Effectiveness of Emamectin Benzoate and Propiconazole for Protecting *Picea engelmannii* (Pinales: Pinaceae) from Mortality Attributed to *Dendroctonus rufipennis* (Coleoptera: Curculionidae) in Wyoming[1]

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Abstract Spruce beetle, *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae), causes significant mortality of mature spruce, *Picea* spp. (Pinales: Pinaceae), in western North America. We evaluated the effectiveness of two formulations of bole injections of emamectin benzoate (TREE-äge® G4 and TREE-äge R10; Arborjet, Inc., Woburn, MA) alone and combined with propiconazole (Propizol®; Arborjet, Inc.) for protecting Engelmann spruce, *Picea engelmannii* Parry ex Engelm., from mortality attributed to colonization by *D. rufipennis*. Treatments were injected 16–19 July 2017, and levels of *Pi. engelmannii* mortality were determined for 2018 and 2019. Each experimental tree ($n = 30$, including an untreated control group) was baited with aggregation pheromone (frontalin) June–September 2018. All surviving treated *Pi. engelmannii* and a second untreated control group were baited June–September 2019. Both TREE-äge G4 and TREE-äge R10 significantly reduced levels of *Pi. engelmannii* mortality compared with the untreated control; however, protection was limited to one field season. Protection was increased to two field seasons by combining TREE-äge G4 and TREE-äge R10 with Propizol. The addition of Propizol, a triazole fungicide, likely reduced the progression of blue stain within treated *Pi. engelmannii* increasing tree health and resistance to *D. rufipennis*.

Key Words Engelmann spruce, fungicides, insecticides, spruce beetle, tree protection

In the western United States, protection of conifers from bark beetles (Coleoptera: Curculionidae) often involves liquid formulations of contact insecticides applied to the tree bole with ground-based sprayers (Fettig et al. 2013). Bole sprays are limited to high-value trees, such as those growing in residential areas, recreational sites (e.g., campgrounds), administrative sites and seed orchards, and applied prior to initiation of the adult flight period of the target bark beetle species. These treatments are very effective when properly applied, but limited to within ~100 m of vehicle access due to the large equipment involved and to >15.2 m from riparian areas due to concerns regarding insecticide drift onto adjacent bodies of

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water and associated nontarget effects (Fettig et al. 2008, 2013). Although several methods exist for protecting conifers from bark beetles (Fettig and Hilszczański 2015), thousands of trees may be treated annually with bole sprays in the western United States during large-scale outbreaks, such as those observed for several bark beetle species during the last two decades (Fettig et al. 2021).

Researchers attempting to find safer, more portable, and longer-lasting alternatives to bole sprays have evaluated injecting systemic insecticides directly into trees. Early research indicated that several methods, active ingredients, and formulations were largely ineffective, but phloem-mobile active ingredients, such as emamectin benzoate, have shown great promise. For example, experimental formulations of emamectin benzoate protected ponderosa pine, *Pinus ponderosa* Douglas ex Lawson (Pinales: Pinaceae), from mortality attributed to western pine beetle, *Dendroctonus brevicomis* LeConte, in California for three field seasons (Grosman et al. 2010). This and other research conducted in the southeastern United States (e.g., Grosman and Upton 2006, Grosman et al. 2009) led to registration of a commercial formulation of emamectin benzoate for protecting conifers from mortality attributed to bark beetles in the United States (TREE-äge®, EPA Reg. No. 100-1309-74578; Arborjet, Inc., Woburn, MA). Later, TREE-äge was demonstrated effective for protecting lodgepole pine, *P. contorta* Dougl., from mortality attributed to mountain pine beetle, *D. ponderosae* Hopkins, in Utah (Fettig et al. 2014) and Engelmann spruce, *Picea engelmannii* Parry ex Engelm. (Pinales: Pinaceae), from mortality attributed to spruce beetle, *D. rufipennis* Kirby, in Utah (Fettig et al. 2017, 2020). *Dendroctonus rufipennis* is one of the more destructive forest insect pests in North America and the most significant threat to mature spruce trees (Fettig et al. 2021). Outbreaks are and have impacted significant areas in Colorado (Colorado Department of Natural Resources 2020), Utah (Utah Department of Natural Resources Forestry, Fire & State Lands 2019), Wyoming (USDA Forest Service 2019), and Alaska (USDA Forest Service 2020).

