FOREST ENTOMOLOGY

Efficacy of Two Systemic Insecticides Injected Into Loblolly Pine for Protection Against Southern Pine Bark Beetles (Coleoptera: Curculionidae)

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ABSTRACT We evaluated the efficacy of systemic insecticides emamectin benzoate and fipronil for preventing mortality of individual loblolly pines, *Pinus taeda* L., as a result of attacks by southern pine bark beetles (Coleoptera: Curculionidae, Scolytinae) for two consecutive years in Mississippi (2005–2006) and Alabama (2006–2007). Trees were injected once in the spring of 2005 (Mississippi) or 2006 (Alabama) and then were baited with species-specific bark beetle lures several weeks later. The southern pine beetle, *Dendroctonus frontalis* Zimmermann, was the target species but was changed to *Ips* spp. in Mississippi (but not Alabama) the second year because of few southern pine beetle attacks on baited trees. Single injections of emamectin benzoate were effective in reducing tree mortality caused by bark beetles compared with untreated checks. Although less effective overall, fipronil also significantly reduced tree mortality from southern pine beetle compared with the checks during the second year in Alabama. Tree mortality continued well after the lures had been removed. Evaluations of bolts taken from experimental trees killed in 2006 indicated that emamectin benzoate effectively prevented parent bark beetle gallery construction and that fipronil significantly reduced lengths of galleries constructed by adult beetles, brood development, and emergence, compared with checks. In contrast, neither insecticide treatment prevented the bark beetles from inoculating blue stain fungi, *Ophiostoma* spp., into treated trees.

KEY WORDS *Dendroctonus frontalis*, *Ips* spp., emamectin benzoate, fipronil, single-tree protection

Bark beetles (Curculionidae: Scolytinae) are responsible for extensive mortality of conifers throughout North America. The southern pine beetle, *Dendroctonus frontalis* Zimmermann, is one of the more important forest pests in the southeastern United States, Mexico, and Central America (Billings et al. 2004) with local and regional outbreaks causing severe economic losses on a nearly annual basis. An unprecedented southern pine beetle outbreak, extending across much of the southeastern United States from 1999 to 2002, caused tree mortality and damage estimated at >$1 billion (Pye et al. 2008). Despite episodic outbreaks, forest stands rated as high hazard for southern pine beetle are widespread across the South (Nowak 2008). Other species of pine bark beetles, including the secondary, less aggressive pests *Ips avulsus* (Eichoff), *Ips grandicollis* (Eichoff), and *Ips calligraphus* (Germar), also are known to cause significant tree mortality particularly during severe drought periods in the southeastern United States (Wilkinson and Foltz 1982).

Bark beetle infestations impact timber and fiber production, water quality and quantity, fish and wildlife populations, recreation, grazing capacity, biodiversity, cultural resources, and other resources (Leuschner 1980). Furthermore, the wildland-urban interface in the southeastern United States is rapidly expanding (Hermansen 2003), thus placing more high-valued residential trees at risk to bark beetle attack. Urban pines may be stressed by a variety of factors, including air pollution and soil compaction, rendering them more susceptible to bark beetle attack (Haverty et al. 1998). Tree losses in recreational, residential, or administrative sites generally result in costs associated with hazardous tree inspections and removal (Johnson 1981), litigation (Johnson 1981), reduced shade, screening, and esthetics (Haverty et al. 1998), and reductions in property values (McGregor and Cole 1985).

Bark beetle management techniques include reducing stand density and promoting tree vigor (prevention), rapid removal or treatment of infestations (suppression), and protection of high-value, individual trees (Goyer et al., 1998, Clarke 2001, Fettig et al. 2007). The latter has historically involved applications of insecticides to the entire bole of the tree using hydraulic sprayers. Benzene hexachloride (BHC), Lindane, fenitrothion (Pestroy), and chlorpyrifos

