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Impact of *Beauveria bassiana* (Ascomycota: Hypocreales) on the Small Southern Pine Engraver (Coleoptera: Scolytidae) in a Loblolly Pine Bolt Assay¹

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Abstract The small southern pine engraver, *Ips avulsus* Eichhoff (Coleoptera: Scolytidae), is one member of a guild of southern pine bark beetles that causes millions of dollars of losses to southern U.S. pine forests annually. Our objective was to determine the impact of a commercial preparation of the entomopathogenic fungus *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Ascomycota: Hypocreales) in protecting pine host plant tissue from colonization and damage by *I. avulsus* and on the *B. bassiana*-induced mortality of *I. avulsus* adults during the first 2 wk of host attack. The field trials were conducted in the Kisatchie National Forest (Rapides Parish, LA) using a small-bolt technique. Results showed that the *I. avulsus* adults successfully colonized both the *B. bassiana*-treated bolts and the control bolts within the first week of field exposure. Although the formulation did not protect bolts from attack, it caused significantly higher mortality of *I. avulsus* adults in *B. bassiana*-treated bolts than in the control, and significantly reduced the number of adults that reemerged. Approximately 84% of *I. avulsus* adults found in the *B. bassiana*-treated bolts died from *B. bassiana* infection and never reemerged, compared to 14% in the control bolts. Although effects from *B. bassiana* were too late to stop *I. avulsus* from entering host bolts, the high rates of beetle mortality observed under the bark suggest potential utility of the formulation for managing pine bark beetles. Successful application strategies and tactics are unknown at this point and will depend on many factors, including deployment techniques.

Key Words bark beetles, biopesticide, forest pest, mycosis, *Pinus*, Scolytinae

The small southern pine engraver, *Ips avulsus* Eichhoff (Coleoptera: Scolytidae), the eastern five-spined engraver, *Ips grandicollis* Eichhoff (Coleoptera: Scolytidae), and the eastern six-spined engraver, *Ips calligraphus* Germar (Coleoptera: Scolytidae) often attack vulnerable trees, including those being attacked by the southern pine beetle, *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae) (Connor and Wilkinson 1998). Annual activity of beetles in this southern pine bark beetle guild causes millions of dollars of losses in southern forests of the United States. Guild members have wide geographic and host ranges, extending at least from Pennsylvania to Florida to Texas and attacking all pine species within that range (Connor and Wilkinson 1998). The southern pine beetle is the most important

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pest in the guild but, for this study, we chose *I. avulsus* because it is nearly ubiquitous in our area, there is an effective, commercially available lure, and it achieves pest status at least intermittently.

Populations of *I. avulsus* can increase rapidly, with up to 10 generations per year, the shortest life cycle of the southern *Ips* species (Eickwort et al. 2006). However, forest management activities that result in injured or weakened trees, or those that leave large amounts of slash on site, can also create suitable breeding sites and favorable conditions for the growth of *Ips* populations (Connor and Wilkinson 1998, Mayfield et al. 2006, Nebeker 2003). Larger-scale events such as drought can also increase *Ips* infestation thereby promoting these species to primary pests (Clarke 2012).

Although chemical insecticide applications may protect high-value pines from bark beetles, including *Ips*, their application can be expensive and limited to particular environments (e.g., away from water), and it can be difficult to adequately reach the tops of trees where *I. avulsus* typically attack. They are also impractical for large areas or forest stands. Therefore, options including the use of entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Ascomycota: Hypocreales), should be explored as part of a larger management strategy. *Beauveria bassiana* is an entomopathogenic fungus with demonstrated activity against a broad range of forest pests, such as the red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae) (Zhang et al. 2011); the European spruce bark beetle, *Ips typographus* L. (Coleoptera: Scolytidae) (Kreutz et al. 2004); the hemlock wooly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) (Reid et al. 2010); the Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) (Maehara and Kanzaki 2014); and the redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) (Carrillo et al. 2015), among others. The fungus has a wide host range and has been commercialized into products that are generally available and considered safe and environmentally friendly (Zimmermann 2007).