Fettig et al. (2017, 2020) evaluated the efficacy of TREE-äge injected in spring (mid-June) and fall (mid-August) the year prior to *Pi. engelmannii* being challenged by *D. rufipennis* by baiting. Treatments were injected using the Tree IV™ system (Arborjet, Inc.) through plugs inserted in the root collar at two spacings (i.e., the number of plugs based on one plug per 7.6 cm or 15.2 cm diameter at breast height [dbh], 1.37 m in height). The authors concluded TREE-äge injected at a narrow spacing (7.6 cm) in spring was the only effective treatment. In general, research has shown that bole injections may be applied at any time of year when trees are actively transpiring; however, sufficient time (weeks to almost 1 yr) is required for the active ingredients to reach the phloem where bark beetles feed. This requires distinct timing of injections in different bark beetle-host systems (reviewed in Table 1 in Fettig et al. 2020 for the western United States), and is particularly important in subalpine forests where cold air and soil temperatures retard transpiration and transport of active ingredients within *Pi. engelmannii* (Fettig et al. 2014).

During host colonization, blue stain fungi are inoculated into trees by *D. rufipennis* (Six and Bentz 2003). These fungi benefit *D. rufipennis* by providing nutrients and detoxifying tree defensive compounds (Davis et al. 2019), among other factors, and may negatively impact tree health. Research by Fettig et al. (2014) demonstrated that combining TREE-äge with propiconazole, a triazole fungicide commonly used in agriculture, increased the efficacy of TREE-äge for protecting *P. contorta* from *D. ponderosae*. Furthermore, Fettig et al. (2014) sampled the amount of blue stain in each experimental tree and found that propiconazole reduced the proportion of cross-sectional area with blue stain (Fettig et al. 2014), with similar results being demonstrated for other blue stain fungi in western white pine, *P. monticola* Dougl. ex D. Don (Doccola et al. 2020, Wyka et al. 2016), and loblolly pine, *P. taeda* L. (Doccola et al. 2011). Based on these studies, Fettig et al. (2020) hypothesized that combining TREE-äge with propiconazole would increase the efficacy of TREE-äge for protecting *Pi. engelmannii* from mortality attributed to *D. rufipennis*. Injections were applied the fall prior to *Pi. engelmannii* being challenged by *D. rufipennis* by baiting, and no difference in efficacy was observed between TREE-äge and TREE-äge + propiconazole. Furthermore, no difference was observed between TREE-äge and TREE-äge + propiconazole in the maximum depth of blue stain in the sapwood. The authors suggested future studies should evaluate combinations of emamectin benzoate and propiconazole injected into *Pi. engelmannii* at least 1 yr before being challenged by *D. rufipennis*.

The objective of this study was to determine the effectiveness of two newer formulations of emamectin benzoate (TREE-äge G4 and TREE-äge R10, the latter an experimental formulation at the time of study; Arborjet, Inc.) alone and combined with propiconazole (Propizol®; Arborjet, Inc.) for protecting *Pi. engelmannii* from mortality attributed to *D. rufipennis* in Wyoming. TREE-äge G4 is a general use systemic insecticide containing 4% emamectin benzoate and was first registered with the U.S. Environmental Protection Agency (EPA) in 2015. TREE-äge R10 is a restricted use systemic insecticide containing 9.7% emamectin benzoate and was first registered with the EPA in 2018. Propizol is a systemic fungicide containing 14.3% propiconazole.

