone ($F_{3,27} = 8.59$; $P < 0.001$) (Fig. 9B). At 3 m, the largest resin ducts were observed in trees that had been injected or sprayed with MJ and significantly smaller resin ducts were observed in trees that had been injected or sprayed with Tween alone ($F_{3,24} = 6.04$; $P = 0.003$). At both 6 m and 7 m, the largest resin ducts were observed in the MJ-injected trees ($F_{3,26} = 13.89$; $P < 0.001$ and $F_{3,19} = 12.86$; $P < 0.001$, respectively). Significantly smaller resin ducts were observed in all other treatments (Fig. 9B).

3.4.3. Growth effects

There was a significant treatment effect on the overall normalized radial growth of trees sprayed or injected with Tween alone, or MJ ($F_{3,127} = 43.04$; $P < 0.001$) (Fig. 9C), but there was no significant height by treatment interaction ($F_{9,15} = 0.83$; $P = 0.592$). When all bole heights were pooled, growth was significantly lower in trees that had been injected with the combination of Tween and MJ than the other treatments ($F_{3,127} = 43.19$; $P < 0.001$) (Fig. 9C). There was a significant difference in growth between trees that had been sprayed verses those that had been injected with MJ (orthogonal contrast, $F_{1,127} = 26.44$; $P < 0.001$). There was a significant difference between trees that had been treated with MJ (sprayed or injected) and those that had been treated with Tween alone (sprayed or injected) (orthogonal contrast, $F_{1,127} = 104.21$; $P < 0.001$), but there was no significant difference in growth between trees that had been sprayed and those that had been injected with Tween alone (orthogonal contrast, $F_{1,127} = 0.00$; $P < 0.997$) (Fig. 9C).

3.4.4. Microscopic visualization of stem samples

Anatomical differences between MJ-treated trees and Tween-treated trees were examined by SEM (90× and 250×) (Fig. 10). Treatment with MJ caused dramatic changes in the structure of the newly formed xylem (Fig. 10A and B), whereas treatment with solvent resulted in typical growth of the xylem tracheids (Fig. 10C and D). We did not investigate the specific nature of the cells surrounding the traumatic resin ducts (e.g., Hudgins et al., 2003) to determine if these were indeed resin producing epithelial cells, but general observations of the copious amounts resin within the ducts (the majority of resin was removed prior to taking the photographs in Fig. 10) suggests that the cellular structure of these elements was similar to that observed by previous authors. In each case, traumatic resin ducts seemed to be limited by the spacing between radial ray parenchyma (Fig. 10A and B), but the more typical axial resin ducts tended to be bordered by only one radial ray parenchyma. Approximately six layers of tracheids were produced prior to the production of the layer of traumatic ducts. After the traumatic duct layer was produced, normal tracheid production resumed until the final part of the growing season, when latewood tracheids formed.

3.5. Condition of study trees

Specific observations of the condition of experimental trees at the time of initial assessment of mortality revealed that 9 of the 10 trees left untreated appeared to be healthy and showed no signs of resinosus or bark beetle attack (Table 4). One tree in this treatment group had been attacked by a small number of *D. rufipennis* and had a thin, yellowing crown. As with the untreated trees, the majority of trees in the Tween-sprayed treatment group showed few symptoms from the treatment. Two of the ten trees in this group had beads of resin exuding at approximately 3 m high on the bole. Tween-injected trees showed no signs of insect attack or resin exudation, and in general, appeared to be healthy and unaffected by the treatment.

The majority (70%) of trees baited with the *I. perturbatus* aggregation pheromone had been mass attacked by *I. perturbatus* and had boring dust at the base of the stem. Three of these also had successful *D. rufipennis* attacks. In addition, 5 of 10 trees baited with the *I. perturbatus* attractant showed signs of resin exudation