The objectives of this study were to (a) evaluate the impact of a commercial formulation of *B. bassiana* (BotaniGard® 22WP, GHA strain, Victor, NY) in protecting host tissue from colonization by *I. avulsus* and (b) determine the effect of the *B. bassiana* formulation on mortality of *I. avulsus* adults during the attack process. BotaniGard 22WP is a mycoinsecticide formulated to control whiteflies, aphids, and thrips. However, if application of the fungus produced significant adult *I. avulsus* mortality during its attack of pine hosts, the information obtained would be useful in evaluating other important aspects of the impacts of *B. bassiana* on the *I. avulsus* life cycle relating to management. To meet these objectives, field trials were conducted using a small-bolt assay method (Strom and Roton 2009) to closely assess the impact of *B. bassiana* on *I. avulsus* during the first 2 wk of host attack.

Materials and Methods

Exposure of small bolts to *I. avulsus* in field trials. All bolts were exposed to *I. avulsus* via their live capture in multiple-funnel traps. Field trials were conducted between late September 2014 and mid-October 2014 in a thinned loblolly pine stand at the Kisatchie National Forest (Rapides Parish, LA). In each trial, two

apparently healthy loblolly pine (*Pinus taeda* L.) trees showing no symptoms of diseases or pest infestations were felled, and cut into 16 small bolts of approximately 10-cm diameter by 11-cm length. Bolts were selected randomly from each of the two trees and assigned to two treatments: (a) the *B. bassiana* treatment (eight replicates) or (b) the control, treated with water (eight replicates). For the *B. bassiana* treatment, a commercial formulation (BotaniGard 22WP, containing 4×10^{13} conidia/kg) was prepared according to the product label direction. Approximately 6 g of wettable powder was dissolved in 300 ml of tap water. Five drops of Silwet 408®, also known as Silwet REACH® (General Electric Company, Waterford, NY) was added as a surfactant. The resulting suspension was sprayed from a 700-ml handheld atomizer (The Bottle Crew, Farmington Hills, MI) directly onto the bark (until saturated) of *B. bassiana*-treated bolts. The preparation of *Beauveria* suspension sprayed contained approximately 5.8×10^7 viable colony forming units (CFUs)/ml estimated by serial dilution assay on potato dextrose agar (PDA) plates (39.0 g of PDA, dissolved in 1 L of distilled water and amended with 0.1 ml/L streptomycin sulfate). For the control treatment, tap water was sprayed onto the bark of assigned bolts from a different 700-ml handheld atomizer. Bolts from both treatments were kept separate to prevent cross-contamination.

Bolts were allowed to air dry for 24 h, at which time they were placed individually into a clean plastic bucket and deployed in the field. Each bucket (containing a bolt) was attached to the bottom of a Lindgren 12-funnel trap (Fig. 1A–C), baited to attract *I. avulsus* using racemic ipsdienol and Lanierone (Synergy Semiochemical Corp., Burnaby, Canada) following methods of Strom et al. (2015). The 16 baited trap–bolt combinations were deployed in a thinned loblolly pine stands in a 4×4 grid pattern, with approximately 7.5 m between adjacent traps, and left in the field for 7 d, after which time the bolts were collected. Individual bolts were removed from the plastic buckets and examined for beetles on the exterior bark, and the remaining content of each bucket, primarily adult insects and frass, was emptied into a sterile glass vial for further evaluation. Bolts were then returned to their container and sealed, being kept separate by treatment (to avoid *B. bassiana* spores cross-contamination), and immediately stored in a refrigerator until subsequent evaluation.