Materials and Methods

This study was conducted on the Bridger-Teton National Forest, Wyoming (first tree: 43.80°N, 110.21°W, 2,593 m elevation; last tree: 43.81°N, 110.24°W, 2,582 m elevation), 2017–2021. In June 2018, surrounding stands had a mean live tree (12.7 cm dbh) density of 43.7 m^2/ha of basal area of which (based on the number of trees) 79.5% was *Pi. engelmannii,* 17.4% was subalpine fir, *Abies lasiocarpa* (Hooker) Nuttall (Pinales: Pinaceae), and 3.1% was *P. contorta* based on 12 0.041-ha circular plots randomly distributed throughout the study area. In these plots, 56.7% of live *Pi. engelmannii* (mean dbh = 38.9 cm) had evidence of colonization by *D. rufipennis* when data were collected. Thirty uninfested *Pi. engelmannii* ($N = 180$) >25 cm dbh were randomly assigned to each of six treatments: (1) TREE-äge G4 (EPA Reg. No. 74578-10) injected at 7.5 ml/2.54 cm dbh (mean dbh ± SEM = 38.6 ± 1.4 cm), (2) TREE-äge G4 injected at 7.5 ml/2.54 cm dbh + Propizol (EPA Reg No. 74578-8) injected at 6 ml/2.54 cm dbh mixed with 6 ml/2.54 cm dbh of distilled water (mean dbh ± SEM = 42.5 ± 2.0 cm), (3) TREE-äge R10 (EPA Reg. No. 74578-12) injected at 3.2 ml/2.54 cm dbh (mean dbh ± SEM = 38.1 ± 1.5 cm), (4) TREE-äge R10 injected at 3.2 ml/2.54 cm dbh + Propizol injected at 6 ml/2.54 cm dbh mixed with 6 ml/2.54 cm dbh of distilled water (mean dbh ± SEM = 37.1 ± 1.1 cm), (5) untreated control (2018) (mean dbh ± SEM = 45.2 ± 2.0 cm), and (6) untreated control (2019) (mean dbh ± SEM = 40.3 ± 1.9 cm). Adjacent experimental trees were separated by >10 m.

Mean temperature and relative humidity during injections were 20.9°C (range = 12.8–27.2°C) and 48.2% (range = 22–80%). Treatments 1–4 were injected using the Tree IV and QUIK-jet AIR® systems (Arborjet, Inc.) through #4 Arborplugs (Arborjet, Inc.) inserted in the root collar at a narrow spacing (numbers of plugs based on one plug per 7.6 cm dbh) on 18–20 June 2017. Of note, the QUIK-jet AIR system employs a pressurized air tank allowing for injecting of products at high pressures (>1,650 kPA) and more quickly than with the Tree IV system. All trees in treatments 1–5 were baited with frontalin released at ~125 μg/d at 20°C (Product #3300, Synergy Semiochemical Corp., Delta, BC, Canada) at ~2 m in height on the north aspect on 13–14 June 2018 (i.e., ~12 months following injections). Baits were removed on 18–19 September 2018. All surviving trees in treatments 1-4 and all trees in treatment 6 were baited in the same manner on 18–19 June 2019. Baits were removed on 9 September 2019.

For 2018, tree mortality was determined based on the presence (dead) or absence (live) of crown fade 18–19 June 2019. Due to COVID-19, we were unable to visit the study site in 2020. As such, for 2019 tree mortality was based on the presence (live) or absence (dead) of green needles 22 June 2021. Proportions of spruce trees that died across treatment groups were compared using two-proportions z-tests (prop.test). Comparisons were made among all systemic treatments (treatments 1–4) and the 2018 untreated control and then for cumulative mortality for systemic treatments (treatments 1–4) and the 2019 untreated control. Analyses were conducted with R Statistical Software (version 3.6.3) via RStudio (version 1.2.5033) using the Base package (R Core Team 2020).

