Evaluations of emamectin benzoate and propiconazole for protecting individual Pinus contorta from mortality attributed to colonization by Dendroctonus ponderosae and associated fungi

Christopher J Fettig, A Steven Munson, Donald M Grosman and Parshall B Bush

Abstract

BACKGROUND: Protection of conifers from bark beetle colonization typically involves applications of liquid formulations of contact insecticides to the tree bole. An evaluation was made of the efficacy of bole injections of emamectin benzoate alone and combined with the fungicide propiconazole for protecting individual lodgepole pine, Pinus contorta Dougl. ex Loud., from mortality attributed to colonization by mountain pine beetle, Dendroctonus ponderosae Hopkins, and progression of associated blue stain fungi.

RESULTS: Injections of emamectin benzoate applied in mid-June did not provide adequate levels of tree protection; however, injections of emamectin benzoate + propiconazole applied at the same time were effective for two field seasons. Injections of emamectin benzoate and emamectin benzoate + propiconazole in mid-September provided tree protection the following field season, but unfortunately efficacy could not be determined during a second field season owing to insufficient levels of tree mortality observed in the untreated control, indicative of low D. ponderosae populations.

CONCLUSION: Previous evaluations of emamectin benzoate for protecting P. contorta from mortality attributed to D. ponderosae have failed to demonstrate efficacy, which was later attributed to inadequate distribution of emamectin benzoate following injections applied several weeks before D. ponderosae colonization. The present data indicate that injections of emamectin benzoate applied in late summer or early fall will provide adequate levels of tree protection the following summer, and that, when emamectin benzoate is combined with propiconazole, tree protection is afforded the year that injections are implemented.

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trees or small-scale stands (e.g. <10 ha)⁴ and preventive applications of insecticides to individual trees.⁵ The latter have historically involved applications of liquid formulations of contact insecticides applied directly to the tree bole with hydraulic sprayers. Operationally, only high-value trees growing in unique environments or under unique circumstances are treated; for instance, trees in residential, recreational or administrative sites. Tree mortality in these environments generally results in undesirable impacts such as reduced shade, screening, aesthetics and visitor use. Dead trees pose potential risks to public safety, requiring routine inspection and eventual removal. Property values may decline as shade trees are lost.⁶ Trees growing in progeny tests, seed orchards or those genetically resistant to forest diseases (e.g. white pine blister rust) may also be considered for treatment, especially if epidemic populations of _D. ponderosae_ are present. During large-scale outbreaks, hundreds of thousands of trees may be treated annually in the western United States.⁵

Fettig _et al._⁷ reported that carbaryl is one of the most effective, economically viable and ecologically compatible insecticides available for protecting individual trees from colonization by _D. ponderosae_. Carbaryl treatments generally provide two field seasons of protection with a single application. However, applications on trees are continually being challenged, most recently on the basis of the toxicity of carbaryl spray deposition to foraging bees. Pyrethroids, such as permethrin and bifenthrin, are also effective, but generally only provide one field season of protection with a single application.⁷,⁸ While these treatments are widely used, they require transporting large equipment into remote areas, which can be problematic.⁵ Furthermore, concerns regarding the potential for spray drift to be deposited onto adjacent bodies of water are common, although evidence suggests drift poses little threat if appropriate no-spray buffers are used.⁹ However, susceptible trees within these buffers are left untreated and are therefore vulnerable to colonization by _D. ponderosae_.

Researchers attempting to find safer, more portable and longer-lasting alternatives to bole sprays have evaluated the effectiveness of injecting systemic insecticides directly into the lower bole of trees for several decades.⁵ Several active ingredients, including acephate,¹⁰ azadirachtin (neem),¹¹ dinotefuran,¹² fipronil,¹³,¹⁴ and oxydemeton methyl,¹⁵ were demonstrated to be ineffective for protecting trees from bark beetles. In more recent years, the efficacy of phloem-mobile active ingredients injected with pressurized systems capable of maintaining high pressures (>275 kPa) have been evaluated for several bark beetle species.⁵ For example, Grosman _et al._¹⁶ examined experimental formulations of emamectin benzoate for protecting individual conifers from mortality attributed to several bark beetle species in the western United States. Small quantities (usually <500 mL tree⁻¹ (total volume) based on tree size) were injected with the Arborjet Tree IV™ microinfusion system (Arborjet Inc., Woburn, MA), and later trees were challenged by baiting. While results for _D. ponderosae_ were inconclusive, a single injection of emamectin benzoate was effective for protecting _P. ponderosa_ from mortality attributed to western pine beetle, _D. brevicomis_ LeConte, for three field seasons,¹⁴ spurring additional research concerning the development of emamectin benzoate for protecting trees from bark beetle attacks in the western United States.⁵

