

Microbial control of *Agrilus planipennis* (Coleoptera: Buprestidae) with *Beauveria bassiana* strain GHA: field applications¹

Houping Liu^{a*} and Leah S. Bauer^{a,b}

^aDepartment of Entomology, Michigan State University, East Lansing, MI, USA; ^bUSDA Forest Service, Northern Research Station, East Lansing, MI, USA

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The effects of *Beauveria bassiana* strain GHA, applied as BotaniGard ES, on newly colonised and well-established populations of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) were evaluated in the field using foliar and trunk sprays in Michigan in 2004–2005. Results from field trials at a newly colonised white ash site showed a 41% reduction in *A. planipennis* population in fungal-treated trees compared with that of untreated controls. In addition, fungal infection was also found in 20% of the larval population within 14 days of incubation under laboratory conditions. At a site with a well-established *A. planipennis* population in green ash trees, larval density was reduced by 47% for trees treated with the fungus compared with that of the controls; 21% of larvae from the current generation were found infected after 14 days of laboratory incubation. Fungal-treated green ash trees also produced fewer adults emerging in the next generation, with a 63% reduction in adult density observed in treated trees compared to that of controls. As a result, fungal-treated trees sustained 42% less crown dieback than did controls. *Agrilus planipennis* larval density was negatively correlated with trunk height above the ground, and positively correlated with log diameter. Results of laboratory leaf bioassays on *A. planipennis* adults showed that fungal conidia persisted well under field conditions, with mortality of 78–100% at 7-days post-exposure for leaves collected between 2 and 264 h after application. Potential strategies for using *B. bassiana* strain GHA for managing *A. planipennis* are discussed.

Keywords: emerald ash borer; *Agrilus planipennis*; *Beauveria bassiana* strain GHA; conidial persistence; field applications; microbial control

Introduction

Agrilus planipennis Fairmaire (Coleoptera: Buprestidae), the emerald ash borer, a sporadic woodboring pest of ash trees (*Fraxinus* spp.) native to areas of northeastern Asia (CAS 1986; Yu 1992; Xu 2003), was first discovered in southeastern Michigan and neighbouring Ontario, Canada, in 2002 (Haack et al. 2002). Despite quarantine regulations and attempted eradication efforts, *A. planipennis* continues to spread throughout areas of Michigan, Ohio, Indiana, Illinois, Maryland, Pennsylvania, West Virginia, and Ontario due to transport of infested ash firewood, timber, nursery stock, and natural dispersal (MDA 2006; USDA-APHIS 2007). High population densities of *A. planipennis* result in

*Corresponding author. Email: liuho@msu.edu

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gradual death of ash trees as larvae feeding in the phloem girdle branches and the main trunk. Crown dieback and epicormic branching of ash trees are two characteristic symptoms of infestation. Tree mortality takes several years; tree condition, site, age, species, transplantation history, and rainfall affect the rate of tree decline and death. *Agrilus planipennis* is expected to adversely impact forest biodiversity, wildlife habitat, quality of riparian areas, ash resources, and urban areas in North America because ash trees are a common and widely distributed species (USDA FS-NERS 2004; MacFarlane and Meyer 2005; Poland and McCullough 2006). While eradication of *A. planipennis* appears unlikely due to limited detection and control methods (USDA APHIS 2006), management tactics are needed to contain, slow the spread, and suppress high field populations of *A. planipennis*.

