Research Article

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

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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.

Keywords: fungicides; insecticides; lodgepole pine; mountain pine beetle; Scolytinae; tree injections

1 INTRODUCTION

Bark beetles (Coleoptera: Curculionidae, Scolytinae) are primary disturbance agents in coniferous forests of the western United States. In particular, outbreaks of mountain pine beetle, *Dendroctonus ponderosae* Hopkins, have been severe, long lasting and well documented.[1] This species ranges throughout British Columbia and Alberta, Canada, most of the western United States, into northern Mexico, and colonizes several pine species, most notably lodgepole pine, *Pinus contorta* Dougl. ex Loud., ponderosa pine, *P. ponderosa* Dougl. ex Laws., sugar pine, *P. lambertiana* Dougl., whitebark pine, *P. albicaulis* Engelm., limber pine, *P. flexilis* James, and western white pine, *P. monticola* Dougl. ex D. Don.[2] Extensive levels of tree mortality associated with *D. ponderosae* outbreaks may result in host replacement by other tree species and plant associations, and may affect timber and fiber production, water quality and quantity, fish and wildlife populations, aesthetics, recreation, grazing capacity, real estate values, biodiversity, carbon storage, endangered species and cultural resources.

Current tactics for managing *D. ponderosae* include tree removals to reduce stand density (thinning),[3] sanitation harvests,[3] applications of semiochemicals to protect individual

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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.*[14] 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*, *D. ponderosae* carries symbiotic fungi (e.g. *Ophiostoma montium* and *Grosmannia clavigera*), primarily in specialized structures of the integument called mycangia.[2] 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 m^2 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-äge®, 4.0% active ingredient (AI), EPA Reg. No. 100-1309-74578; Arborjet Inc.] applied on 16–18 June 2009 at 10 mL 2.54 cm dbh^{-1} undiluted (mean dbh ± SEM = 23.8 ± 0.7 cm); (2) bole injections of emamectin benzoate (10 mL 2.54 cm dbh^{-1}) combined in solution with propiconazole (Alamo®, 14.3% AI, EPA 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); (3) bole injections of emamectin benzoate applied on 15–19 September 2009 (as above for treatment 1) (mean dbh ± SEM = 22.9 ± 0.5 cm); (4) bole injections of emamectin benzoate + propiconazole applied on 15–19 September 2009 (as above for treatment 2) (mean dbh ± SEM = 22.1 ± 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 ($F_{6,203} = 1.4$; $P = 0.21$), which is known to influence the susceptibility of *P. contorta* to attack by *D. ponderosae*.[2,3] Treatments 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

	Total	*Pinus contorta*	*Picea engelmanni*	*Abies lasiocarpa*
Alive[a]				
Basal area (m^2 ha^{-1})	26.6 ± 1.48	18.9 ± 4.3	7.6 ± 2.3	0.2 ± 0.1
Trees ha^{-1}	889.6 ± 293.4	696.8 ± 283.0	182.9 ± 55.6	9.9 ± 6.1
Mean dbh (cm)	18.4 ± 0.6	20.4 ± 1.7	21.3 ± 2.6	15.5 ± 0.8
Dead[b]				
Basal area (m^2 ha^{-1})	—	5.5 ± 3.5	—	—
Trees ha^{-1}	—	103.8 ± 54.4	—	—
Mean dbh (cm)	—	23.2 ± 3.6	—	—

[a] Values are mean ± SEM, all trees ≥8.9 cm dbh (1.37 m height).
[b] 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.[38]

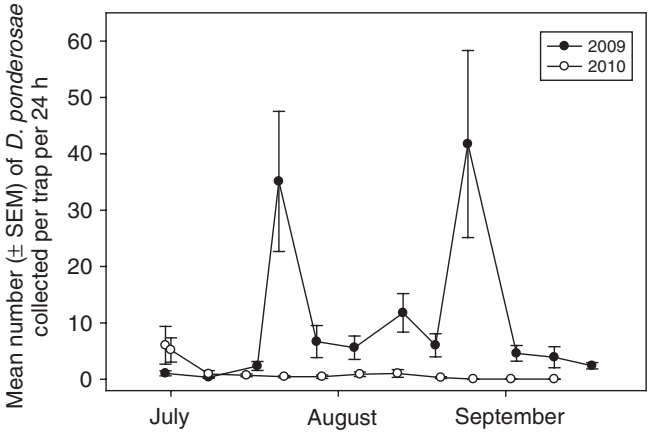

Figure 1. Mean number (± SEM) of *Dendroctonus ponderosae* captured in five 16-unit multiple-funnel traps baited with *D. ponderosae* lures, Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, Utah (40.643° N, 110.933° W; ~2865 m elevation), 2009–2010.