The two treatments were evaluated in the following sequence of events after each field trial: (1) assessment of host tissue colonization by *I. avulsus* adults, using a “compass point” method (Strom and Roton 2011), a day after the field trial was completed; (2) assessment of *B. bassiana* spore viability from spray residue collected from bark samples; (3) assessment of reemerged *I. avulsus* adults that accumulated in the sealed container over a period of 5 d; and (4) assessment of the *I. avulsus* adults found inside the phloem tissue of dissected whole bolt in the two treatments.

Extent of bolt utilization by *I. avulsus*. Refrigerated bolts were removed from storage after 2 to 5 d, and assessed for signs of *I. avulsus* activity (i.e., presence of beetle galleries) or host tissue colonization, using a “compass point” method (Strom and Roton 2011) to obtain phloem–bark samples. The aim was to evaluate the phloem tissue utilization and the extent of beetle colonization on each bolt (Fig. 1D). The resulting phloem–bark samples, 1.27-cm-diameter round punch disks (15 to 18 per bolt depending on bolt circumference; Fig. 1D) were examined and scored as yes or no for *I. avulsus* activity, and the proportion of disk with activity was recorded.



Fig. 1. (A) Site of field trials at the Kisatchie National Forest, Rapides Parish, LA. (B) Lindgren funnel trap baited for *Ips avulsus* with ipsdienol and lanierone. (C) Loblolly pine bolt placed in a plastic bucket. (D) Phloem disks (1.27-cm diameter removed by punching holes in the bark of bolts, then placed on sterile moistened filter paper to evaluate mycosis from *Beauveria bassiana* infection. (E) Eead *I. avulsus* exhibiting symptoms of *B. bassiana* infection (mycosis). (F) Dead *I. avulsus* adult on moistened sterile filter paper after 72 h. (G) Visual assessment of phloem tissue utilization on an untreated control bolt (4U) versus a *B. bassiana*-treated bolt (7B).

Ips avulsus adults (alive or dead) extracted from the disk samples were placed onto a sterile moistened filter paper in petri dishes and incubated at room temperature (~25°C) in the laboratory so that visual evaluation of *I. avulsus* cadavers could be conducted for signs of *B. bassiana* infection (mycosis) after 72 h (Fig. 1E–F).

***Beauveria bassiana* spore viability.** The *B. bassiana* spores from spray residue (white granules) on the bark of *B. bassiana*-treated loblolly bolts were identified using a dissecting microscope. Random samples of residue were collected from the bark of representative bolts, and inoculated directly on PDA culture plates with the aid of a sterilized insect pin (BioQuip Products, Rancho Dominguez, CA). Morphology of isolates' colonies was assessed on inoculated

plates after 72 h at ambient room temperature (~25°C). The viability of spores isolated from the spray residue was confirmed as either viable (positive) or nonviable (negative) from mycelia growth or colony morphology on the PDA plates and microscopic examination of spores.

Reemerged adults. Each examined bolt from the above assessments was returned to its exact plastic bucket, sealed, and kept in full shade but in otherwise ambient, outside conditions for 5 d to allow the *I. avulsus* adults to reemerge from each bolt for evaluation. Reemerged beetles were reemerging adults and not the new-generation beetles. The short duration of the experiment would be insufficient for completion of a new generation. The total number of *I. avulsus* adults found inside each plastic bucket was counted and recorded. All adults were placed onto sterile moistened filter paper in petri dishes, counted, placed into two categories (alive or dead), and incubated at room temperature (~25°C) in the laboratory. Visual assessment of cadavers was conducted after 72 h to evaluate mycosis from *B. bassiana* infection.

Adults extracted from the whole bolt. The number of *I. avulsus* adults extracted from each bolt (i.e., the whole-bolt bark tissue) was counted and recorded, and the number of dead *I. avulsus* adults showing symptoms of *B. bassiana* infection (mycosis) was confirmed by visual assessments on sterile moistened filter as previously described. In addition, *I. avulsus* adults and larvae found in the phloem tissue of each bolt during extraction of adults were examined for *B. bassiana* infection.