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(Dursban) had been registered with the U.S. Environmental Protection Agency (EPA) for this use, but all three have been withdrawn because of concerns about toxicity and/or human exposure in residential sites. In 2003, bifenthrin (Onyx) was registered by the EPA for protection of ornamental trees against several *Dendroctonus* species, including southern pine beetle, but so far this product has not been widely available to consumers. Fettig et al. (2006) indicated that the registered rate (0.06% [AI]) may be too low for efficacy against some western bark beetle species. Two other products, carbaryl (Sevin) and permethrin (Astro), are registered and effective against western bark beetle species, but they have proven ineffective against southeastern bark beetle species (Zhong et al. 1994). Spray applications may result in drift (Fettig et al. 2008), which can be detrimental to natural enemies (Billings 1980), and generally require the use of large equipment which can limit the ability to reach and treat target trees. The current abundance of susceptible trees and forests underlines the need to develop new methods to protect individual trees from bark beetle attacks.

Systemic insecticides have been suggested as a potentially useful tool for the protection of individual trees or forested areas from bark beetles. These largely water-soluble chemicals can be applied to the soil for absorption through the roots or direct injection into the phloem or xylem tissue of the trunk and are absorbed into the tissues of a plant to repels or kills most or some kinds of the insects that feed upon it (http://www.answers.com/topic/systemic-insecticide). Trunk injection studies have been conducted using acephate (Orthene) (Crisp et al. 1979, unpublished data in Billings 1980), fenitrothion (Pestroy) dicrotophos (Bidrin) (Dalusky et al. 1990), and azadirachtin (neem) (Dothie-Holt and Borden 1999). Although attack success and tree mortality were not prevented in any of the studies, reductions in brood development or production was reported. Oxdydementon methyl (Metasystox-R) applied by Mauget injectors (Inject-a-cide) into the trunk, is registered for use against several western bark beetle species but has been relatively ineffective for protecting individual ponderosa pines, *Pinus ponderosa* Dougl. ex Laws., from mortality attributed to the western pine beetle, *D. brevicomis* LeConte (Haverty et al. 1996).

When trunk injected, the insecticide emamectin benzoate (Syngenta Crop Protection, Greensboro, NC) was found to be highly effective for 3 yr against pine wood nematode, *Bursaphelenchus xylophilis* (Takai et al. 2000, 2001, 2003a,b) and 6 yr against coneworms, *Dioryctria* spp. (Grosman et al. 2002; D.M.G., unpublished data). Another insecticide, fipronil (BASF, Florham Park, NJ), was efficacious against coneworms and the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock) (D.M.G., unpublished data). Trunk injections of emamectin benzoate and fipronil prevented both the successful colonization of treated bolts and mortality of standing trees by *Ips* bark beetles 3 and 5 mo after treatments were applied (Grosman and Upton 2006). Given these successes, trials were initiated to determine the efficacy of emamectin benzoate and fipronil for protecting individual trees from southern pine beetles.

Materials and Methods

Two separate evaluations of emamectin benzoate and fipronil were conducted in Mississippi (2005–2006) and Alabama (2006–2007). The Mississippi trial was conducted on the Chickasawhay Ranger District of the DeSoto National Forest (31.43° N, 88.64° W, 96-m elevation). The Alabama trial was conducted on the Oakmulgee Ranger District of the Talladega National Forest (32.88° N, 87.04° W, 100-m elevation). There were three treatments: 1) emamectin benzoate (EB) injection at 0.08 g (AI) per cm diameter at breast height (dbh) (1.37 m in height); 2) fipronil (FIP) injection at 0.08 g (AI) per cm dbh; and 3) untreated check, used to assess beetle pressure.

In both study areas, experimental trees were located in areas reporting southern pine beetle activity (infestations) during the year before study establishment. The treatments were evaluated using a randomized complete block design with groups of loblolly pine, *Pinus taeda* L., serving as blocks. All blocks of trees were located within 75 m of an access road to facilitate treatment and were selected to limit potential for infestation expansion to nonstudy trees. Thirty-five replications of the three treatments were installed for each trial, with one to three replications per block. The DBH of test trees ranged from 15 to 45 cm. The spacing between adjacent tree blocks was >100 m to ensure that a sufficient number of beetles would be in the vicinity of each tree block to rigorously test the efficacy of these treatments.

