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Source: Journal of Entomological Science, 48(4):270-278.
Published By: Georgia Entomological Society
https://doi.org/10.18474/0749-8004-48.4.270
URL: http://www.bioone.org/doi/full/10.18474/0749-8004-48.4.270

Efficacy of Abamectin and Tebuconazole Injections to Protect Lodgepole Pine from Mortality Attributed to Mountain Pine Beetle (Coleoptera: Curculionidae) Attack and Progression of Blue Stain Fungi[1]

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J. Entomol. Sci. 48(4): 270-278 (October 2013)

Abstract Bark beetles (Coleoptera: Curculionidae, Scolytinae) are important tree mortality agents in western coniferous forests. Protection of individual trees from bark beetle attack has historically involved applications of liquid formulations of contact insecticides to the tree bole using hydraulic sprayers. More recently, researchers have examined the effectiveness of injecting small quantities of systemic insecticides directly into trees, but early efforts were largely unsuccessful. In this study, we determine the efficacy of fall (16 - 18 September) injections of abamectin (Abacide™ 2Hp; Mauget Inc., Arcadia, CA) alone and combined with tebuconazole (Tebuject™ 16, Mauget Inc.) for protecting individual lodgepole pine, *Pinus contorta* Dougl. ex Laws., from mortality attributed to mountain pine beetle, *Dendroctonus ponderosae* Hopkins. Both abamectin and abamectin + tebuconazole were efficacious for one field season, whereas results from a second field season were inconclusive due to insufficient beetle pressure. To our knowledge, this is the first demonstration of the successful application of a systemic insecticide for protecting *P. contorta* from mortality attributed to *D. ponderosae*.

Key Words *Dendroctonus ponderosae*, fungicides, insecticides, *Pinus contorta*, tree injections

Bark beetles (Coleoptera: Curculionidae, Scolytinae), a large and diverse group of insects consisting of ~550 species in North America, are primary disturbance agents in coniferous forests of the western U.S. Population levels of a number of species (<1%) oscillate periodically, often reaching densities that result in extensive levels of tree mortality when favorable climatic and forest conditions coincide (Fettig et al. 2007, Bentz et al. 2010). In particular, recent outbreaks of mountain pine beetle, *Dendroctonus ponderosae* Hopkins, have been severe, long-lasting and well documented (Bentz et al. 2009). This species ranges throughout British Columbia and Alberta, Canada, most of the western U.S., 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.

[1]Received 27 Novemeber 2012; accepted for publication 24 February 2013.
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ex D. Don. (Gibson et al. 2009). Extensive levels of tree mortality associated with *D. ponderosae* outbreaks may result in host replacement by other tree species and plant associations that may impact timber and fiber production, water quality and quantity, fish and wildlife populations, aesthetics, recreation, grazing capacity, real estate values, biodiversity, carbon storage, threatened and endangered species, and cultural resources. About 8% of forests in the U.S. are classified at high risk (defined as >25% of stand density will be lost in the next 15 years) to insect and disease outbreaks, and *D. ponderosae* is ranked most damaging of all mortality agents considered (Krist et al. 2007).

Dendroctonus ponderosae initiates and concentrates attacks in the lower tree bole, facilitating host colonization through the use of aggregation pheromones (Pitman et al. 1968, Pitman and Vité 1969, Ryker and Libbey 1982). Like several species of *Dendroctonus*, *D. ponderosae* carries symbiotic blue-stain fungi [e.g., *Ophiostoma montium* (Rumbold von Arx) and *Grosmannia clavigera* (Robinson-Jeffrey & R.W. Davidson)], primarily in specialized structures of the integument called mycangia. These fungi are inoculated into the tree upon colonization by the beetle, and rapidly spread throughout the phloem and sapwood (Solheim 1995). This causes blue pigmentation or "blue staining" of the sapwood whereas the heartwood is unaffected due to its lower moisture content being incompatible with fungal growth. Developing larvae and new adults obtain vital nutrients by feeding on associated fungal structures (Six and Paine 1998), but scientists debate the contribution of blue stain fungi in the death of trees attacked by *D. ponderosae* (Six and Wingfield 2011).