In Michigan, *A. planipennis* has a 1- or 2-year life cycle, with the four larval instars capable of overwintering in the outer sapwood or outer bark (Cappaert, McCullough, Poland, and Siegert 2005; Petrice and Haack 2006). Entomopathogenic fungi were identified as an important cause of mortality in field populations based on field surveys in Michigan (Bauer, Liu, Haack, Petrice, and Miller 2004). Fungal infections were most prevalent in mature larvae, which may be exposed to the environment due to bark splits that form over their galleries. In earlier studies, we found *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin was highly virulent against *A. planipennis* (Liu and Bauer 2006). Subsequent greenhouse and field trials with *B. bassiana* strain GHA demonstrated its lethal effects on both emerging adults and larvae of *A. planipennis* (Liu and Bauer 2008). Sublethal effects on *A. planipennis* adult longevity, female fecundity, and larval development were also observed for this fungus (Liu and Bauer 2008). In this study, we evaluated the impact of *B. bassiana* strain GHA, formulated as the registered biopesticide BotaniGard ES (Emerald BioAgriculture Corp., Lansing, MI), on newly colonised and well-established *A. planipennis* populations through field applications. Using BotaniGard ES, we also tested conidial persistence of *B. bassiana* strain GHA under field conditions through laboratory leaf bioassays.

Materials and methods

Study sites

Two sites were selected in southeastern Michigan in 2004, one near the Ann Arbor Airport in Ann Arbor and the other at the southwestern corner of Fox Hills Country Club in Plymouth. The Airport site (42°13'23"N, 83°44'44"W) consisted of 60, 6-year-old white ash trees (*F. americana* L. var. 'autumn purple') with diameters at breast height (DBH) of 4–5 cm and heights of 4–6 m. We planted these trees (roots were balled and burlapped) in four rows (15 tree/row) with a 2 × 2-m (row × column) spacing in the early summer of 2003; they were in good growing condition and showed no external symptoms of *A. planipennis* infestation (adult exit holes, epicormic shoots, bark splits, or crown dieback). To the north, four *A. planipennis* infested green ash trees were found in a woodlot adjacent to the field and a disposal site for *A. planipennis*-infested ash logs was located ca. 10 m south of this site.

The Fox Hills site (42°21'17"N, 83°36'06"W), consisted of a 20-year even-aged plantation with ca. 180 green ash (*F. pennsylvanica* Marsh.) trees, ranging from 7 to 20 cm in DBH and 8 to 12 m in height. We estimated the trees in this plantation became infested with *A. planipennis* about 3 years prior to our study, and the population densities of *A. planipennis* were highly variable between trees. The trees were planted in 17 rows

(12–17 trees/row) with a 2.5 × 2.5-m spacing (row × column). A 4-m wide seasonal road through the middle of the plantation divided the site into two roughly equal areas, with trees on the west side slightly larger than those to the east.

Airport site: effects on A. planipennis colonisation

To determine the effects of *B. bassiana* strain GHA on *A. planipennis* colonisation of ash trees, a total of 14 trees from the southwestern corner of the Airport site were used for treatment, and 13 trees from the northeastern corner were used as untreated controls. BotaniGard ES, an emulsifiable suspension of live *B. bassiana* strain GHA conidia, was diluted using tap water to 3×10^8 conidia/mL and applied to the trees using a CO₂ backpack sprayer (R&D Sprayers, Opelousas, LA) equipped with a nozzle # 8001 EVS; spray pressure was set at 275,790 Pa (40 p.s.i.) for a delivery rate of 330 mL/min. The trees were treated individually with ca. 600 mL of suspension each for an application rate of ca. 30×10^{13} conidia/ha. Two, 80-cm extension tubes were used for the sprayer to reach the tree tops. A total of four sprays were made by treating the trees every 2 weeks between 25 June and 5 August, 2004, to cover the entire adult flying season in the field. Control trees were not treated. All trees were felled, cut into 60-cm log sections in October 2004, placed in individual plastic bags, and returned to the laboratory for dissection. The control logs were dissected first, and work surfaces, tools, and utensils were surface sterilized with 70% ethyl alcohol before and after each use to minimise cross contamination and secondary exposure of *A. planipennis* to entomopathogenic fungi. After dissection from the logs, *A. planipennis* were incubated individually in 24-well plastic plates under saturated conditions; mycosis was determined 14 days later. Larval density and fungal exposure level for each tree were used for analysis, with 14 treatment and 13 control replicates.