The primary objectives of this study were (1) to determine the efficacy of bole injections of emamectin benzoate alone and combined with the fungicide propiconazole for protecting individual _P. contorta_ from mortality attributed to _D. ponderosae_ and progression of associated blue stain fungi, and (2) to determine whether timing of injection (mid-June versus mid-September) influences levels of efficacy. Like several species of _Dendroctonus_ and _Ophiostoma montium_ and _Grosmannia clavigera_, primarily in specialized structures of the integument called mycangia.⁷ These fungi are inoculated into the tree upon colonization by the beetle and rapidly spread throughout the phloem and sapwood.¹⁶ This causes ‘blue staining’ of the sapwood, while the heartwood is unaffected owing to its lower moisture content being incompatible with fungal growth. By combining emamectin benzoate with propiconazole higher levels of tree protection may occur. Emamectin benzoate is a macrocyclic lactone insecticide derived from avermectin B1 (= abamectin) by fermentation of the soil actinomycete _Streptomyces avermitilis_ (Burg _et al._) that disrupts neurotransmitters, causing irreversible paralysis.⁵ Propiconazole is a triazole fungicide that inhibits the 14-alpha demethylase enzyme and arrests cellular growth.¹⁷

### 2 MATERIALS AND METHODS

#### 2.1 Study area

This study was conducted in the Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, Utah, United States (40.643° N, 110.933° W; ~2865 m elevation) in 2009–2012. Site selection was based on aerial and ground surveys indicating that _D. ponderosae_ infestations were active in the area.¹⁸ Trees were located in forests with a live mean stand density of 26.6 m² basal area ha⁻¹, of which 71.1% was _P. contorta_ with a mean diameter at breast height (dbh, 1.37 m above ground level) of 20.4 cm. The remainder was represented by Engelmann spruce, _Picea engelmannii_ Parry ex Engelm., and subalpine fir, _Abies concolor_ (Hooker) Nuttall (Table 1). About 13.0% of standing _P. contorta_ and 22.5% of _P. contorta_ basal area had been killed by _D. ponderosae_ during the 3 years preceding initiation of this study (Table 1).

#### 2.2 Treatments and experimental design

Along a forest road (FS 80416), 210 live, uninfested _P. contorta_, 15–30 cm dbh, were selected for this study, with over 10 m between trees. Thirty randomly selected trees were assigned to each of seven treatments: (1) bole injections of emamectin benzoate (TREE-¨age No. 100–741; Syngenta Crop Protection Inc., Greensboro, NC) applied on 16–18 June 2009 at 10 mL 2.54 cm dbh⁻¹; (2) bole injections of emamectin benzoate (10 mL 2.54 cm dbh⁻¹) combined in solution with propiconazole (Alamo®; 14.3% AI, EPA Reg. No. 100–174; Syngenta Crop Protection Inc., Greensboro, NC) applied on 16–18 June 2009 at 10 mL 2.54 cm dbh⁻¹ undiluted (mean dbh ± SEM = 23.8 ± 0.7 cm); (3) bole injections of emamectin benzoate (10 mL 2.54 cm dbh⁻¹) combined in solution with propiconazole (Alamo®; 14.3% AI, EPA Reg. No. 100–741; Syngenta Crop Protection Inc., Greensboro, NC) applied on 16–18 June 2009 at 10 mL 2.54 cm dbh⁻¹ diluted in 30 mL of distilled water (mean dbh ± SEM = 22.6 ± 0.4 cm); (4) bole injections of emamectin benzoate + propiconazole applied on 15–19 September 2009 (as above for treatment 1) (mean dbh ± SEM = 22.9 ± 0.5 cm); (5–7) three separate untreated controls (mean dbh ± SEM = 22.8 ± 0.5, 22.4 ± 0.4 and 22.4 ± 0.4 cm respectively). One control group was used to assess _D. ponderosae_ population pressure during each field season (2009–2011), based on levels of tree mortality observed. There were no significant differences in tree dbh among treatments (F₆,203 = 1.4; P = 0.21), which is known to influence the susceptibility of _P. contorta_ to attack by _D. ponderosae_.³⁴ Treating 1 to 4 were injected directly into the tree bole at eight points ~0.3 m
Table 1. Stand conditions within the study area, Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, Utah (40.643° N, 110.933° W; ~2865 m elevation), 2009