The total number of *I. avulsus* adults from each bolt (i.e., those extracted from the phloem disks, the reemerged adults found inside the plastic bucket, and those extracted from under the whole-bolt bark tissue) was recorded. *Monochamus*, *Thanasimus*, and *Temnoscheila* species found in the plastic buckets at the completion of the field experiment were evaluated visually for signs of *B. bassiana* infection and mycelia growth.

Statistical analysis. The *B. bassiana* treatment and the control bolts were compared based on the average number of *I. avulsus* adults collected from (a) the phloem disk samples (Fig. 1D), (b) the number of adults inside the plastic trap-bucket at the time of collection (Fig. 1C), and (c) the number of adults in the whole-bolt tissue (Fig. 1G). Counts of larvae and infected larvae were also recorded. The mortality (%) of *I. avulsus* adults (attributed to the *B. bassiana* infection based on mycosis on cadavers) was calculated as the number of infected *I. avulsus* adults divided by the total number of *I. avulsus* adults collected multiplied by 100 ($[(\text{infected}/\text{total}) \times 100]$). All statistical analyses from the two field trials were conducted using the software package JMP® (V.11, SAS Institute, Cary, NC).

Due to nonnormal data distribution in the proportion of *I. avulsus* colonization of the phloem tissue (from disk evaluation), the adult count, the adults infected, and the adult mortality from infection, the nonparametric Wilcoxon test (rank sum test for one-way analysis of variance) was performed to assess differences between the *B. bassiana* treatment and the control, with trial used as the block effect. The mean and the standard error of mean (mean \pm SEM) were presented for comparison of treatment effects. Statistical significance was determined using a *P* value of 0.05.

Results

Exposure of small bolts to *I. avulsus* in field trials. Weather data obtained from the nearest weather station, located at the Kisatchie National Forest, Stuart Seed Orchard, Louisiana (N 31°30'08", W 92°27'42"), approximately 11 km northeast of the trial site, showed the average daily air temperature was higher (24.2°C) during the first trial compared to the second (22.5°C) trial. Bolts in both treatments were successfully colonized.

Beauveria bassiana-treated and control bolts were compared based on the (a) evaluation of bolt disks, used to assess utilization of host tissue; (b) *B. bassiana* spore viability, used to assess longevity of spore in spray residue after the field trial; (c) reemerged adults in trap-bucket, used to assess treatment effects on the reemerged *I. avulsus* adults from bolts and their infection by *B. bassiana*; and (d) evaluation of whole-bolt bark tissue, used to measure the impact of *B. bassiana* on *I. avulsus* in the phloem tissue, and the mortality of adults in the bolt.

Extent of bolt utilization by *I. avulsus*. The lower temperature may have reduced *Ips* activity during the second trial relative to the first; however, the proportion of bolt samples colonized in the first trial ($97.6 \pm 1.2\%$) was not significantly different ($\chi^2 = 2.36$, $df = 1$, $P = 0.1242$) from the second trial ($92.6 \pm 2.3\%$). Similarly, there was not a significant difference in phloem colonization by *Ips* adults in the *B. bassiana*-treated bolts ($95.8 \pm 1.8\%$) than the control ($94.4 \pm 2.1\%$) bolts ($\chi^2 = 0.24$, $df = 1$, $P = 0.6239$).

The average number of *I. avulsus* adults extracted from disk samples was significantly higher in the *B. bassiana* treatment (15.9 ± 2.9 adults) than in the control (9.1 ± 1.9 adults), however, the difference was not statistically significant ($\chi^2 = 3.15$, $df = 1$, $P = 0.076$). The number of dead *I. avulsus* adults on the phloem disk samples (attributed to *B. bassiana* infection) was higher in the *B. bassiana* treatment (8.1 ± 2.3 adults) than in the control (0.7 ± 0.6 adult) (Table 1), with 51% and 8% *I. avulsus* adult mortality, respectively (Fig. 2). The difference between treatments was statistically significant ($\chi^2 = 12.27$, $df = 1$, $P = 0.0005$). Reduced adult mortality in the control bolts indicated adults survived through bark colonization and likely exited the bolt without being trapped by infection from *B. bassiana* spores.