Fox Hills site: effects on well-established A. planipennis population

The effect of foliar and trunk applications of *B. bassiana* strain GHA, applied as BotaniGard ES, on a well-established field population of *A. planipennis* was evaluated in the green ash plantation at the Fox Hills site. Pre-treatment crown conditions were rated for each tree after full leaf flush on 7 June 2004 using an estimate of crown dieback ranging from 0 to 100%. A tree with no crown dieback was rated as 0% while a tree with no live foliage was recorded as 100%. For data analysis, we divided the trees into three levels of dieback based on their crown ratings: low (0–24%), medium (25–50%), and high (>50%). Post-treatment crown conditions were rated on 31 May 2005 for all trees that remained in the treatment and control.

Pre-treatment larval density of *A. planipennis* was estimated by sampling a 50-cm long section along the main trunk between 200 and 250 cm above the ground from two randomly selected trees from each side. On 31 May 2004, these trees were felled, dissected, and the number of *A. planipennis* were recorded for each section.

A conidial suspension of *B. bassiana* strain GHA, formulated as BotaniGard ES, was sprayed at the concentration of 3.2×10^8 conidia/mL on the trunk and crown of each tree individually in the east side of this site using a truck-mounted hydraulic sprayer. The trees were sprayed every 2 weeks from 23 June to 3 August 2004, a total of four times. Eight to 11 L of BotaniGard suspension was applied to each tree to achieve a deposition of approximately 30×10^{13} conidia/ha. Trees on the west side were not treated.

A total of 50 trees (25 from each side) were felled between December 2004 and April 2005 for evaluation, with 15, 5, and 5 trees from low, medium, and high levels of crown

dieback, respectively. After the trees were felled, the main trunks were cut into 100-cm log sections from the base up to the point where diameter measured ca. 2 cm. Each 100-cm log section was cut again into a 30-cm long lower trunk portion and a 70-cm long upper trunk portion and returned to the laboratory in dark plastic bags. The lower 30-cm portions from each log were dissected immediately for *A. planipennis* larvae and prepupae, which were incubated individually as described before to determine the level of fungal exposure. The 70-cm upper portions of all logs from 20 trees with low crown dieback (10 from each treatment) were stored at 4°C. The following summer 2005, adults were reared from these logs after incubation in individual cardboard rearing tubes at room temperature (20–25°C) for 8 weeks according to the methods described by Liu and Bauer (2006). *Agrilus planipennis* adults were collected daily from each rearing tube, killed by 2-h exposure to –20°C, and incubated individually in 24-well plastic plates under saturated conditions for fungal infection; mycosis was confirmed 14 days later.

The efficacy of BotaniGard ES treatments in managing *A. planipennis* was measured by analysing differences between treatment and control trees including: (a) pre- and post-treatment crown condition; (b) post-treatment larval density for *A. planipennis*; (c) fungal exposure level of *A. planipennis* larvae based on 14 days of laboratory incubation; (d) post-treatment adult density for *A. planipennis*; and (e) fungal exposure level of *A. planipennis* adults based on 14 days of laboratory incubation.

Conidia persistence in the field using laboratory bioassays

Field persistence of *B. bassiana* strain GHA conidia, formulated as BotaniGard ES, was evaluated through leaf bioassays following the last fungal application at the Airport site. Four bioassays were conducted with white ash leaves collected from control and fungal-treated ash trees at 2, 96, 168, and 264 h after application. Two healthy leaves were collected from the mid-crown of each tree, with one leaf from the south side and one from the north side; five trees were sampled at each time, and control trees were sampled before treatment trees to avoid contamination. Each sample consisted of a leaf pinnately compounded with seven to nine leaflets and ranged from 20 to 30 cm in length. Upon collection, each leaf was sealed individually in a plastic zip-lock bag to prevent possible cross-contamination and returned to the laboratory in a cooler.