Current tactics for managing *D. ponderosae* infestations include treatments that reduce stand density (thinning) and presumably host susceptibility (Fettig et al. 2007), sanitation harvests that remove infested trees (Fettig et al. 2007), applications of semiochemicals to protect individual trees or small-scale stands (e.g., <10 ha) (Gillette and Munson 2009), and applications of insecticides to protect individual trees (Fettig et al. 2013). Protecting individual trees from *D. ponderosae* has historically involved applications of liquid formulations of contact insecticides applied directly to the tree bole using hydraulic sprayers. In an operational context, only high-value trees growing in unique environments or under distinct circumstances are treated with insecticides. These may include trees in residential, recreational (e.g., campgrounds) or administrative sites. Tree losses in these environments generally result in undesirable impacts such as reduced shade, screening, aesthetics and visitor use. Dead trees pose potential hazards to public safety, requiring routine inspection (Johnson 1981) and eventual removal. Furthermore, property values may be significantly impacted (McGregor and Cole 1985). In addition, trees growing in progeny tests, seed orchards, or those genetically resistant to forest diseases (e.g., white pine blister rust) may be considered for preventative treatments, especially if epidemic populations of *D. ponderosae* exist in the area. During large-scale outbreaks, hundreds of thousands of trees may be treated annually with insecticides (Fettig et al. 2013).

Fettig et al. (2006) reported that carbaryl remains as one of the most effective, economically viable, and ecologically-compatible insecticides available for protecting individual trees from *D. ponderosae* attack, and generally provides 2 field seasons of protection with a single application. However, uses on trees are continually being challenged, most recently on the basis of the toxicity of carbaryl spray deposition to foraging bees. Bole applications of pyrethroids, such as permethrin and bifenthrin, are also effective, but may only provide 1 field season of protection with a single application. Whereas these treatments are widely used, they require transporting large equipment into remote areas, which can be problematic. This is an important concern when treating *P. contorta* at high elevations (>2400 m) in the western U.S. where snow loads in May-June often preclude access, preventing treatment prior to the initiation of flight activity by *D. ponderosae*. Furthermore, concerns regarding the potential for spray drift to be deposited onto adjacent bodies of water are common, although recent evidence suggests drift poses little threat if appropriate no-spray buffers are used (Fettig et al. 2008). However, trees within these buffers are often left untreated and 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 small quantities of systemic insecticides directly into the lower bole. Early work indicated that several methods, active ingredients and formulations were ineffective (Fettig et al. 2013). In 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 in the western U.S. For example, Grosman et al. (2010) examined experimental formulations of emamectin benzoate and fipronil for protecting individual trees from mortality attributed to several bark beetle species. Small quantities [usually <500 ml tree (total volume) based on tree size] were injected with the Arborjet Tree IV™ microinfusion system (Arborjet Inc., Woburn, MA), and subsequently challenged by baiting. Whereas results for *D. ponderosae* were inconclusive, a single injection of emamectin benzoate protected *P. ponderosa* from western pine beetle, *D. brevicomis* LeConte, for 3 field seasons (Grosman et al. 2010). To our knowledge, this was the first successful application of an injected systemic insecticide used to protect individual trees from bark beetle attack in the western U.S. Other research confirmed bole injections of fipronil were ineffective for protecting *P. contorta* from mortality attributed to *D. ponderosae* (Fettig et al. 2010).

Conditions such as lower ambient air and soil temperatures and higher soil moistures may help explain the lack of efficacy observed for *D. ponderosae* as these factors may slow product uptake and translocation within *P. contorta* in high-elevation forests. As such, failures for protecting *P. contorta* from mortality attributed to *D. ponderosae* were initially attributed to inadequate distribution of the active ingredient following injections made several wks prior to trees coming under attack by *D. ponderosae* (Grosman et al. 2010, Fettig et al. 2013). Based on this and the efficacy of abamectin demonstrated in the southern U.S. for a complex of engraver beetles (D.G.M., unpubl. data), we examined the efficacy of fall (16 - 18 September) injections of abamectin (Abacide™ 2Hp; Mauget Inc., Arcadia, CA) alone and combined with tebuconazole (Tebuject™ 16, Mauget Inc.) for protecting individual *P. contorta* from mortality attributed to *D. ponderosae* attack and the inoculation and spread of blue stain fungi. Abamectin (= avermectin B1) is a natural fermentation product of a soil actinomycete, *Streptomyces avermitilis* (Burg et al.), and acts on insects by interfering with neural and neuromuscular transmission, similar to emamectin benzoate. Tebuconazole is a triazole fungicide used to treat plant pathogenic fungi.