<table>
<thead>
<tr>
<th></th>
<th>Pinus contorta</th>
<th>Picea engelmanni</th>
<th>Abies lasiocarpa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>26.6 ± 1.48</td>
<td>18.9 ± 4.3</td>
<td>7.6 ± 2.3</td>
</tr>
<tr>
<td><strong>Basal area (m² ha⁻¹)</strong></td>
<td>889.6 ± 293.4</td>
<td>696.8 ± 283.0</td>
<td>182.9 ± 55.6</td>
</tr>
<tr>
<td><strong>Trees ha⁻¹</strong></td>
<td>20.4 ± 1.7</td>
<td>21.3 ± 2.6</td>
<td>15.5 ± 0.8</td>
</tr>
<tr>
<td><strong>Mean dbh (cm)</strong></td>
<td>18.2 ± 0.6</td>
<td>18.4 ± 0.6</td>
<td>18.5 ± 0.5</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM, all trees ≥ 8.9 cm dbh (1.37 m height). Values are mean ± SEM, trees ≥ 8.9 cm dbh killed by *Dendroctonus ponderosae* within the last 3 years. Years since death based on crown condition.14

2.3 Tree mortality

Tree mortality was estimated initially on the basis of the presence, condition, distribution and density of *D. ponderosae* attacks (none, unsuccessful attack, strip attack and mass attack based on pitch tubes and boring dust)² on the tree bole in September of the year of baiting (e.g. 16–17 September 2009 for trees baited in 2009). However, mortality was based on the presence or absence of crown fade, an irreversible symptom of tree mortality, the following year (2010–2012). The only criterion used to determine the effectiveness of insecticide and insecticide + fungicide treatments was whether individual trees died as a result of colonization by *D. ponderosae*.19 Treatments were considered to have sufficient *D. ponderosae* pressure if ≥60% of the untreated, baited control trees died from *D. ponderosae* colonization.19 Treatments were considered to be efficacious when fewer than seven trees died as a result of *D. ponderosae* colonization while ≥60% of the untreated, baited control trees died (see Shea et al.19 for a complete description). This experimental design serves as a standard for such evaluations in the western United States and provides a very conservative test of efficacy.57

2.4 Blue stain

Blue stain was sampled in each experimental tree at ~1.37 m height on the northern aspect with an increment borer (4.3 mm; Haglof Co., Langsele, Sweden). The length of blue stain visible on each core was recorded from the phloem to the pith, and the area colonized by blue stain was calculated as a proportion of the cross-sectional area of each tree (see Fettig et al.28 for a complete description). Samples were collected at the end of the study (11–12 September 2012) to negate any impact on tree health during the study. It is important to note that the growth of blue stain fungi ceases within the first year of successful colonization by *D. ponderosae* owing to substantial declines in sapwood moisture content.21 A one-way analysis of variance (treatment) was performed on the proportion of cross-sectional area with blue stain, with α = 0.05 (SigmaStat v.12.0; Systat Software Inc., San Jose, CA). Data were tested for normality using the Shapiro–Wilk test and analyzed with non-parametric statistics (Kruskal–Wallis one-way analysis on ranks; SigmaStat v.12.0).