Results and Discussion

No phytotoxic effects were observed for any systemic treatment, which agrees with previous studies evaluating emamectin benzoate and propiconazole for protecting conifers from mortality attributed to bark beetles. The time required for uptake of formulations ranged from 3 min (3 trees; TREE-äge G4 and TREE-äge R10 injected with the QUIK-jet AIR system) to 44 min (1 tree; TREE-äge G4 + Propizol injected with the Tree IV system). Considerable time is often required for uptake of systemic injections in conifers, which depends on the rate of transpiration and is influenced by air and soil temperatures, short-wave radiation, relative humidity, and soil moisture content as these factors affect stomatal conductance (Collatz et al. 1991). The rate of uptake in this study was faster than observed in earlier studies of *Pi. engelmannii* (e.g., Fettig et al. 2017, 2020), especially considering the large volumes injected in the TREE-äge G4 + Propizol and TREE-äge R10 + Propizol treatments (max = 656 ml/tree). TREE-äge G4 and TREE-äge R10 required the least amount of time to complete injections (mean ± SEM, 5.7 ± 0.2 and 6.0 ± 0.5 min, respectively), but most of the replicates for these treatments were injected with the QUIK-jet AIR system.

In 2018, the proportion of *Pi. engelmannii* killed by *D. rufipennis* ranged from 3.3% (TREE-äge G4 + Propizol and TREE-äge R10 + Propizol) to 56.7% (untreated control). Significantly lower levels of *Pi. engelmannii* mortality were observed in all systemic treatments compared with the untreated control ($\chi^2 > 10.0$, df = 1, $P < 0.001$ in all cases) (Fig. 1). No other significant differences were observed among