Materials and Methods

This study was conducted on the Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, UT (40.38° N, 110.56° W; ~2,865 m elevation), 2009 - 2012. Site selection was based on aerial and ground surveys indicating *D. ponderosae* infestations were active in this area (Blackford et al. 2010). One hundred twenty (120) apparently-healthy *P. contorta*, 15 - 30 cm diameter at breast height (dbh, 1.37 m above ground level) were selected along a forest road with a minimum of 10 m between adjacent trees. Thirty randomly-selected trees were assigned to each of 4 treatments: (1) bole injections of Abacide™ 2Hp [1.9% active ingredient (a.i.); EPA No. 7,946 - 30; Mauget Inc.] applied at 20 ml per 2.54 cm dbh (mean dbh ± SEM = 22.4 ± 0.6 cm); (2) bole injections of Abacide™ 2Hp (as above) combined in solution with Tebuject™ 16 (16.0% a.i.; EPA No. 7,946 - 28; Mauget Inc.) applied at 6 ml per 2.54 cm dbh (mean dbh ± SEM = 21.3 ± 0.6 cm); and 2 separate untreated controls (3 and 4) (mean dbh ± SEM = 22.4 ± 0.4 cm for both). There were no significant differences in tree dbh among treatments ($F_{3,116} = 0.8$; $P = 0.49$), which is known to influence the susceptibility of *P. contorta* to *D. ponderosae* (Gibson et al. 2009). Treatments 1 and 2 were injected directly into the tree bole at 8 points (locations) ~0.3 m above the ground using the Arborjet Tree IV™ microinfusion system (Arborjet Inc.) during 16 - 18 September 2009. Treated trees were allowed ~37 wks to translocate active ingredients prior to baiting. One commercially-available tree bait [*trans*-verbenol (1.2 mg/d) and *exo*-brevicomin (0.3 mg/d); Contech Inc., Delta, BC] was stapled to the bole of each tree at ~2 m in height on the northern aspect on 15 June and removed 28 September 2010. In 2011, all surviving trees in treatments 1 and 2, and the second group of untreated controls (treatment 4) were baited from 16 June to 16 September. The manufacturer estimates the life expectancy of these baits is ~100 - 150 d depending on weather conditions (www.pherotech.com/page194.htm), which covers the major flight activity period of *D. ponderosae* in this area (C.J.F. et al., unpubl. data). One control group was used to assess beetle pressure during the first field season (2010), and the second used to assess beetle pressure the second field season (2011).

Blue stain was sampled in each experimental tree at 1.37 m in height on the northern aspect with an increment borer (4.3 mm; Haglof Co., Langsele, Sweden). The length of blue stain visible on each sample 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. Samples were collected at the end of the study (11 - 12 September 2012) to negate impacting tree health during the study. It is important to note that the growth of blue stain fungi ceases within the first year of successful attack by *D. ponderosae* due to substantial declines in sapwood moisture content (Kim et al. 2005). Tree mortality was initially estimated based on the presence, distribution and density of *D. ponderosae* attacks (none, unsuccessful attacks, strip attack and mass attack) (Gibson et al. 2009) in September of the year of baiting (e.g., 27 - 28 September 2010 for trees baited in 2010), however mortality was based on the presence or absence of crown fade, an irreversible symptom of tree mortality, the following year (2011 and 2012).

A one-way analysis of variance (treatment) was performed on the proportion of cross-sectional area with blue stain using α=0.05 (SigmaStat Version 12.0, Systat Software, Inc., San Jose, CA). Data were tested for normality using the Shapiro-Wilk test, and analyzed with nonparametric statistics (Kruskal-Wallis One Way Analysis on Ranks; SigmaStat Version 12.0) when appropriate. The only criterion used to determine the effectiveness of treatments was whether individual trees succumbed to attack by *D. ponderosae*. Treatments were considered to have sufficient beetle pressure if ≥60% of the untreated, baited control trees died of *D. ponderosae* attack. Treatments were considered efficacious when <7 trees died as a result of bark beetle attack if ≥60% of the untreated, baited control trees died (Shea et al. 1984 for a complete description). This experimental design serves as a standard for such evaluations in the western U.S. and provides a very conservative test of efficacy (reviewed in Fettig et al. 2013).