2.5 Propiconazole residue levels

Residue levels of propiconazole were determined in phloem (i.e. the target tissue where *D. ponderosae* feeds and fungal spores are inoculated) from ten randomly selected trees treated with emamectin benzoate + propiconazole in mid-June and ten randomly selected untreated controls (treatment 5). Samples (bark with phloem attached) were collected on 21–22 July 2009 and 16–17 September 2009 by punching a 2.54 cm hole through the outer bark with a leather drive punch at four aspects on the bole at 1, 2 and 3 m above ground level. Samples collected in September were obtained ~15 cm below those collected in July. All samples were placed in individual ziplock bags and shipped in coolers containing blue ice by overnight carrier to the Agricultural and Environmental Services Laboratory at The University of Georgia for further processing. Samples were received at the Agricultural and Environmental Services Laboratory within 48 h of field collection and assigned individual identification numbers. Samples were stored in a freezer until extraction was initiated, cores were cut in half and weighed and the half-core was placed in a 40 mL vial with 5 mL of ethyl acetate. The mixture was sonicated for 1 min, and the vial was placed in the refrigerator overnight. The following...
morning, the ethyl acetate extract was filtered through a glass wool plug and analyzed by gas chromatography. The PerkinElmer gas chromatograph was equipped with an NP detector and a ZB5 Megabore (0.53 mm) 30 m column. The column oven was programmed from 135 to 275 °C at 5 °C min⁻¹. A fortified sample and reagent blank were included with each set of samples. No interfering or compounds co-eluting with propiconazole were observed in the reagent blank or untreated samples. The recovery rates for wood chips fortified with 10 μg and allowed to dry varied from 65 to 80%. The propiconazole was quantitated by external standardization with a lower detection limit of 0.5 ppm following a three-point calibration. The chromatography is a modification of EPA Drinking Water Method 507 adapted to current laboratory analytical systems. A two-way analysis of variance (sample date and height) was performed on the concentration of propiconazole, with α = 0.05 (SigmaStat v.12.0).

2.6 Flight periodicity of Dendroctonus ponderosae

Five 16-unit multiple-funnel traps were baited with one D. ponderosae lure [trans-verbenol (~1.2 mg day⁻¹), exobrevicomin (~0.3 mg day⁻¹) and myrcene (~270 mg day⁻¹; Contech Inc.] and randomly dispersed throughout the study area to assess the flight activity of D. ponderosae. Traps were hung on 3 m metal poles with collection cups 80–100 cm above the ground. A Prozap Pest Strip (2,2-dichlorovinyl dimethyl phosphate; Loveland Industries Inc., Greeley, CO) was placed in the collection cup to kill arriving insects and reduce damage or loss to predacious insects. Traps were first deployed each year on 18 June 2009 and 16 June 2010, but trapping was discontinued in 2011 owing to the declining numbers of D. ponderosae collected (Fig. 1). Twelve collections were made each year on an approximate weekly basis. Specimens were later tallied and identified using available keys and voucher specimens.

2.7 Temperatures

Three HOBO data loggers (Onset Computer Corp., Bourne, MA) were attached to trees within the study area for accumulation of ambient and soil temperatures every 30 min during the first year following injections (from 16 June 2009 to 16 June 2010). Ambient temperatures were measured at a height of 1 m above ground level, and soil temperatures were measured at a depth of 10 cm.

3 RESULTS AND DISCUSSION

3.1 Phytotoxicity

No symptoms of phytotoxicity associated with bole injections of emamectin benzoate or emamectin benzoate + propiconazole were observed. Fettig et al. reported that phytotoxic effects were observed in one P. contorta injected with abamectin + tebuconazole, the latter a triazole fungicide commonly used to treat plant pathogenic fungi, but that the crown recovered the following year. However, this tree was the smallest in their sample population (dbh = 15 cm), a potential confounding factor. Doccola et al. evaluated several fungicides for toxicity against the blue stain fungus Ophiostoma minus (Hedgcock) Sydow & P. Sydow, which was artificially inoculated into loblolly pine, P. taeda L., by inserting a single 0.5 cm plug of malt agar contaminated with O. minus against the sapwood at breast height. In nature, P. taeda is inoculated with O. minus upon colonization by the southern pine beetle, D. frontalis Zimmermann, much in the same way following colonization of P. contorta by D. ponderosae and its fungal symbionts. They reported that trees injected with mono- and dipotassium salts of phosphorous acid exhibited phytotoxic effects, but that after 83 days the crowns recovered. Trees injected with propiconazole (even at rates twice that used in the present study) and 2-(4-thiazolyl) benzimidazole did not exhibit phytotoxic effects. Phytotoxicity has not been reported following injections of emamectin benzoate in pine, but few studies have been published.