Each insecticide treatment was injected into the tree trunk at four cardinal points 0.3 m above the ground with the Arborjet Tree IV microinfusion system (Arborjet, Inc., Woburn, MA). In Mississippi, treatments were applied 12–13 April 2005, 1 d after a 6.7-cm rainfall and high temperatures were 24–26°C (75–79°F). In Alabama, treatments were applied from 25 to 27 April 2006 when maximum temperature ranged from 24 to 28°C (75–82°F). This site had good moisture conditions, receiving 4.8 cm of rainfall during the week before treatment application. To encourage attack by southern pine beetle, all test trees and untreated checks were baited with two vials of racemic frontalin (99% cp; 400-μl centrifuge vial; 3.3 μg/d @ 20°C) and Hercules turpentine (190 g of UHR polysleeve; 2–3 g/d) (Synergy Semiochemicals Corp., Burnaby, BC, Canada). The injected trees were allowed 4–5 wk to translocate chemicals before baiting. Despite baiting, southern pine beetle populations in Mississippi declined during 2005 and in April and May 2006 few experimental trees were attacked by southern pine beetle. Accordingly, the target bark beetle species in Mississippi was switched in 2006 from southern pine beetle to *Ips* spp. On 21 May 2006, frills were cut with a hatchet into the sapwood between the injection points near the base of all remaining live experimental trees. A cellulose sponge was inserted into each cut and loaded with 10 ml of a 4:1 mix of sodium *N*-methyldithiocarbamate (Vapam, Osmose Inc., Buffalo, NY) plus dimethyl sulfoxide (DMSO) (Aldrich Chemical, Milwaukee, WI). This treatment nearly ceases resin flow in 1–2 wk and has been used to induce attacks by *Ips* spp. (Roton 1987, Strom et al. 2004). Pheromone packets of racemic ipsdienol (97% cp, 100-mg bubble cap; 500 μg/d), racemic ipsenol (90% cp, 100-mg bubble cap; 500 μg/d), and *cis*-verbenol (94% cp, 150-mg bubble cap; 600 μg/d) (Synergy Semiochemicals Corp.) were attached to hardwoods or shrubs near each tree group to attract *Ips* spp. The baits were changed every 4 wk until 13 July 2006.

In Alabama, relatively few southern pine beetle pitch tubes were observed on most trees on 16 June 2006, 3 wk after baiting. At this time, one endo-brevicomin lure (96% cp; 40-mg bubble cap; 400 μg/d) (Synergy Semiochemicals Corp.) was deployed 4 m from each tree block (Sullivan et al. 2007) to enhance bark beetle attraction to the frontalin and turpentine lures. Six weeks later, the lures were replaced, and all lures were removed 16 August 2006. A similar baiting scheme was used in 2007.

At each site, if a check tree was killed during the first year, a replacement was randomly selected and added to the replicate for the second year if one or both injected trees were alive. Any injected pines killed the first year were not replaced.

Data Analysis. The EB and FIP formulations were tested as treatments to prevent tree mortality by southern pine bark beetles. Therefore, methodology to evaluate treatment efficacy required that two criteria be met to demonstrate effective tree protection: 1) trees had to be challenged by beetles and 2) trees must not die (Shea et al. 1984; Haverty et al. 1996, 1998; Strom et al. 2004). Treatments were considered to have sufficient beetle pressure if at least 60% of the untreated control trees died from beetle attack (Shea et al. 1984). Insecticide treatments were considered efficacious if $\geq$80% treated trees survived after bark beetle attack. These criteria were established based on a sample size of 35 trees per treatment and the test of the null hypothesis, Ho:S (survival $\geq$90%). These parameters provided a conservative binomial test (α = 0.05) to reject Ho when more than six trees died. The power of this test, that is the probability of having made the correct decision in rejecting Ho, is 0.84 when the true protection rate is 70%. Based on the above-mentioned error rate consideration, we failed to reject the null hypothesis (90% survival) for any treatment when no more than seven of 35 trees died as a result of bark beetle attack (Shea et al. 1984).