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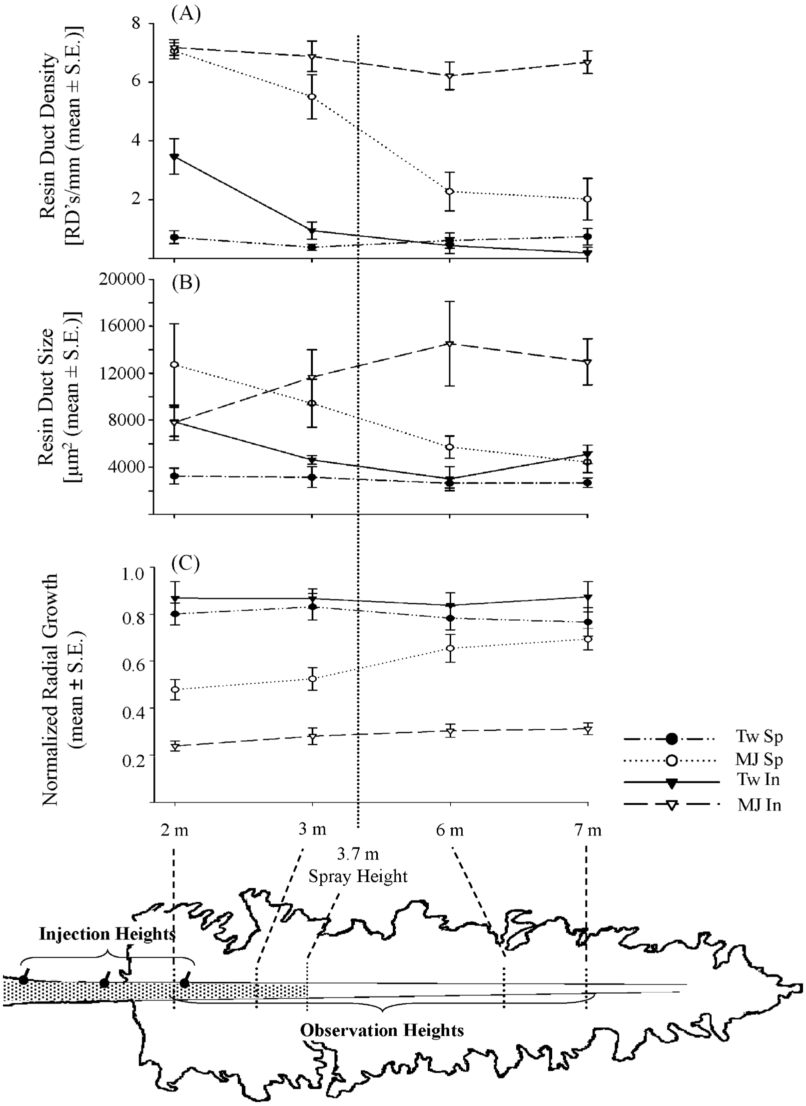

Fig. 9. Effects of MJ and Tween or Tween alone (sprayed or injected) on (**A**) resin duct density, (**B**) resin duct size, and (C) normalized radial growth in *Picea* × *lutzii* trees near Silvertip Creek, Kenai Peninsula, AK (*N* = 8–10/height).

Table 4
Observations of treated trees near Silvertip Creek, Kenai Peninsula, AK, 18 August 2005

Treatment	Other subcortical insects	Resin exudation	Foliage characteristics	Parasitism
Unbaited	1 of 10[a]	0 of 10	1 of 10[b]	0 of 10
Tween-sprayed	0 of 10	2 of 10	0 of 10	0 of 10
Attractant-baited	3 of 10[a]	5 of 10	4 of 10[b]	3 of 10[c]
Attractant-baited, verbenone, *trans*-conophthorin	1 of 10[a]	4 of 10	0 of 10	0 of 10
Attractant-baited, MJ-sprayed	3 of 10[a] 1 of 10[d]	9 of 10	2 of 10[b]	0 of 10
Attractant-baited, MJ-sprayed, verbenone, *trans*-conophthorin	5 of 10[a] 1 of 10[e]	9 of 10	0 of 10	0 of 10
MJ-sprayed	2 of 10	8 of 10	0 of 10	0 of 10
Verbenone, *trans*-conophthorin	2 of 10[a] 1 of 10[e] 1 of 10[f]	3 of 10	0 of 10	0 of 10
MJ-sprayed, verbenone, *trans*-conophthorin	6 of 10[a]	8 of 10	0 of 10	0 of 10
Attractant-baited, MJ-injected	2 of 10[a] 2 of 10[e]	9 of 10	8 of 10[b]	2 of 10[c]
Tween-injected	0 of 10	0 of 10	0 of 10	0 of 10

[a] Attacks by *Dendroctonus rufipennis*.
[b] Needle discoloration (light green, yellow, purple, or brown) or crown dieback.
[c] High levels of parasitism of *Ips perturbatus* larvae.
[d] Longhorned beetle (Coleoptera: Cerambycidae) boring dust.
[e] Attacks by *Polygraphus* spp.
[f] Attacks by *Dryocoetes* spp.