3.2 Residue analyses

To the authors’ knowledge, no studies have examined propiconazole residues following bole injections in P. contorta. Residues were detected in phloem tissue at all three sample heights (1, 2 and 3 m) shortly (~4.5 weeks) after injections were implemented (Fig. 2), indicating that propiconazole had translocated within the tree from the point of injection (~0.3 m height). However, only four of ten trees sampled positive at ~4.5 weeks. All ten trees tested positive for propiconazole 3 months after injections (Fig. 2), and significantly higher concentrations were detected compared with those ~4.5 weeks after injection (F₁,5₄ = 6.2, P = 0.016). Sample height had no effect on residue concentrations (F₂,₅₄ = 0.35, P = 0.71).

Takai et al. analyzed twigs from Japanese black pine, P. thunbergii Palatore, and Japanese red pine, P. densiflora Siebold & Zuccarini, at 3, 15 and 27 months following injection with emamectin benzoate, and reported that levels sufficient for control of pine wood nematode, Bursaphelenchus xylophilus (Steiner & Buhler) Nickle, were present on each sample date. Two P. densiflora were also injected through one hole 0.5 m above the ground, and after 5 months each tree was felled and discs 10 cm thick were collected at 2, 4, 6 and 8 m above the injection point. They reported that emamectin benzoate spirals upwards counterclockwise in the sapwood. Their data suggest that emamectin benzoate concentrations decrease with increasing distance from the point of injection.

3.3 Progression of blue stain

All trees that died in this study sampled positive for the presence of blue stain, with the exception of one tree injected with emamectin benzoate + propiconazole in mid-September (Table
2). While blue stain was not recovered from this tree, it is possible that blue stain was present and not detected by the sampling method used. Blue stain was not detected in any tree treated with emamectin benzoate + propiconazole in mid-September (Table 2), but seven trees treated with emamectin benzoate + propiconazole in mid-June sampled positive (Table 2). Twelve and three trees treated with emamectin benzoate in mid-June and mid-September, respectively, sampled positive (Table 2). Twenty-three trees sampled positive for blue stain (26.4% of trees with blue stain) that were attacked by *D. ponderosae* at levels insufficient to cause tree mortality. There were many trees (123) attacked by *D. ponderosae* at sublethal levels from which blue stain was not detected. In conifers, induced defenses result in the formation of lesions surrounding the point of attack that contain high concentrations of secondary compounds toxic to the beetle, which also inhibit growth of symbiotic fungi.27

Injections of emamectin benzoate + propiconazole, regardless of treatment in mid-June or mid-September, resulted in a significant reduction in the proportion of cross-sectional area with blue stain compared with the 2009 and 2010 untreated controls (*H = 86.1, df = 6, P < 0.001*) (Fig. 3). However, only emamectin benzoate + propiconazole applied in mid-September was significantly different from the 2011 untreated control (Fig. 3). Injections of emamectin benzoate alone were not significantly different from those including propiconazole (Fig. 3), suggesting that effects may be an artifact of the large numbers of trees killed (and therefore containing blue stain) in the 2009 and 2010 untreated controls (Table 2). Furthermore, when analyzing only those trees that contained blue stain within each treatment (Table 2), no significant treatment effect was observed (*H = 7.1, df = 5, P = 0.216*). In this analysis, values ranged from 33.9 ± 4.5% (emamectin benzoate injected in mid-September) to 48.7 ± 3.1% (untreated control 2011) of the cross-sectional area colonized by blue stain.

### 3.4 Levels of tree mortality

In 2009, *D. ponderosae* pressure was sufficient to challenge treatments, as 80% of the untreated controls died (Table 2). Injections of emamectin benzoate + propiconazole applied in mid-June were effective for protecting individual *P. contorta* from mortality attributed to *D. ponderosae*; however, emamectin benzoate was ineffective (Table 2). *Dendroctonus ponderosae* pressure was also sufficient to challenge treatments in 2010, as 60% of the untreated controls died (Table 2). Mid-June injections of emamectin benzoate + propiconazole and mid-September injections of emamectin benzoate and emamectin benzoate + propiconazole were efficacious (Table 2). During 2011, no trees were killed in the emamectin benzoate and emamectin benzoate + propiconazole treatments (Table 2); however, *D. ponderosae* pressure was insufficient to challenge treatments, as only 2/30 control trees died (Table 2), precluding any determination of efficacy.19