Tree mortality was assessed every 2–8 wk between June and December. A tree was recorded as dead based on fading of the foliage from green to yellow or red, an irreversible symptom of tree mortality. During the course of the experiment, one tree was felled prematurely because of misidentification of tree fade, and this tree was deleted from the analysis. Treatment efficacy was evaluated once 60% of check trees had been killed. The cumulative mortality of trees killed per treatment each year was also compared at both sites by analysis of variance by using the StatView statistical program (SAS Institute 1999).

In 2006, study trees that had been killed were felled and a section of lower bole ($\approx$60 cm) was taken from each tree at $\approx$5 m height. In the laboratory, two 10- by 50-cm bark samples (total, 1,000 cm^2) were removed from each bolt to determine the cause of tree mortality and to confirm the presence of southern pine beetle or *Ips* engraver beetles and success of attacks. The following measurements were recorded: 1) number of bark beetle attacks, 2) presence of bark beetle galleries >2.5 cm in length, 3) presence or absence of bark beetle brood, 4) presence or absence of bark beetle brood emergence holes, 5) number of cerambycid egg niches, 6) number cerambycid larval galleries >2.5 cm in length, and 7) presence or absence of blue stain fungi. Cerambycid colonization of logs, particularly checks, was extensive and larval feeding often obliterated most bark beetle galleries. Therefore, bark beetle data collected from under the bark was ranked based on the relative present or absent of beetle galleries, brood, emergence holes, and blue stain fungi (1, many or all; 0.5, about half; 0, few or none). Data were analyzed by the nonparametric Kruskal–Wallis test using the StatView statistical program.

Results

Mississippi Trial. In 2005, the southern pine beetle pressure proved to be quite low as only eight of 35 untreated control trees were killed by bark beetles (Fig. 1A). In comparison, four FIP- and two EB-treated trees died. A cursory evaluation of bark beetle success indicated that galleries were almost always <2.5 cm in length, and no brood was produced in treated trees. However, all trees had been infected with blue stain fungi.

After Vapam/DMSO treatment and deployment of *Ips* pheromone baits near each tree group in 2006, control tree mortality exceeded 60% (designated threshold) by 3 August, $\approx$6 wk after initial tree baiting (Fig. 1B). At that time, 30% (nine of 30) of the FIP-treated trees and 18% (six of 33) of EB-treated trees had faded, indicating the EB treatments were efficacious. By the end of the year and well after the removal of the baits, 67% (22 of 33) of the check trees were dead, compared with 53% (16 of 30) of FIP-treated and 33% (11 of 33) of EB-treated trees. The EB treatment significantly reduced tree mortality compared with the check (F = 10.76; df = 1, 18; P = 0.002), but the FIP treatment did not (F = 1.77; df = 1, 18; P = 0.191).

Evaluation of logs collected from faded (dead) trees indicated that a similar number of *Ips* engraver beetles attacked the study trees regardless of treatment (Table 1). However, the success of the *Ips* beetles in constructing galleries (>2.5 cm) or producing brood was significantly less for both injection treatments compared with the checks. Similarly, the number of cerambycid egg niches was similar for all treatments, but there were significantly fewer larval galleries in