One- to 6-day-old laboratory-reared *A. planipennis* adults were used in the bioassays. The adult beetles were reared from naturally-infested ash trees (10–20 cm DBH range) and fed on greenhouse-raised evergreen ash, *F. uhdei* (Wenzig) Lingelsh according to the methods of Liu and Bauer (2006). The source of the infested trees was Lower Huron Metropark, Belleville, MI (42°09'48"N, 83°24'46"W) in April 2004. After being felled by chainsaw, the trunk of each tree was cut into 60-cm logs and stored in a cold room at 4°C until needed for rearing adults.

For bioassays, a total of five adults were exposed for 48 h to a field-collected control or treatment white ash leaf in a 15-cm plastic Petri dish. To keep each leaf hydrated, its petiole was inserted in a hole punched into the top of a 2-mL vial filled with tap water; the vials were refilled with water every other day. Following the exposure period, the adults were transferred to a fresh Petri dish with a greenhouse-raised evergreen ash leaf. This leaf was replaced with a fresh one every 6 days. Mortality was monitored daily for 14 days, with those still moving beyond that recorded as alive. Dead adults were removed from the Petri dish, and cultured individually in a chamber consisting of a 60-mm plastic Petri dish lined with moist sterile filter paper. Mycosis of adult cadavers was verified after 7 days. There

were five adults per replicate and 10 replicates per assay, for each of the four assay dates in this study. Adult mortality, time-to-death, and cadaver mycosis were used in the analysis.

Data analysis

Percent mortality, fungal exposure level, and percent defoliation rate were subjected to angular (arcsin square root) transformation before analysis. Analysis of variance (ANOVA) ($\alpha=0.05$) was used to analyse data on post-treatment larval density, fungal exposure level, and cadaver mycosis at the Airport site; adult mortality and time-to-death for laboratory leaf bioassays; and pre-treatment larval density at the Fox Hills site. Student–Neuman–Keul’s test ($\alpha=0.05$) followed if significant differences were detected between treatments (PROC GLM) (SAS Institute 2004). ANOVA with repeated measures was used to analyse crown defoliation data before and after treatment, while sub-sampling model was used to analyse post-treatment larval density, fungal exposure level based on 14 days of laboratory incubation, and adult density data from the Fox Hills site (PROC GLM) (SAS Institute 2004). Least squares means was used to separate the means if ANOVA indicated significant difference (PROC GLM) (SAS Institute 2004). PROC REG (SAS Institute 2004) was used to analyse the correlation between larval density and trunk height above the ground, as well as average log diameter for data collected at the Fox Hills site.

Results

Airport site: effects on *A. planipennis* colonisation

Agrilus planipennis undergoes a 1- or 2-year life cycle in southeastern Michigan, so fourth-instar larvae dissected out of the trees cut from this site by October 2004 were from eggs laid in 2003; younger larvae (first to third instars) were from eggs laid in 2004 (Liu and Bauer, unpublished data). Both control and treated trees contained fourth instar larvae from the previous generation, with more found in the treated trees (Table 1). Larvae from the current generation (first to third instars) accounted for 24 and 8% for the control and treated trees, respectively (Table 1). While there was no significant difference in larval density for the current generation, trees treated with *B. bassiana* strain GHA contained 41% fewer young larvae (first to third instars) than did the controls (Table 1). While no fungal infection occurred in young larvae, *Beauveria bassiana* strain GHA was effective against larger *A. planipennis* larvae from the previous generation (fourth instar). Results showed that 20% of larvae on trees from the treated area were infected after 14 days of laboratory incubation (Table 1), ranging from 0 to 38% in different log sections; no significant difference in fungal exposure level between different log sections was detected ($F_{6,75}=1.11$, $P=0.365$). No larvae died of fungal infection in the control (Table 1).