Results and Discussion

During this study, we observed no definitive evidence of external symptoms of phytotoxicity associated with any treatment. However, 1 tree (#470, abamectin + tebuconazole) with only limited evidence of unsuccessful attacks by *D. ponderosae* that sampled negative for blue stain may have suffered some phytotoxic effects based on the unusual pattern of fade observed in the lower crown. However, whereas these symptoms were visible in September 2010 and June 2011, the crown recovered shortly thereafter and symptoms were not visible in September 2011 and 2012. This tree was the smallest in the study (dbh = 15 cm), which may have been a contributing factor given that all trees were injected at 8 points around the bole resulting in higher levels of injury for this tree (per unit size) than others injected with abamectin + tebuconazole (mean dbh = 21.3 cm). Average uptake time (i.e., the amount of time required for trunk injected solutions to completely enter the tree) was ~12 min.

In 2010, *D. ponderosae* pressure was sufficient to adequately challenge these treatments as 60% of the untreated controls died (Table 1). Fall injections of both abamectin and abamectin + tebuconazole were effective for protecting individual *P. contorta* from mortality attributed to *D. ponderosae* as no mortality occurred in either treatment (Table 1). Unfortunately, *D. ponderosae* pressure was insufficient to adequately challenge these treatments in 2011 as only 2/30 control trees died (Table 1) preventing us from determining efficacy during the second field season. Similar to our

Table 1. Effectiveness of bole injections of abamectin and abamectin + tebuconazole for protecting *Pinus contorta* from mortality attributed to colonization by *Dendroctionus ponderosae*, Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, UT (40.38° N, 110.56° W; ~2,865 m elevation), 2009 - 2012.

Treatment*	2010 Mortality**	2011 mortality†	Cumulative mortality	Trees with bluestain§	Live trees with bluestain§
Abamectin	0/30	4/30	4/30	6/30	2/26
Abamectin + tebuconazole	0/30	0/30	0/30	3/30	3/30
Untreated control (2010)	18/30	4/12‡	22/30	25/30	3/8
Untreated control (2011)	-	2/30	2/30	13/30	11/28

* Abamectin and abamectin + tebuconazole were injected directly into the tree bole using the Arborjet Tree IV™ microinfusion system (Arborjet Inc., Woburn, MA) during 16 - 18 September 2009.

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

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

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

§ Samples were collected at 1.37 m in height on the northern aspect with an increment borer in 2012.

observations, DeBlander et al. (2012) reported a ~70% reduction in tree mortality attributed to *D. ponderosae* throughout much of the area in 2011. Grosman et al. (2010) stated that in high-elevation forests injecting trees in the fall and allowing for an extended period of translocation (over several months) prior to baiting could perhaps increase efficacy, which appears to be supported by our data. Future work should consider this when planning bole injections in *P. contorta* forests.

Forty-seven (of 120) trees exhibited blue stain including several (19 trees, 40.4% of trees with blue stain) that had been attacked by *D. ponderosae* at levels insufficient to cause tree mortality. Alternatively, there were many trees (73) attacked by *D. ponderosae* at sublethal levels from which blue stain was not detected. A significant treatment effect was observed ($H = 38.9$, $df = 3$, $P < 0.01$). Fall injections of abamectin + tebuconazole resulted in a significant reduction in the proportion of cross-sectional area with blue stain compared with both untreated controls, but were not significantly different from abamectin alone (Fig. 1). Abamectin was only significantly different from the 2010 untreated control, which had a much higher proportion of trees killed compared with the 2011 untreated control (Table 1, Fig. 1). However, when analyzing only those trees that contained blue stain within each treatment (Table 1), no significant treatment effect was observed ($F_{3,43} = 2.48$, $P = 0.074$), which may be an artifact of the low statistical power (0.354) associated with the test. In this analysis, trees treated