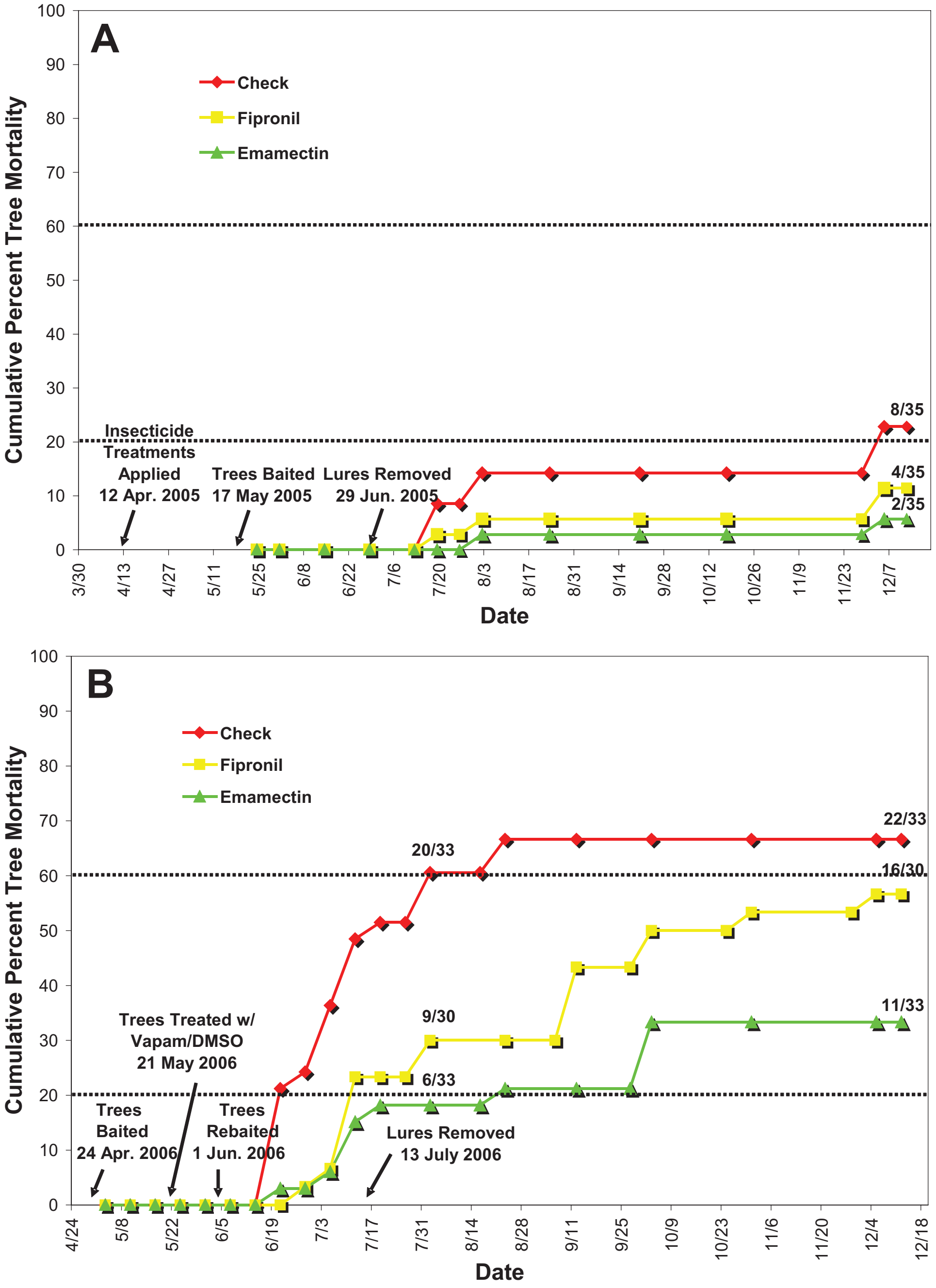

Fig. 1. Cumulative first- (A) and second-year (B) mortality of emamectin benzoate- and fipronil-treated and untreated loblolly pine after attack by southern pine beetle (2005) and *Ips* engraver beetles (2006), Chickasawhay Ranger District, DeSoto National Forest, MS. The dashed line at 60% cumulative mortality is the level of tree mortality considered necessary for a valid test (Shea et al. 1984); the dashed line at 20% cumulative mortality is the maximum allowable mortality for treatments to be considered efficacious. (Online figure in color.)

the injected logs. All logs were infected with blue stain fungi (Table 1).

Alabama Trial. Beetle pressure was low in 2006, and check tree mortality only reached the 60% (21 of 35) threshold by 4 October—20 wk after initial lure deployment and 7 wk after lure removal (Fig. 2A). Mortality of FIP-treated trees exceeded 28% (10 of 35) at this time, whereas only 14% (five of 35) of the EB-treated trees had died, below the 20% threshold defined as effective control. By the end of the calendar

Table 1. Effects of emamectin benzoate (EB) and fipronil (FIP) injection treatments on mean (± SEM) success of bark beetle adult attack, brood development, and emergence, presence of blue stain fungi, and success of cerambycid larvae in logs taken from faded study trees in Mississippi and Alabama, 2006