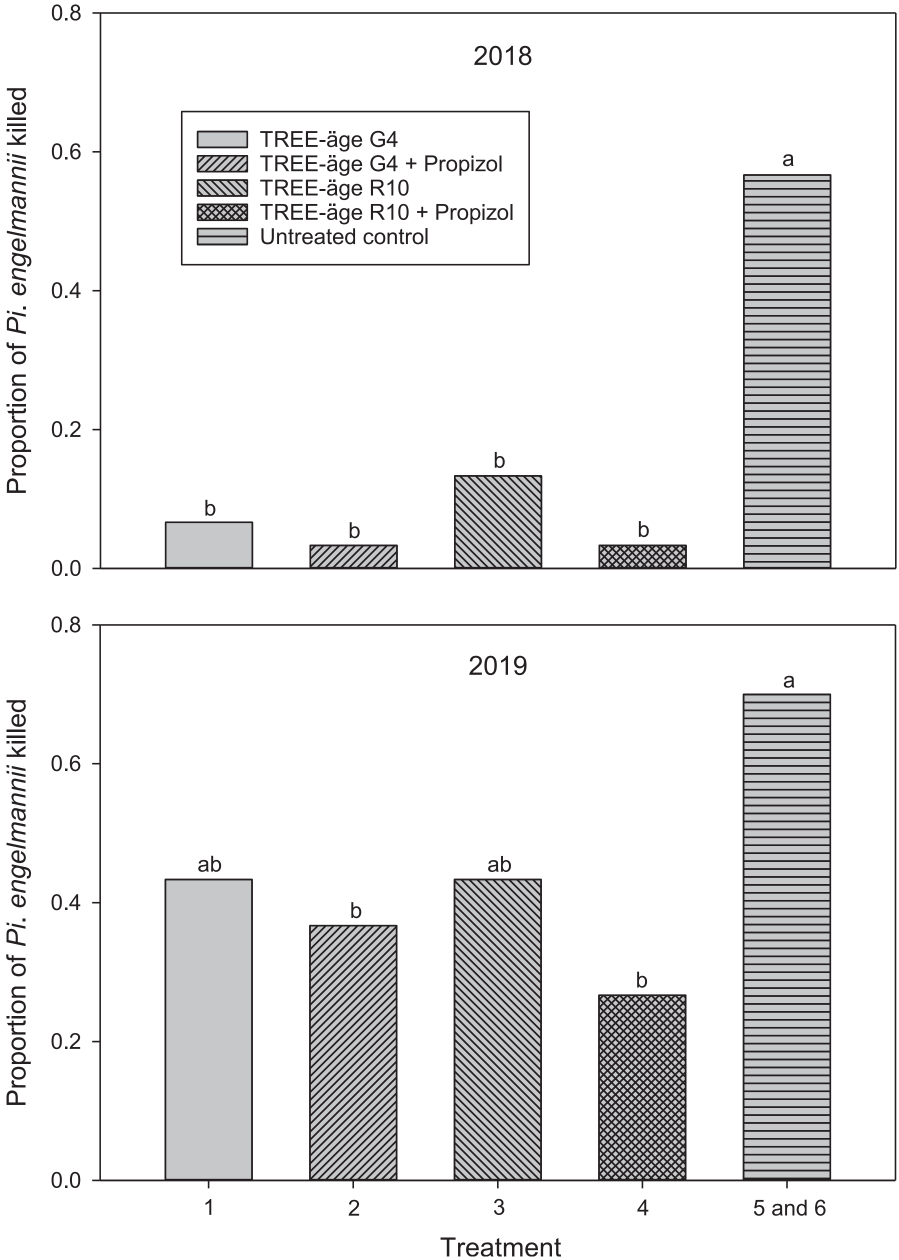

Fig. 1. Proportion of *Picea engelmannii* killed by *Dendroctonus rufipennis* following injections of emamectin benzoate (TREE-äge G4 and TREE-äge R10) alone and combined with propiconazole (Propizol), Bridger-Teton National Forest, Wyoming, 2018 and 2019. Proportions followed by the same letter within year are not significantly different ($P > 0.05$).

treatments (Fig. 1). In 2019, the proportion of *Pi. engelmannii* killed by *D. rufipennis* increased substantially and ranged from 26.7% (TREE-äge R10 + Propizol) to 70.0% (untreated control). Significantly lower levels of *Pi. engelmannii* mortality were observed in TREE-äge G4 + Propizol and TREE-äge R10 + Propizol than the untreated control ($\chi^2 = 5.4$, df = 1, $P = 0.02$ and $\chi^2 = 9.6$, df = 1, $P = 0.002$, respectively) (Fig. 1). No other significant differences were observed among treatments (Fig. 1). Although treatments were randomly allocated among experimental trees, there was a significant difference in tree dbh among treatments ($F_{5,\ 174} = 3.4$, $P = 0.006$). Trees in the untreated control (2018) were larger than trees in the TREE-äge R10 and TREE-äge R10 + Propizol treatments. No other significant differences in tree dbh were observed among treatments. The dbh of *Pi. engelmannii* influences susceptibility to colonization by *D. rufipennis* (Jenkins et al. 2014); however, the differences observed in our study were relatively small (<8.2 cm, based on mean values) and likely exerted little influence. Furthermore, all experimental trees were of sizes considered susceptible to *D. rufipennis* (>25 cm dbh) (Schmid and Frye 1976).

We conclude that both TREE-äge G4 and TREE-äge R10 are effective for protecting *Pi. engelmannii* from mortality attributed to *D. rufipennis* when injected in mid-June the year prior to being challenged by *D. rufipennis*, but protection is limited to one field season (Fig. 1). Protection is increased to two field seasons by combining TREE-äge G4 and TREE-äge R10 with Propizol (Fig. 1). Previously, we demonstrated that TREE-äge injected in spring at a narrow spacing (7.6 cm) was effective for protecting *Pi. engelmannii* from mortality attributed to *D. rufipennis* in Utah (Fettig et al. 2020). Unlike TREE-äge G4 and TREE-äge R10, TREE-äge provided two field seasons of protection in that study. Of note, an advantage of TREE-äge G4 is that it is available for use by the general public.

Given the data presented herein, we recommend use of TREE-äge G4 + Propizol or TREE-äge R10 + Propizol when more than one field season of protection is desired. We reiterate the importance of applying injections the spring prior to the year that protection of *Pi. engelmannii* is desired (e.g., in spring 2021 for 2022) and at a relatively narrow injection spacing. At time of this writing, a significant *D. rufipennis* outbreak is occurring in Alaska in white spruce, *Pi. glauca* (Moench) Voss, and Lutz spruce, *Pi.* x *lutzii* Little. We caution the reader that preliminary evaluations of similar systemic treatments indicate these treatments are not effective for protection of *Pi. glauca* and *Pi.* x *lutzii* in south-central Alaska (C.J.F. et al., unpubl. data).

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