above the ground with the Arborjet Tree IV™ microinfusion system. The length of time to treat each tree, including the time required for all of the product to enter the tree, was 78 ± 8 min ($n = 60$), which is higher than reported in previous studies.[14]

One commercially available tree bait [*trans*-verbenol (~1.2 mg day^{-1}) and *exo*-brevicomin (~0.3 mg day^{-1}); Contech Inc., Delta, BC, Canada] was stapled to the bole of each tree in treatments 1, 2 and 5 at ~2 m height on the northern aspect on 21 July 2009 (i.e. not immediately upon injection, in order to allow chemicals time to translocate within the tree prior to being challenged by baiting[14]) and removed on 16 September 2009. All trees in treatments 1 to 4 that were alive in 2010 and the second group of untreated controls (treatment 6) were baited from 15 June to 28 September 2010. Similarly, all trees that did not succumb to mortality in 2009 and 2010 in treatments 1 to 4 and the third group of untreated controls (treatment 7) were baited from 16 June to 16 September 2011. The manufacturer estimates that the life expectancy of these baits is 100–150 days, depending on weather conditions, covering most of the flight activity period of *D. ponderosae* in this area (Fig. 1).

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.[5,7]

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.*[20] 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 $\alpha = 0.05$ (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.[22] A two-way analysis of variance (sample date and height) was performed on the concentration of propiconazole, with $\alpha = 0.05$ (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}), *exo*-brevicomin (~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[23] 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.*[20] 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.[20] Doccola *et al.*[24] evaluated several fungicides for toxicity against the blue stain fungus *Ophiosoma 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,[25] much in the same way following colonization of *P. contorta* by

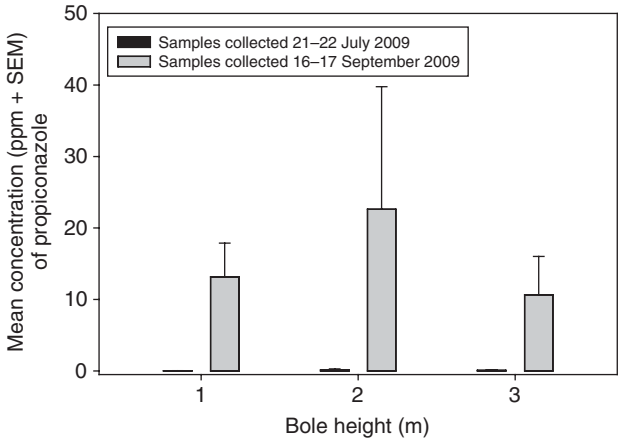

Figure 2. Mean concentration (+ SEM) of propiconazole in phloem tissue collected at three heights along the bole following injection of emamectin benzoate + propiconazole (14.3% AI) applied on 16–18 June 2009 at 10 mL 2.54 cm dbh^{-1} diluted in 30 mL of distilled water, Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, Utah (40.643° N, 110.933° W; ~2865 m elevation).

D. ponderosae and its fungal symbionts.[2] 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.[24] Phytotoxicity has not been reported following injections of emamectin benzoate in pine,[5] 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 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 ($F_{1,54} = 6.2$, $P = 0.016$). Sample height had no effect on residue concentrations ($F_{2,54} = 0.35$, $P = 0.71$).