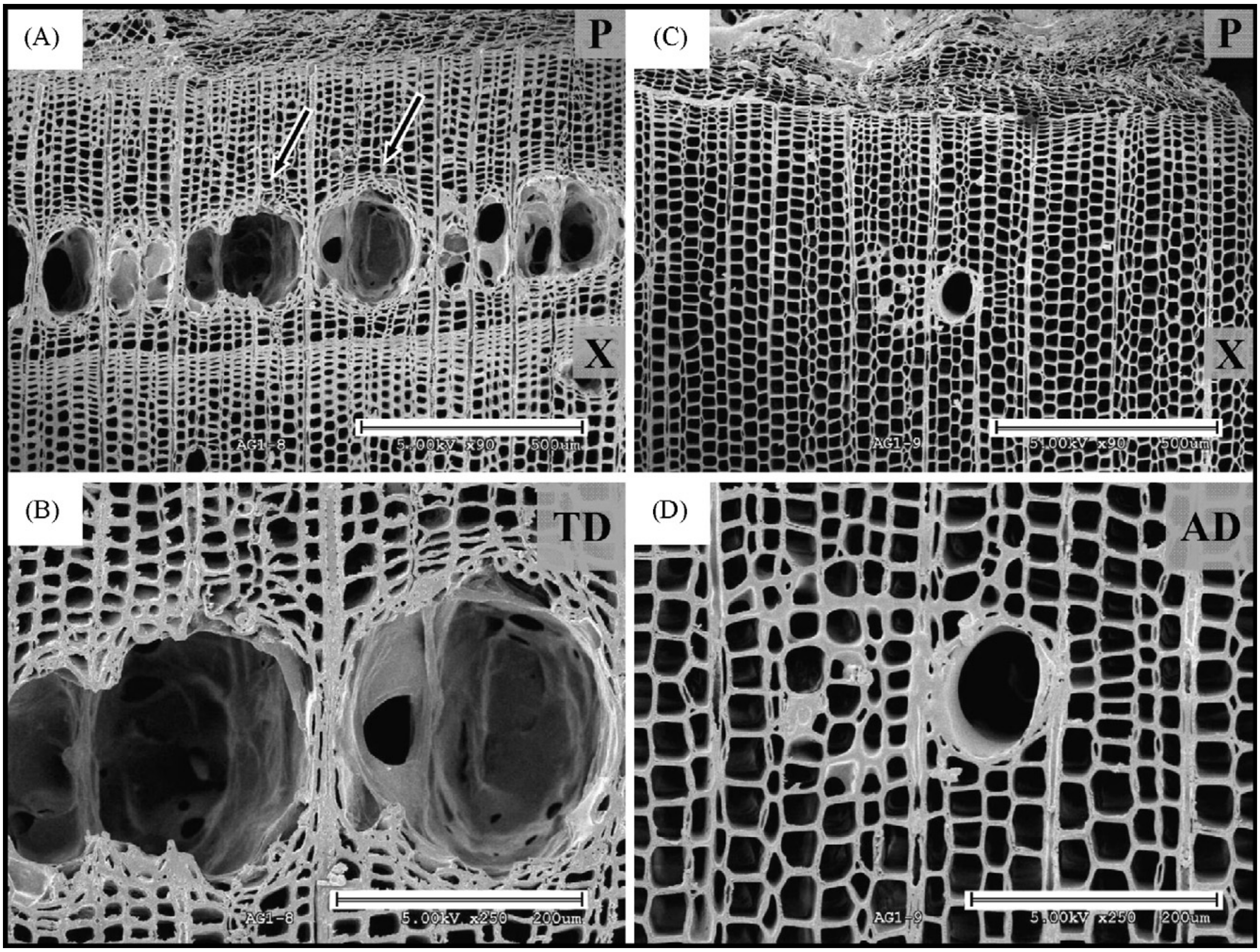

Fig. 10. Induction of traumatic resin duct formation in the outer growth ring (P, phloem; X, xylem; TD, traumatic duct and AD, axial duct) of treated *Picea* × *lutzii* trees. Trees were sprayed either with the methyl jasmonate (MJ) dissolved in Tween 20 or with Tween 20 alone on 18 May, and again on 3 June 2005. Trees were felled and sectioned the following year (17 May 2006). The MJ-sprayed sample (panels (A) and (B)) was removed from a section 2.5 m in height along the bole, whereas the Tween 20-treated (panels (C) and (D)) sample was removed from a section 0.5 m in height. Traumatic resin ducts are apparent (arrows in (A)) in a section from a MJ-sprayed tree (bar = 500 μm) and the same sample at a higher magnification (bar = 200 μm). In Tween 20-treated trees (panels (C) and (D)) traumatic resin ducts are absent, although a lone axial resin duct is present. Scales are in panels (C) and (D) the same as in panels (A) and (B), respectively.

along the stem, although to a lesser degree than trees that had been treated with MJ (see below). In contrast, trees baited with the attractant and the interruptant showed signs similar to unbaited trees, except that four trees showed symptoms of slight resin accumulation on the bark surface. Trees that had been sprayed with MJ and baited with the attractant generally resembled those baited with the attractant alone. However, 9 of 10 trees in this treatment group showed signs of resinosus on the surface of the bole. Exuded resin on the surface appeared to form a physical barrier against attacking beetles by encapsulating them on the bark. Resin emanated from areas where beetles attempted attacks and elsewhere on the bark surface where no wounds were present. We peeled a small section of bark away and noticed that a number of the attacks were aborted and the galleries were compartmentalized by necrotic, resin-soaked phloem tissue. Although many of these trees were heavily attacked only two showed symptoms of needle decline (yellowing or necrosis). Trees sprayed with MJ and treated with the attractive and interruptive semiochemicals showed resin exudation in 9 of the 10 trees, and five of these had unsuccessful attacks by *D. rufipennis*. One of the trees had resin exuding from as high as 6 m on the bole and was heavily attacked by *Polygraphus* spp.