Fox Hills site: effects on well-established *A. planipennis* population

In 2004, the average initial crown dieback of the green ash trees at the Fox Hills site was 27% ($n=95$) and 20% ($n=78$) for the control and treatment trees, respectively, with no significant difference observed between them ($t_{171}=1.96$, $P=0.052$). By 2005, significant effect was observed for treatment ($F_{1,121}=27.30$, $P<0.0001$), time ($F_{1,121}=226.88$, $P<0.0001$), and the interaction between treatment and time ($F_{1,121}=15.48$, $P<0.0001$) when crown dieback of all remaining trees (70 for controls and 53 for treatments) was

Table 1. Larval density and fungal exposure level of *A. planipennis* when treated by *B. bassiana* strain GHA (BotaniGard ES) through foliar and trunk applications on white ash trees at the Airport site, Ann Arbor, MI, 2004.

Treatment (conidia/ha)	Reps	No. of larvae		Density (/m ²) (Mean ± SEM) ^a		No. of larvae infected ^b	Infection rate (Mean ± SEM) ^a
		Total	1 st -3 rd	Total	1 st -3 rd		
0	13	89	21	14.7 ± 4.6A	2.7 ± 0.8A	0	0 ± 0A
30 × 10 ¹³	14	178	15	20.5 ± 4.0A	1.6 ± 0.6A	36	19.6 ± 6.1B

^aMeans followed by the same upper case letter within a column are not significantly different (Student–Neuman–Keul test, $\alpha = 0.05$). ^bLarvae were incubated individually in 24-well plastic plates under saturated conditions in the laboratory for 14 days after being dissected out from the logs; mycosis was examined for all larvae but only confirmed on fourth instars.

considered. By 2005, the average post-treatment crown dieback increased among trees in both control and treated trees (Table 2). *Beauveria bassiana* strain GHA treated trees, however, suffered less crown dieback than did the controls, with an increase of 34% dieback for the treated trees compared with an increase of 57% for the control trees (Table 2). Fungal treatments significantly reduced crown dieback in this heavily infested green ash stand.

Pre-treatment population levels of *A. planipennis* were similar between the two plots at the Fox Hills site; average larval densities were 154 and 171 larvae/m² for the controls and treatments, respectively, with no significant difference between them ($F_{1,2} = 0.02$, $P = 0.908$) (Table 3). Overall, the *A. planipennis* larval population decreased during summer 2004 for trees on both sides as a portion of larvae from the previous generation matured and emerged as adults during this period. For trees treated with *B. bassiana* strain GHA, however, post-treatment larval density was almost half that in control trees, with average densities of 55 and 103 larvae/m², respectively (Table 3). A significant treatment effect was observed for *B. bassiana* strain GHA based on *A. planipennis* larval density, with less larvae per unit area found in treated trees than in control trees ($F_{1,28} = 9.33$, $P < 0.0001$) (Table 3).

No significant effect of the pre-treatment crown dieback rate was found on post-treatment *A. planipennis* larval density ($F_{2,46} = 2.27$, $P = 0.114$), although higher larval density was found in trees with light and medium crown dieback compared to those with heavy dieback (Figure 1). The average post-treatment larval density for trees with light, moderate, and heavy dieback was 128, 114, and 84 larvae/m² for controls, respectively, compared to 71, 103, and 38 larvae/m² for the treatments, respectively (Figure 1). No

Table 2. Effect of *Beauveria bassiana* strain GHA (BotaniGard ES) treatments on crown dieback caused by *A. planipennis* in green ash trees at the Fox Hills site, Plymouth, MI, 2004–2005.

Treatment	No. of Trees ^a	Crown dieback (%) (Mean ± SEM) ^b	
		2004	2005
Control	70	26.4 ± 3.0A	83.8 ± 3.5A
<i>BbGHA</i>	53	15.4 ± 2.8A	49.0 ± 5.5B

^aOnly trees remained standing in 2005 were included. ^bRepeated measures ANOVA was used to test treatment effects. Means followed by the same upper case letter within a column are not significantly different (Least Squares Means, $\alpha = 0.05$).