***Beauveria bassiana* spore viability.** All *B. bassiana* spores collected from spray residue samples from representative bolts in the *B. bassiana* treatment were viable based on microscopic assessment of colonies on PDA. Results indicate that the *B. bassiana* spores from spray residues were viable beyond the duration of the field experiment following the application on the bark.

Reemerged adults. The number of reemerged *I. avulsus* adults was significantly ($\chi^2 = 6.16$, $df = 1$, $P = 0.0131$) lower in the *B. bassiana* treatment (4.2 ± 1.8 adults) than in the control (19.8 ± 7.3 adults), indicating that more adults were contained within the treated bolts and never reemerged. In addition, the average number of dead adults in the trap-bucket (attributed to *B. bassiana* infection) was significantly ($\chi^2 = 10.21$, $df = 1$, $P = 0.0014$) higher in the *B. bassiana* treatment (2.1 ± 0.4 adults) than the control (0.4 ± 0.3 adult) (Table 1), with 50.7% and 1.9% adult mortality, respectively (Fig. 2).

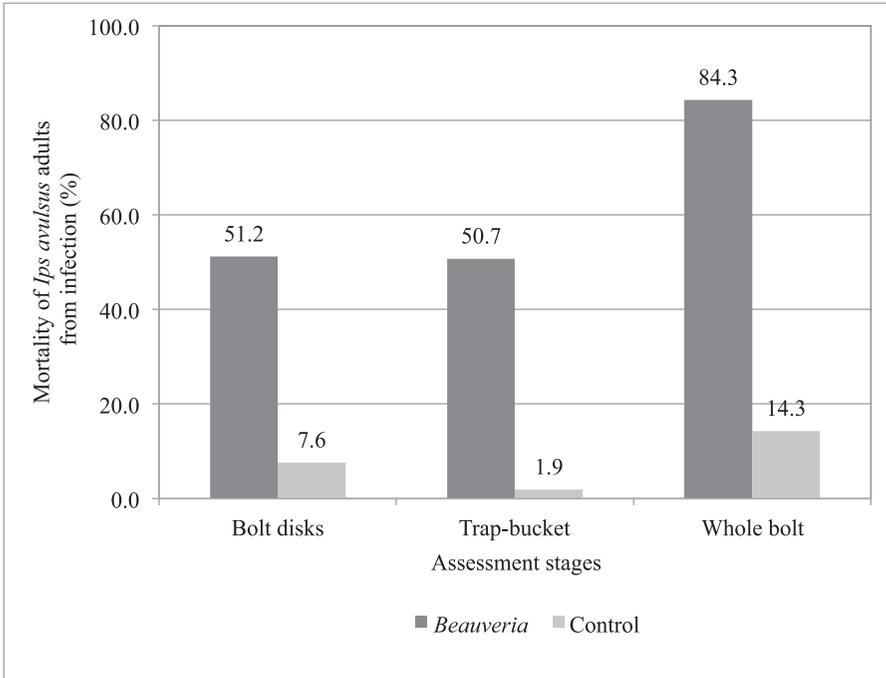


Fig. 2. Mortality attributed to the infection by the commercial formulation of *Beauveria bassiana* on the small southern pine engraver *Ips avulsus* adults at three assessment stages following a loblolly pine-bolt assay field experiment.