Nearly all (eight of 10) trees sprayed with MJ alone had resin exuding along the bole of the tree; two of these had been attacked by *D. rufipennis*. On trees treated with the interruptant alone, one showed signs of *D. rufipennis* attack and another had been attacked by *Dryocoetes* spp., but none were attacked by *Ips* spp. When the interruptant was combined with the MJ spray, nearly all trees (eight of 10) produced large amounts of resin; six of these trees had been attacked by *D. rufipennis*.

Trees that were injected with MJ and then baited with the attractant showed clear symptoms of extreme resinosus in all but one case. In many cases the resin originated above where attacks were obvious and was noticeable up to 15 m on the bark surface. Nine of ten trees were heavily attacked by *I. perturbatus* and many of these attacks were supplemented by attacks from associated subcortical insects. Two of these trees had been attacked by *D. rufipennis;* two others by *Polygraphus* spp. A few trees that had been injected with MJ and attacked by bark beetles showed signs of blue-staining caused by *Ophiostoma* spp. (fungi commonly associated with subcortical insects of spruce). Hymenopteran pupal cases left under the bark suggested a considerable amount of parasitism of scolytids, specifically in trees that had been injected with MJ and subsequently baited with attractant. Four of the ten

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MJ-injected trees died during the 10.6 week between treatment and evaluation.

4. Discussion

4.1. Verbenone and conophthorin: effects on bark beetle colonization and tree mortality

Semiochemical-based tree protection from bark and ambrosia beetles, either at the single tree or stand level, has been attempted for nearly 20 years by applying verbenone to trees in bubblecap or pouch release devices (Amman et al., 1989; Lindgren et al., 1989; Shore et al., 1992; Progar, 2005); in a grid pattern on trees or stakes in pouches (Bentz et al., 2005); or broadcast in beads (Shea et al., 1992); or in flakes (Gillette et al., 2006). Some of these studies (i.e., Bentz et al., 2005; Progar, 2005) tested verbenone over multiple years, but results showed that the efficacy was not reliable over time. With the discovery of other bark beetle repellent semiochemicals such as (*E*)-2-and (*Z*)-3-hexen-1-ol (Wilson et al., 1996), 1-hexanol, benzaldehyde, benzyl alcohol, and nonanal (Borden et al., 1998), and guaiacol, hexanal, (*E*)-2-hexanal, salicylaldehyde, and conophthorin (Borden et al., 2003) from various non-hosts, land managers have had a larger toolkit of potential interruptants with which to manage bark beetles.