Site	Treatment	*N*	No. bark beetle attacks per 1000 cm^2	Ranking (% with)				No. cerambycid egg niches per 1,000 cm^2	No. cerambycid larval galleries per 1,000 cm^2
				Bark beetle galleries (length >2.5 cm) present	Bark beetle brood present	Bark beetle emergence holes present	Blue stain fungi present		
				Ips engraver beetles					
Mississippi	EB	11	9.3 ± 1.5a	0 ± 0a	0 ± 0a	0 ± 0a	100 ± 0a	22.8 ± 4.5a	0.5 ± 0.5a
	FIP	16	9.8 ± 1.0a	22 ± 4b	6 ± 4a	6 ± 4a	100 ± 0a	20.3 ± 1.9a	1.9 ± 0.9a
	Check	22	9.5 ± 0.7a	100 ± 0c	100 ± 0b	100 ± 0b	100 ± 0a	16.9 ± 2.3a	11.3 ± 0.7b
				Southern pine beetle					
Alabama	EB	7	11.0 ± 1.8a	0 ± 0a	0 ± 0a	0 ± 0a	100 ± 0a	17.8 ± 5.1b	0.4 ± 0.4a
	FIP	12	11.8 ± 1.8a	45 ± 11b	46 ± 11b	36 ± 12b	100 ± 0a	11.5 ± 1.5a	4.8 ± 1.3b
	Check	24	12.3 ± 1.0a	100 ± 0c	100 ± 0c	100 ± 0c	100 ± 0a	8.9 ± 0.8a	10.6 ± 5.2c

Means followed by the same letter in each column of the same site are not significantly different at the 5% level based on Fisher's protected LSD (counts) or Kruskal–Wallis (ranked).

year, 66% (24 of 35) of the check trees were attacked and killed. In contrast, 31% (12 of 35) of FIP-treated trees were dead, but no additional EB-treated trees had died.

A similar number of adult bark beetles (southern pine beetle) attacked the study trees regardless of treatment (Table 1). The success of the bark beetles in constructing galleries (>2.5 cm) or producing brood was significantly less for the both injection treatments compared with the checks. Similarly, the number of cerambycid egg niches was nearly the same for all treatments, but there were significantly fewer larval galleries in the injected logs. All of the logs were infected with blue stain fungi (Table 1).

In 2007, 60% (18 of 30) of check trees had red crowns within 8 wk of initial baiting (31 May) (Fig. 2B). In sharp contrast, mortality of FIP- and EB-treated trees was well below the 20% threshold—13% (three of 22) and 0% (0 of 28), respectively, at this time. Tree mortality at the end of the year was 87% (26 of 30) for checks, 43% (10 of 22) for FIP-treated trees, and 37% (11 of 28) for EB-treated trees (Fig. 2B). Cumulative mortality during the 2-yr period was 93% (50 of 54), 65% (22 of 35), and 51% (18 of 35) for check, FIP, and EB trees, respectively. The treatments significantly reduced tree mortality compared with the checks (FIP: $F = 14.02$; df = 1, 30; $P = 0.0004$ and EB: $F = 29.32$; df = 1, 30; $P < 0.0001$).

Discussion

This is the first published report documenting the successful application of a systemic insecticide for protecting individual *P. taeda* from mortality attributed to *D. frontalis.* To that end, EB injections successfully prevented mortality of standing pines from pine bark beetles at both sites for two consecutive years after treatment. The lack of bark beetle galleries, brood, and emergence holes in EB-treated pines indicates that this systemic is an effective deterrent to bark beetle colonization.