### 3.4.1 Potential effect of blue stain

The contribution of blue stain fungi to the death of *P. contorta* colonized by *D. ponderosae* is under debate27 and has yet to be fully determined. It is clear that developing larvae and new adults obtain vital nutrients by feeding on associated fungal structures,23,29 Furthermore, some studies have shown that fungi associated with *D. ponderosae* are capable of causing direct tree mortality,21,30 but others have failed to demonstrate such an effect.31 The present data suggest that the addition of propiconazole to emamectin benzoate may have limited the progression of blue stain in some trees, but that the effect is masked by the proportion of trees killed, as already discussed. Doccola et al.24 reported that injections of propiconazole resulted in the smallest lesions surrounding areas where *O. minus* had been inoculated in *P. taeda*, suggesting that propiconazole was most effective for limiting the within-tree growth of *O. minus* among the fungicides assayed. The effect persisted for more than 2 years after treatment,24 likely influencing tree health. It is interesting to note that in the present study no trees treated with emamectin benzoate + propiconazole in mid-September sampled positive for blue stain, and that the same treatment applied in mid-June was effective for protecting trees from mortality attributed to *D. ponderosae* for two field seasons (Table 2).

### 3.4.2 Potential effects of temperature on tree physiology and timing of injection

Physiological responses of trees to temperature vary considerably; however, metabolic activity depends on the availability and transport of water, which ceases when tissues are frozen.32 Some photosynthesis may occur at temperatures below freezing (e.g. by conifers on sunny days in winter) when needles are unfrozen, but activity is limited owing to reduced or retarded absorption and transport of nutrients and water. Soil temperature is a major factor controlling root growth,33 but, because of the inherent difficulties of studying effects in forest trees, much of what is known is limited to studies on seedlings. Related work has demonstrated a root-zone threshold temperature of between 8 and 12 °C, above which root growth and normal physiological functioning occur in many conifers, including *Pi. engelmannii*, which was present in the area investigated in this study (Table 1), and *P. contorta*.33 –36 It appears that root growth starts at ~5 °C in *P. contorta*, increases rapidly at temperatures above 10 °C, attains maximum values at 20 °C and declines substantially at temperatures above 30 °C.35 Running
and Reid studied the influence of root-zone temperatures on *P. contorta* seedlings, and reported that root resistance was 67% of total plant resistance at 7 °C and 93% at 0 °C.

Several authors have speculated that low ambient and soil temperatures may explain the lack of efficacy observed with bole injections in high-elevation forests, as these factors may slow product uptake and transport. As such, failures in *P. contorta* were initially attributed to inadequate distribution of emamectin benzoate following injections made several weeks before trees were attacked by *D. ponderosae*. This argument is supported by the present data, as trees injected with emamectin benzoate in mid-June 2009 and challenged by baiting that field season (2009) suffered high levels of tree mortality, while the same treatments implemented in mid-September 2009 and challenged in 2010 did not (Table 2). Furthermore, only three trees that were treated with emamectin benzoate in mid-June 2009 and survived that year were killed the following (Table 2), suggesting that a higher level of protection was afforded in 2010 than in 2009.

Fettig et al. demonstrated that fall injections of abamectin alone and combined with tebuconazole were effective for protecting individual *P. contorta* from mortality attributed to *D. ponderosae*. Interestingly, injections of emamectin benzoate + propiconazole applied in mid-June provided adequate levels of tree protection for two field seasons (2009, 2010) when sufficient levels of *D. ponderosae* pressure occurred to make definitive conclusions regarding efficacy (Table 2). This suggests that the addition of fungicide may be essential if efficacy is desired the same year treatments are implemented.

Mean ambient and soil temperatures ranged from −28.2 ± 0.3 °C to 26.3 ± 1.2 °C and from −3.2 ± 0.2 °C to 11.1 ± 0.6 °C the year after injections were applied (n = 3) (Fig. 4). During this time, mean soil temperatures were over 5 °C on 107 days (Fig. 4, dashed line), but occurrence was primarily limited to July, August and September. Assuming that a mean soil temperature of 5 °C represents a threshold value of metabolic activity suitable for effective transport of product following injection in *P. contorta* (but see above) may explain the lack of efficacy observed with injections applied in mid-June. It is likely that initial *D. ponderosae* attacks occurred around 30 June 2009, based on the numbers of beetles collected on 16–30 June 2009 (Fig. 1), the occurrence of ambient temperatures above the flight threshold of year when the tree is actively translocating, adequate time is needed to allow for full distribution of the active ingredient within the tree before being attacked by *D. ponderosae*. Under optimal