We showed that a simple, two-component interruptant system combining verbenone with a single non-host volatile (conophthorin) can significantly reduce attack density and mortality caused by *I. perturbatus* on *P.* × *lutzii* trees in Alaska. The treatment offered a full year of protection in two different studies. Although the *I. perturbatus* interruptant has not been tested as long as in some other studies (i.e., Bentz et al., 2005; Progar, 2005), two consecutive years of use in the same stand yielded similar results. Thus, we found no indication that treatment efficacy would be reduced over time. To our knowledge, this is the first study to demonstrate that individual trees can be protected from attack by *Ips* spp. by using interruptive semiochemicals when trees are challenged with a highly attractive pheromone. Studies with behavioral chemicals in Europe (reviewed in Jakuš et al., 2003) have proven equivocal in effectively protecting Norway spruce, *Picea abies*, from *I. typographus*. In some of these studies, significant results may have been obtained had trees been adequately challenged with an attractive bait.

The number of bark beetle attacks required to overcome the defense system of a conifer is a variable, but critical and interesting parameter. It has been referred to as "the threshold of successful attack" [first introduced by Thalenhorst (1958) as cited in Mulock and Christiansen, 1986] or more recently as "the critical density" (Guérard et al., 2000). Once the density of *Ips* spp. entrance holes reaches a specific level, initiation of new galleries ceases (Martinek, 1961; Bakke, 1983). Several studies have presented results on the kinetics of *Ips* spp. mass attack (Anderson, 1948; Wood and Vité, 1961; Sartwell, 1970; Byers, 1984; Hedgren and Schroeder, 2004), but rarely for standing trees (Hedgren and Schroeder, 2004). Our measure of the time course of colonization for *I. perturbatus* suggests that the synthetic aggregation pheromone is efficacious in eliciting a complete mass attack on standing trees. We showed that following placement of the attractant (25 May 2004 and 18 May 2005), the number of bark beetle entrance holes increased rapidly until a maximum was reached for new attacks per day. Guérard et al. (2000) reported that ~8.5 *I. acuminatus* attacks/dm^2 was the "critical density" to observe 95% mortality in a stand of Scots pine, *Pinus sylvestris*, in France. We found that ~4.5 attacks/dm^2 caused 60%, whereas ~11.3 attacks/dm^2 caused 100% mortality. Therefore, we estimate that the "critical density" for *I. perturbatus* attacking standing spruce in Alaska is between 4.5 and 11.3 attacks/dm^2.

An objective standard for classifying tree protectants as efficacious was proposed by Hall et al. (1982) and later refined by Shea et al. (1984) in studies focused on insecticide efficacy against western pine beetle, *Dendroctonus brevicomis* LeConte. Shea et al. (1984) surmised that if 18 of 30 (60%) of untreated trees died after being baited with a pheromone that adequate beetle "pressure" had been applied in the experiment. We achieved this level of mortality in both years of our study with 100% mortality of trees that had been baited with the attractant in year 1 and 60% in year 2. The second criterion, and ultimate determinant of efficacy, was that no more than 20–23% of the protectant-treated trees should be killed (Shea et al., 1984). In our first study, 30% of attractant + interruptant-treated trees were killed and therefore did not meet this criterion. Conversely, in our second study, none of the attractant + interruptant-treated trees was killed. Based on the results from the second study, the spruce trees were protected equivalent to a successful insecticide trial (Shea et al., 1984; Fettig et al., 2006).