The results from the FIP trials were unexpected, because Grosman and Upton (2006) reported that an identical application rate of FIP protected all injected pines from *Ips* bark beetles in Texas. FIP was only efficacious the second year in Alabama against southern pine beetle infestation. However, southern pine beetle pressure also was much higher in the second year of the study in Alabama, as evidenced by the reduced time frame required to reach the 60% threshold of tree mortality among checks and number of southern pine beetle infestations reported on the Oakmulgee District in the Southern Pine Beetle Information System database (0 in 2006; 47 in 2007). The success of the FIP injections in 2007 under higher southern pine beetle pressure suggests that the insecticide may not have had sufficient time to move within the pines the first year before the baits were applied and the trees were challenged. As the attack densities of bark beetles did not vary significantly among treatments, it was apparent that injected insecticides treatments did not prevent attack. The pioneer gender (male *Ips* and female southern pine beetle) that initiated attacks was either deterred or killed upon penetration into the phloem layer and exposure to these active ingredients. This mode of activity illustrates the need for widespread transport of the active ingredients throughout the tree before it is challenged by bark beetles. Climatic factors, such as temperature and precipitation, will affect the time necessary for the systemic to circulate within the tree. Emamectin benzoate was shown to provide near complete protection against *Ips* engraver beetle attack on *P. taeda* logs 1 mo after injection, whereas fipronil required nearly 3 mo to distribute enough to provide an equal level of protection (Grosman and Upton 2006). The amount of time required for these systemic insecticides to adequately translocate within the tree under variable climatic conditions should be studied. In Japan, EB is injected during the dormant season to protect against pinewood nematode (Takai et al. 2000, 2001, 2003a).

The number and position of the injection ports also could influence insecticide transport. The dead trees usually were attacked by ambrosia beetles, *Platypus* spp., and boring dust was evident around the base of the trees. Treated trees were attacked below the in-