Takai *et al.*[26] 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 & Buhrer) 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.[26]

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

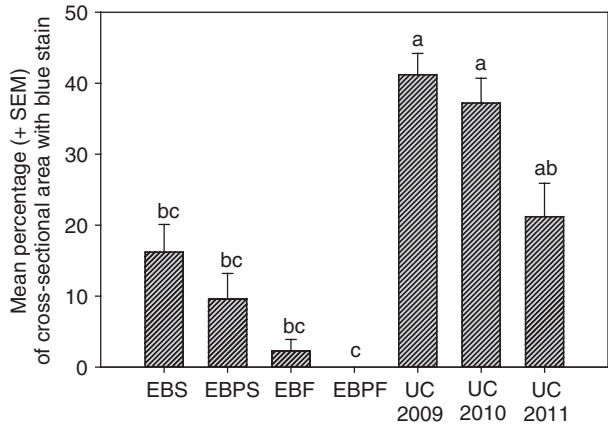

Figure 3. Mean percentage (+ SEM) of cross-sectional area with blue stain. Samples were collected at 1.37 m height on the northern aspect with an increment borer, Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, Utah (40.643° N, 110.933° W; ~2865 m elevation), 2012. Means followed by the same letter are not significantly different (Tukey's HSD; $P > 0.05$). Emamectin benzoate (EB) and emamectin benzoate + propiconazole (EBP) were injected during 16–18 June (S) and 15–19 September 2009 (F).

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] Beginning in 2011, a collapse in *D. ponderosae* populations and a reduction in associated levels of tree mortality were reported for much of the Uinta-Wasatch-Cache National Forest,[28] which confirms the present field observations and coincides with the large declines in trap catches observed the previous year (Fig. 1).

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 debate[27] 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.[27,29] 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

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

Treatment[a]	2009 mortality[b]	2010 mortality[c]	2011 mortality[d]	Cumulative mortality	Trees with blue stain[f]	Live trees with blue stain[f]
EBS	7/30	3/27	0/20	10/30	12/30	2/20
EBPS	1/30	4/29	0/25	5/30	7/30	2/25
EBF	—	1/30	0/29	1/30	3/30	2/29
EBPF	—	1/30	0/29	1/30	0/30	0/29
Untreated control (2009)	24/30	0/6	0/6	24/30	27/30	3/6
Untreated control (2010)	—	18/30	4/12[e]	22/30	25/30	3/8
Untreated control (2011)	—	—	2/30	2/30	13/30	11/28

[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.

and Reid[33] 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.[5,14] 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*.[5,14] 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.*[20] 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.[14] 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

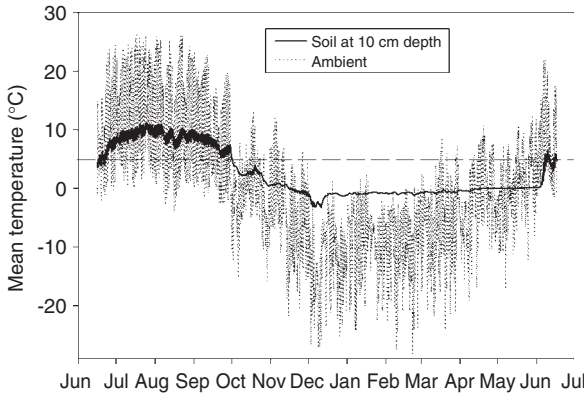

Figure 4. Mean ambient and soil temperatures recorded at half-hour intervals on three data loggers, Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, Utah (40.643° N, 110.933° W; ~2865 m elevation), 2009–2010.

value of 18.3 °C[37] (Fig. 4) and previous field observations in nearby areas (Munson AS, unpublished). In this context, trees injected in mid-June had ~2 weeks to transport product to the phloem during a period of time when soil temperatures were around 5 °C (Fig. 4), suggesting that only limited transport occurred. Treatments injected in mid-September had ~41 weeks for transport to occur, with several additional weeks when soil temperatures were over 5 °C (Fig. 4). The importance of the relationship between soil temperature, timing of injection and transport of product to the phloem is further substantiated by the detection of higher residues of propiconazole in phloem several months, as compared with several weeks, after injection (Fig. 2).

4 CONCLUSION

The present data indicate that emamectin benzoate is effective for protecting *P. contorta* from mortality attributed to *D. ponderosae*, but that it is critical for injections to occur the year before tree protection is required. While injections can be applied at any time 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

conditions (e.g. adequate soil moisture, moderate temperatures and good overall tree health), this takes ∼4 weeks in some systems.[14] 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.[5] 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)[5,14] 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. *Pi. engelmanni*) where previous attempts using bole injections for tree protection have been unsuccessful.

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