Extracted adults from whole bolts. Assessments of bark tissue from whole bolts showed that *I. avulsus* adults successfully colonized both the *B. bassiana*-treated bolts and the control bolts. The average number of *I. avulsus* adults extracted from the bark tissue of each individual bolt was significantly ($\chi^2 = 5.73$, $df = 1$, $P = 0.0167$) higher in the *B. bassiana* treatment (133.6 ± 19.0 adults) than in the control (90.1 ± 12.8 adults). The number of dead adults attributed to *B. bassiana* infection was also significantly ($\chi^2 = 20.47$, $df = 1$, $P < 0.0001$) higher in the *B. bassiana* treatment (112.6 ± 18.8 adults) than in the control (12.9 ± 9.0 adults) (Table 1), with approximately 84% and 14% adult mortality, respectively (Fig 2.). The higher infection mortality may help to explain the similar or higher number of adults collected from disk samples in the *B. bassiana*-treated bolts.

There was no significant difference ($\chi^2 = 0.24$, $df = 1$, $P = 0.6239$) between the average number of *I. avulsus* larvae found in the phloem tissue of the *B. bassiana*-treated bolts (19.7 ± 1.8 larvae) and the control bolts (20.2 ± 3.3 larvae). However, the average number of larvae infected was significantly ($\chi^2 = 4.3778$, $df = 1$, $P = 0.0364$) higher in the *B. bassiana* treatment (7.3 ± 2.1 larvae) than in the control (2.4 ± 1.5 larvae) (Table 1).

Visual assessments of bolts in the *B. bassiana* treatment and the control revealed similarity in the severity of symptoms following *I. avulsus* attacks. The *B.*

Table 1. Mean comparisons between the *Beauveria bassiana* treated and the control bolts using the nonparametric Wilcoxon test, to assess the effect of a commercial formulation of *B. bassiana* on the small southern pine engraver *Ips avulsus* in a loblolly pine-bolt assay field experiment.

Stages	Assessment	Treatment	n	Mean ± SEM	χ^2	DF	Prob > ChiSq*
Bolt disks	Colonization (%)	<i>Beauveria</i>	16	95.8 ± 1.7	0.24	1	0.6239
		Control	16	94.4 ± 1.9			
	Adults counted	<i>Beauveria</i>	16	15.9 ± 2.9	3.15	1	0.0760
		Control	16	9.1 ± 1.9			
Trap-bucket	Adults infected	<i>Beauveria</i>	16	8.5 ± 2.4	12.27	1	0.0005**
		Control	16	0.8 ± 0.7			
	Adults infected, dead	<i>Beauveria</i>	16	8.1 ± 2.3	12.27	1	0.0005**
		Control	16	0.7 ± 0.6			
Trap-bucket	Adults counted	<i>Beauveria</i>	16	4.2 ± 1.8	6.16	1	0.0131**
		Control	16	19.8 ± 7.3			
	Adults infected	<i>Beauveria</i>	16	2.1 ± 0.4	10.21	1	0.0014**
		Control	16	0.4 ± 0.3			
Trap-bucket	Adults infected, dead	<i>Beauveria</i>	16	2.1 ± 0.4	10.21	1	0.0014**
		Control	16	0.4 ± 0.3			

Table 1. Continued.

Stages	Assessment	Treatment	n	Mean ± SEM	χ^2	DF	Prob > ChiSq*
Whole bolt	Adults counted	<i>Beauveria</i>	16	133.6 ± 19.0	5.73	1	0.0167**
		Control	16	90.1 ± 12.8			
	Adults infected	<i>Beauveria</i>	16	115.5 ± 18.7	20.48	1	<0.0001**
		Control	16	13.4 ± 9.1			
	Adults infected, dead	<i>Beauveria</i>	16	112.6 ± 18.8	20.47	1	<0.0001**
		Control	16	12.9 ± 9.0			
	Larvae counted	<i>Beauveria</i>	16	19.7 ± 1.8	0.24	1	0.6239
		Control	16	20.2 ± 3.3			
	Larvae infected	<i>Beauveria</i>	16	7.3 ± 2.1	4.38	1	0.0364**
		Control	16	2.4 ± 1.5			

* Prob > χ^2 = The Wilcoxon test probability value (P value) determined at level of 0.05.