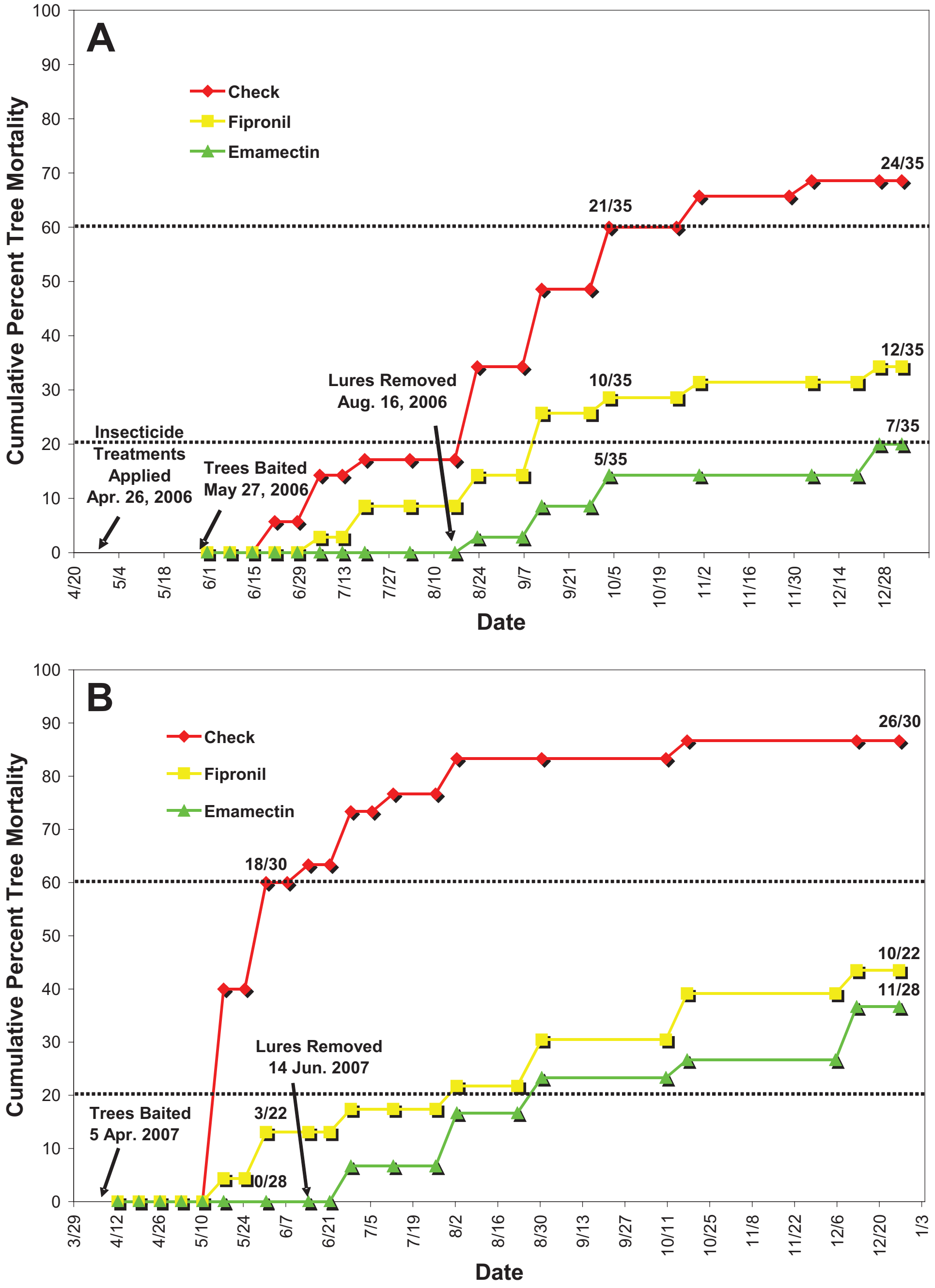

Fig. 2. Cumulative first- (A, 2006) and second-year (B, 2007) mortality of emamectin benzoate- and fipronil-treated and untreated loblolly pine after attack by southern pine beetle, Oakmulgee Ranger District, Talladega National Forest, AL. The dashed line at 60% cumulative mortality is the level of tree mortality considered necessary for a valid test (Shea et al. 1984); the dashed line at 20% cumulative mortality is the maximum allowable mortality for treatments to be considered efficacious. (Online figure in color.)

jection ports. These beetles attack trees that are dead or dying. Unlike southern pine beetle and *Ips* spp., the ambrosia beetles bore straight into the heartwood and do not tunnel in the cambial layer. These observations suggest that the injected insecticides were not translocated toward the roots or that the ambrosia beetles did not contact enough of insecticide as they bored into the tree to deter or kill them.

The efficacy evaluation was made once 60% of check trees had been killed, but tree mortality continued long after the baits had been removed and the check mortality threshold had been reached. Although mortality of treated trees eventually exceeded 20% at the end of year on occasion, the evaluations of the tree bolts indicated that gallery construction and girdling by the bark beetles were not responsible for a majority of the deaths of treated trees. All dead study trees were infected with blue stain fungi. Most bark beetles have complex associations with fungi species, including nonstaining *Ceratocystiopsis* spp. (carried in the southern pine beetle mycangium) and staining *Ophiostoma* and *Ceratocystis* spp. (carried on the external body surface) (Paine et al. 1997). As the beetle bores into the phloem tissue under the bark, spores of the staining fungi, largely *O. minus,* are inoculated and serve to help beetle colonization by reducing host resistance. The fungi on their own can disrupt water transport and cause tree death (Nelson and Beal 1929). Although the live study trees were not sampled for blue stain, bark beetles were largely unsuccessful in their gallery construction in dead treated trees, particularly with EB. Thus, it seems likely that the numerous blue stain fungi infections and perhaps ambrosia beetle infestation were the primary causes of tree mortality.

The experimental design used (Shea et al. 1984) in these studies is regarded as a conservative test of efficacy and serves as a standard to determine the effectiveness of insecticide bole sprays (e.g., carbaryl, bifenthrin) for individual tree protection in the western United States (Fettig et al. 2006). With bole sprays, bark beetles that land on target trees immediately contact the active ingredient before initiating host colonization. However, injections of systemic insecticides require bark beetles to initiate host colonization and penetrate the phloem before coming in contact with the active ingredient. We therefore feel that this experimental design is very conservative for determining the efficacy of tree injection treatments. In addition, experimental trees were baited with species specific lures for lengthy periods at levels that rarely occur naturally in the field, leading to extended opportunities for fungal inoculations. The success of EB at both sites and FIP in the second year in Alabama under this type of challenge indicates these two insecticides have utility in tree protection. Further work should evaluate the efficacy of these treatments applied at different times of the year and for protecting western conifers from bark beetle attack. In addition, there is a need to evaluate the contributions made by bark beetles and their associated fungi toward tree mortality and the efficacy of combining systemic insecticides with a fungicide for complete single tree protection.

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