### Table 2. Effectiveness of injections of emamectin benzoate and emamectin benzoate + propiconazole for protecting *Pinus contorta* from mortality attributed to colonization by *Dendroctonus ponderosae*, Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, Utah (40.643° N, 110.933° W; ~2865 m elevation), 2009–2012

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>2009 mortalityb</th>
<th>2010 mortalityc</th>
<th>2011 mortalityd</th>
<th>Cumulative mortality</th>
<th>Trees with blue staine</th>
<th>Live trees with blue stainf</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBS</td>
<td>7/30</td>
<td>3/27</td>
<td>0/20</td>
<td>10/30</td>
<td>12/30</td>
<td>2/20</td>
</tr>
<tr>
<td>EBPS</td>
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<td>0/25</td>
<td>5/30</td>
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<td>EBPF</td>
<td>—</td>
<td>1/30</td>
<td>0/29</td>
<td>1/30</td>
<td>0/30</td>
<td>0/29</td>
</tr>
<tr>
<td>Untreated control (2009)</td>
<td>24/30</td>
<td>0/6</td>
<td>0/6</td>
<td>24/30</td>
<td>27/30</td>
<td>3/6</td>
</tr>
<tr>
<td>Untreated control (2010)</td>
<td>18/30</td>
<td>4/12b</td>
<td>1/12a</td>
<td>22/30</td>
<td>25/30</td>
<td>3/8</td>
</tr>
<tr>
<td>Untreated control (2011)</td>
<td>—</td>
<td>2/30</td>
<td>2/30</td>
<td>13/30</td>
<td>11/28</td>
<td></td>
</tr>
</tbody>
</table>

a Emamectin benzoate (EB) and emamectin benzoate + propiconazole (EBP) were injected directly into the tree bole using the Arborjet Tree IV™ microinfusion system during 16–18 June (S) and 15–19 September 2009 (F).

b Mortality was based on the presence (dead) or absence (live) of crown fade in 2010.

c Mortality was based on the presence (dead) or absence (live) of crown fade in 2011.

d Mortality was based on the presence (dead) or absence (live) of crown fade in 2012.

e Four trees that were mass attacked during 2010 but with green foliage at the time of treatment evaluation in 2011, faded by 2012.

f Samples were collected at 1.37 m height on the northern aspect with an increment borer in 2012.
conditions (e.g. adequate soil moisture, moderate temperatures and good overall tree health), this takes ~4 weeks in some systems. However, it appears to take much longer in high-elevation forests where low soil temperatures (Fig. 4) retard absorption and transport of nutrients and water. By combining emamectin benzoate with propiconazole, efficacy is afforded the same year if injections are applied before beetle flight (i.e. as soon as snow melt permits access to the site).

These findings are promising, as bole injections represent essentially closed systems that eliminate drift and reduce non-target effects and applicator exposure. Accordingly, the authors suspect that bole injections will become more common for protecting *P. contorta* from mortality attributed to colonization by *D. ponderosae*, particularly in areas where bole sprays are desired but impractical (e.g. along property lines or within no-spray buffers). However, in the case of emamectin benzoate and emamectin benzoate + propiconazole, an obstacle that may affect use is the amount of time required to inject pines (78 ± 8 min in this study, but which appears to be an outlier) compared with bole sprays (<10 min tree^-1) primarily associated with uptake of the solution into the tree (i.e. the injection system can be installed and removed in several minutes). Future research should concentrate on the development of tools or tactics that will reduce the amount of time required for injections in *P. contorta*, and on alternative timings of injection in other high-elevation conifers (e.g. *P. engelmannii*) where previous attempts using bole injections for tree protection have been unsuccessful.

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REFERENCES

13. Fettig CJ, DeGomez TE, Gibson KE, Dabney CP and Borys RR, Effectiveness of permethrin plus-C (Masterline®) and carbyl (Sevin® SL) for protecting individual, high-value pines from bark beetle attack. *Arbor Urban For* **32**:201–207 (2008).

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