** Significant value as defined above.

bassiana application showed no noticeable inhibitory impact on the development and progression of *Ophiotoma* blue stain fungus in both treatments (Fig. 1G). *Monochamus titillator* F. (Coleoptera: Cerambycidae) and *Thanasimus dubius* F. (Coleoptera: Cleridae) found inside the plastic bucket in the *B. bassiana* treatment showed no mycosis.

Discussion

In this study, we examined the effect of a commercial *B. bassiana* formulation (BotaniGard) applied prophylactically as a bark spray to prevent attacks by *I. avulsus* in a pine-bolt bioassay. Results, however, showed that *I. avulsus* adults successfully attacked and colonized *B. bassiana*-treated bolts. But, the mortality of *I. avulsus* adults attributed to *B. bassiana* infection was significantly higher in the *B. bassiana*-treated bolts (84%) than in the controls (14%). Furthermore, the mean number of *I. avulsus* adults that eventually emerged from the attacked bolts (found in the trap-bucket) was significantly higher from the control than from the *B. bassiana*-treated bolts. A significant number of *I. avulsus* adults found in the *B. bassiana*-treated bolts were infected and killed by *B. bassiana*. This further suggests that any lethal effects of the prophylactic use of the commercially formulated *B. bassiana* in managing *I. avulsus* is through exposure of beetles attacking the trees and eventual infection and mortality of the insects once they have invaded the wood and, thus, emergence of beetles may be significantly reduced. The lag time between exposure to the fungal conidia and infection or death of the target beetle nullifies the use of *B. bassiana* as a treatment to prevent successful attack and infestation of the wood by *I. avulsus*. However, use of this entomopathogenic fungus might offer long-term management utility in other ways including, but not restricted to, targeting an active *I. avulsus* population with the goal of exposing a significant number of adults to lethal doses of viable spores or employing a trap-tree method (Copoly and Morris 1972).

Kreutz et al. (2004) found that the horizontal transmission of *B. bassiana* in the spruce bark beetle, *I. typographus*, was very effective. A single contact between a male treated with 2.0×10^5 conidia and a female that was not treated proved sufficient to transmit a lethal dose of the fungus to the untreated female. Indeed, we observed that a significant number of *I. avulsus* adults never emerged from the treated bolts due to high under-bark mortality from infection following contact with *B. bassiana* spores on the bark of treated bolts during the attack process. We also noticed that some emerging *I. avulsus* adults in the *B. bassiana*-reated bolts may have been infected, and became too weak to sustain efficient dissemination of a lethal dose of *B. bassiana* spores via horizontal transmission. Liu and Bauer (2008) found that the efficacy of *B. bassiana* strain GHA on emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), improved in the field when formulations were sprayed directly onto bark surfaces of infested host trees prior to adult emergence. Castrillo et al. (2010) reported sufficient inoculum persisted on bark treated with *B. bassiana* strain GHA for 7 to 14 d and during that period caused 40% to 57% mortality of emerging *A. planipennis*. Interestingly, we found that viable *B. bassiana* spores were recoverable from loblolly pine bark up to 16 wk after

application with BotaniGard in a separate field study conducted in east Texas (R.O. unpubl. data).

On the other hand, ultraviolet (UV) radiation (Costa et al. 2001, Fargues et al. 1996, Inglis et al. 1995, Jaronski and Goettel 1997, Moreley-Davies et al. 1996), mycoparasitism (Posada et al. 2004), and temperature (Martin et al. (2000) may reduce conidia viability and overall efficacy. Multiple applications to circumvent the issue of persistence would increase management costs. Yet, Bextine and Thorvilson (2002) demonstrated that the application of UV-reflective dyes incorporated into alginate pellets of *B. bassiana* against the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), effectively extended the persistence of the fungus and allowed for monitoring of ant foraging behavior. A similar approach might be developed for monitoring the longevity and survival of *B. bassiana* spores in pine bolts and on the bark or for tracking the movement of conidia transmitted by adults within the gallery beneath the bark until emergence.

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