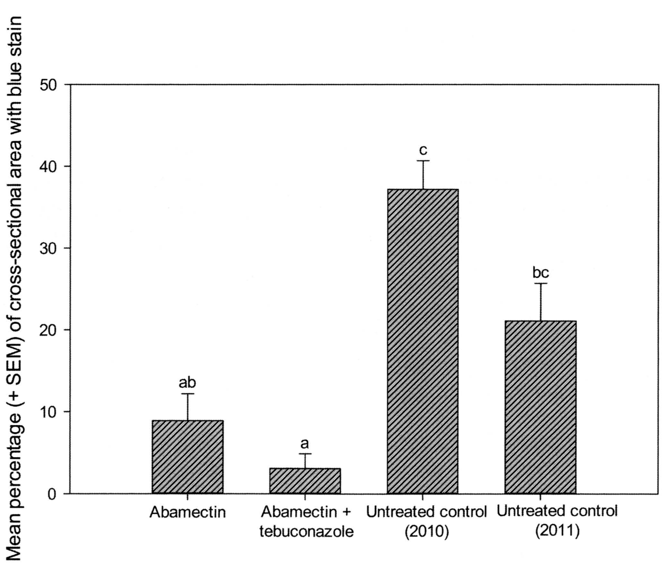

Fig. 1. Mean percentage (+ SEM) of cross-sectional area with blue stain. Samples were collected at 1.37 m in height on the northern aspect with an increment borer. The length of blue stain visible from the phloem to the pith was recorded for each sample, Heber-Kamas Ranger District, Uinta-Wasatch-Cache National Forest, UT (40.38° N, 110.56° W; ~2,865 m elevation), 2012. Means followed by the same letter are not significantly different (Tukey's HSD; $P > 0.05$).

with abamectin + tebuconazole had 31.2 ± 8.5% (mean ± SEM) of their cross-sectional area colonized by blue stain compared with 44.6 ± 3.1, 44.7 ± 1.8, and 48.7 ± 3.1% for abamectin, the 2010 untreated control and the 2011 untreated control, respectively. Given these data, it appears the addition of tebuconazole to abamectin may have limited the progression of blue stain in some trees attacked by *D. ponderosae*, but that the effect is masked by the proportion of trees killed. That is, in our study all trees that died sampled positive for blue stain, but only a small portion (20.6%) of trees that survived *D. ponderosae* attacks had blue stain (Table 1). Some studies have shown that fungi associated with *D. ponderosae* are capable of causing direct tree mortality (Yamaoka et al. 1995, Kim et al. 2008). It is interesting to note that 13.3% of the trees treated with abamectin died during 2011, whereas no mortality was observed in trees treated with abamectin + tebuconazole (Table 1).

To our knowledge, this is the first demonstration of a successful application of an injected systemic insecticide for protecting *P. contorta* from mortality attributed to *D. ponderosae*. Both abamectin and abamectin + tebuconazole were efficacious for 1 field season, whereas results from the second field season were inconclusive. Other recent advances in methods and formulations for individual tree injection are promising (reviewed by Fettig et al. 2013), as tree injections represent essentially closed systems that eliminate drift, and reduce nontarget effects and applicator exposure. Accordingly, we suspect tree injections will become a more common tool for protecting trees from bark beetle attack in the near future, particularly in areas where bole sprays are not practical (e.g., along property lines or within no-spray buffers). Finally, the use of all bark beetle management tools should be considered in an integrated approach.

Acknowledgments

The authors thank C.P. Dabney and S.R. McKelvey (Pacific Southwest Research Station, USDA Forest Service), V. DeBlander, L. Dunning, C. Hayes and J. Neumann (Forest Health Protection-Intermountain Region, USDA Forest Service), J. Runyon (Rocky Mountain Research Station, USDA Forest Service), and W. Upton (Texas A&M Forest Service) for technical assistance. In addition, the authors thank J. Gibson of the Uinta-Wasatch-Cache National Forest (Utah) for providing access to the study site, and M. Waindle (Mauget Inc.) for her helpful insights. This research was supported, in part, by a grant (FS agreement 10-CO-11272164-006) from Mauget Inc., by in-kind contributions from Arborjet Inc., and by the USDA Forest Service (Pacific Southwest Research Station and Forest Health Protection), and Texas A&M Forest Service (Forest Health).

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides in the United States must be registered by appropriate State and/or Federal agencies before they can be recommended.

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