The moderate material cost of using this simple application was approximately $20.00/tree (excluding labor), 2005 USD [(verbenone pouch ($6.28) and conophthorin ($13.68)], but costs may decline in the future if demand for conophthorin increases and the semiochemical industry responds with increased production. Cost may vary in warmer areas, where it may be necessary to use multiple applications/year to maintain adequate release rates of the interruptant during the flight period of the pest. The limited environmental impact of the treatment should be considered when evaluating and comparing costs with conventional insecticide treatments [$10.80 (Onyx) or $14.00 (Sevin SL)/tree; excluding labor (Fettig et al., 2006)].

4.2. Methyl jasmonate: effects on bark beetle attraction, host colonization, and mortality

This is the first study to assess the flight response of a bark beetle to methyl jasmonate. Flight response of *I. perturbatus* to traps containing the attractant was not significantly increased by the addition of two release rates of MJ. Because MJ alone elicited responses similar to unbaited traps it is unlikely that MJ plays a role in the host finding behavior of *I. perturbatus*, unless it is perceived in combination with other behavioral chemicals. In addition, eight other associated subcortical scolytids showed no preference for traps baited with MJ alone or in combination with the *I. perturbatus* attractant. The natural release level of methyl jasmonate from a mature *P.* × *lutzii* tree is unknown, though studies with sagebrush, *Artemisia tridentata* ssp. *tridentata* (Preston et al., 2004), and mouseear cress, *Arabidopsis thaliana* (Meyer et al., 2003), show release rates around 26 and 25 ng/(g h), respectively from undamaged plants and up to 1129 and 1156 ng/(g h), respectively from damaged plants. The release rates that we used [~240 ng/h (low release rate) and ~0.171 mg/h (high release rate), Table 1] were likely considerably lower than what we might expect to be the natural release rate from a mature tree and it is possible that higher release rates of MJ could elicit behavioral activity.

We also found that MJ did not influence the colonization of standing *P.* × *lutzii* by *I. perturbatus* when challenged with an attractive bait. In a study that tested the effects of MJ topical application on *I. typographus* colonization of Norway spruce, *P. abies* (Erbilgin et al., 2006), the density of entrance holes was lower on MJ-treated bark than on control bark. Erbilgin et al. (2006) applied MJ to one side of the tree and colonization density was assessed at two heights with the untreated side of the tree serving as the control. However, due to the volatility of MJ, the spiral grain in some species of Pinaceae (Bannan, 1966), or potential planar and lateral movement of absorbed MJ or secondary signals, it is unlikely that the effects of MJ could be confined to a single side of the tree. Our results, based on a uniform application of MJ, showed that there was no effect of MJ alone on the colonization density of *I. perturbatus*. It is possible that MJ-treated trees, when challenged with the attractant, were initially able to defend themselves from attack with increased resin production, but ultimately the cost of this production reduced tree health to the degree that mortality was inevitable following heavy attack.

Preliminary observations (Table 4) suggest that *D. rufipennis* may have been attracted at low levels to the MJ-treated trees, particularly through interaction with the interruptant. It is likely that this behavioral effect is due to the combination of host terpenes in the resin on the surface of the bark with verbenone, but this hypothesis needs to be explored further through experimentation.

4.3. Methyl jasmonate: effects on tree anatomy and physiology

Multiple studies have shown that exogenous application of MJ to conifers can induce the production of resin ducts (Francheschi et al., 2002; Martin et al., 2002; Hudgins et al., 2003, 2004; reviewed in Franceschi et al., 2005; Erbilgin et al., 2006). This investigation is one of the first to evaluate the systemic potential of topical applications of MJ to large, standing trees in a wildland forest and the first to evaluate the systemic potential of MJ *via* injection. We observed copious exudation of resin along the stem of MJ-treated trees. Some of the resin exuded from the entrance holes of bark beetles (in attractant-baited trees); in other numerous instances beads of resin were forced through the surface of the unwounded outer bark (in baited and unbaited trees). We also observed resin duct formation 4 m above the point of injection, but did not see a similar translocative effect in trees that had been sprayed with MJ. MJ-spray significantly increased the density of resin ducts produced by *P.* × *lutzii* trees in Alaska at the lower three heights (2–6 m), but the effect declined as measurements were taken along the length of the bole and was not different than the control trees at the highest point assayed (7 m) (Fig. 9A). Conversely, the density of resin ducts observed in MJ-injected trees was not different from control trees at the lowest observation height (2 m), but increased throughout the length of bole and was significantly greater than all other treatment groups at the highest observation height (7 m) (Fig. 9B). Erbilgin et al. (2006) described an increase in the number of resin ducts in MJ-treated trees based on a comparison between treatments of the percentage of sapwood circumference containing resin ducts. Because we measured the actual number of resin ducts, a direct contrast is not possible, though the proportional change between treatments in our study and Erbilgin et al. (2006) are similar. Hudgins et al. (2003) studied the effects of MJ and wounding in various conifers including 12–15-year-old Colorado spruce, *P. pungens*, and observed resin duct densities of six to eight resin ducts per mm. This closely resembles the densities we observed in both MJ-sprayed and MJ-injected stems (~8 per mm) (Fig. 9A). In addition, based on our robust sampling, we showed that MJ (sprayed or injected) significantly increased the size of resin ducts produced in these trees. Interestingly, Hudgins et al. (2003) observed larger resin ducts in wounded trees, whereas we observed no difference near the injection site in trees injected (wounded) with Tween or MJ.

Trees that had been treated with MJ showed a significant reduction in growth, which provides insight into the trade-off between inducible defense and tree growth (reviewed in Herms and Mattson, 1992; discussed in Franceschi et al., 2005). Herms and Mattson (1992) discuss the physiological trade-off that occurs in plants when faced the "dilemma... of whether... to grow or defend." This hypothesis might explain the high level of mortality in the MJ-treated trees. Heijari et al. (2005) observed a similar reduction in growth in MJ-treated *Pinus sylvestris* and the response was dose-dependent, but only significant at the 100 mM concentration of MJ. The key question is whether a balance can be obtained between the production of resin ducts and defensive mechanisms that will provide protection while retaining long-term tree health. At the extreme, MJ-injected trees showed both reduced growth and high mortality, suggesting that the high concentration of MJ injected directly into the sapwood might have magnified the impact on growth to the point of phytotoxicity.

Finally, we did not attempt to investigate the specific pathways that may play a role in the production of traumatic resin (i.e., Hudgins and Franceschi, 2004), but it is possible that activation signaling among cells *via* ethylene emission is governed by the quantity and distance of MJ from the cell. This might explain why trees injected with MJ showed production of resin ducts throughout the stem and those sprayed with MJ showed more localized effects.

Future studies in Alaska will be needed to determine the optimal dose of MJ that ensures the proper balance of resin duct production and tree health. Also a timing study is needed in which different groups of trees are brought under attack at various periods following MJ treatment. This type of study should yield information about the time needed for adequate production of resin ducts, oleoresin, and recovery from treatment with MJ. The time between treatment and tree baiting may even extend as long as 12–15 months after application. In a practical sense, a land manager would need to know the amount of time needed, prior to insect flight, at which MJ would have to be applied for protection of trees. The long-term implications of treatment of trees with MJ is also important, so multi-year studies will be needed to evaluate impacts on treated trees and possibly on neighboring trees that might be affected directly by MJ application or by increases in endogenous production of MJ. We attempted to determine the impact of co-opting the energy of the trees for exaggerated defense response *via* MJ application by measuring the radial growth of the tree. Other parameters may give a better measure of this trade-off between production of potential defensive measures and tree growth and survival.

5. Conclusion

This research represents a valuable demonstration of the efficacy of a relatively inexpensive and environmentally benign combination of a host-/insect-produced compound (verbenone) and non-host compound (conophthorin) for the protection of single trees. The behavioral interruptant may also have efficacy as an area-wide treatment to protect susceptible spruce stands, but this application will require further research. Applying methyl jasmonate to mature live spruce trees can induce the production of resin ducts and copious amounts of resin (proof of concept). These spruce trees rarely form pitch tubes in response to colonization by *I. perturbatus*, but in our investigation, exuded resin on the surface of MJ-sprayed and attractant-baited trees appeared to form a physical barrier against attacking *Ips* by encapsulating them on the bark. Although we were not able to demonstrate the efficacy of coupling these phenomena of interruptive insect behavioral chemistry (verbenone and conophthorin) and induced host resistance (by MJ) as an environmentally sound tree protection strategy for *I. perturbatus*, future refinement of the application of MJ may result in an effective tool for tree protection in this and other bark beetle/host systems.

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