Table 3. Larval density and fungal exposure level of *A. planipennis* before and after treatment of *B. bassiana* strain GHA (BotaniGard ES) at 30×10^{13} conidia/ha through foliar and trunk application on green ash trees at the Fox Hills site, Plymouth, MI, 2004–2005.

Sampling date	Treatment	Reps	Sub-samples ^a	No. of larvae found	Density (m^2) (Mean \pm SEM) ^b	No. of larvae infected ^c	Infection rate (Mean \pm SEM) ^b
May 2004 (Pre-treatment)	Control	2	2	43	154.0 \pm 50.8A	0	0
	<i>BbGHA</i>	2	2	38	171.2 \pm 121.1A	0	0
Dec. 04–April 05 (Post-treatment)	Control	25	216	1933	102.5 \pm 6.1a	16	0.4 \pm 1.4a
	<i>BbGHA</i>	25	180	885	54.6 \pm 6.8b	306	20.9 \pm 1.6b

^aEach replicate (tree) contained 1–11 subsamples. ^bMeans followed by the same upper case letter within a column for pre-treatment are not significantly different (Student–Neuman–Keul test, $\alpha = 0.05$). Means followed by the same low case letter within a column for post-treatment are not significantly different (Least Squares Means, $\alpha = 0.05$). ^cLarvae were incubated individually in 24-well plastic plates under saturated conditions in the laboratory for 14 d after being dissected out from the logs; mycosis was examined for all larvae.

significant difference in larval density was found between different levels of crown dieback in the control ($F_{2,22} = 0.97$, $P = 0.40$) or in the treatment ($F_{2,22} = 2.20$, $P = 0.13$). However, for trees with light dieback at the beginning of the study, post-treatment larval density was significantly higher in control trees compared with larval density in the trees treated with *B. bassiana* strain GHA, indicating the efficacy of fungus against this pest (Figure 1).

Larval density was negatively correlated with the trunk height above the ground for treated and control trees; i.e. the higher the position along the tree trunk, the lower the larval density (Figure 2). Significant regressions were observed for the control ($F_{1,9} = 65.46$, $P < 0.001$) and fungal treatment ($F_{1,9} = 27.61$, $P < 0.001$), with linear regression equations

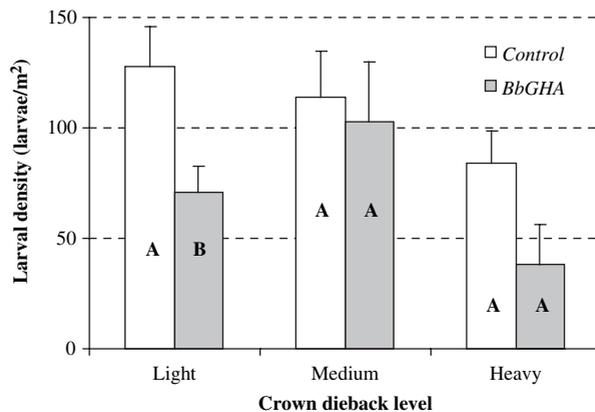


Figure 1. Larval density for *A. planipennis* on green ash trees at different levels of crown dieback after applications of *B. bassiana* strain GHA (BotaniGard ES) at the Fox Hills site, Plymouth, MI, 2004–2005. Each bar represents the average larval density (Mean \pm SEM) of 15 (Light = 0–24%), 5 (Medium = 25–50%), and 5 (Heavy >50%) trees. Bars with the same upper case letter within the same crown dieback level are not significantly different (Student–Neuman–Keul test, $\alpha = 0.05$). The average crown dieback rate for light, medium, and heavy levels at the beginning of the trial was 11, 33, and 67% for control trees, respectively, and 9, 33, and 81% for treated trees, respectively.

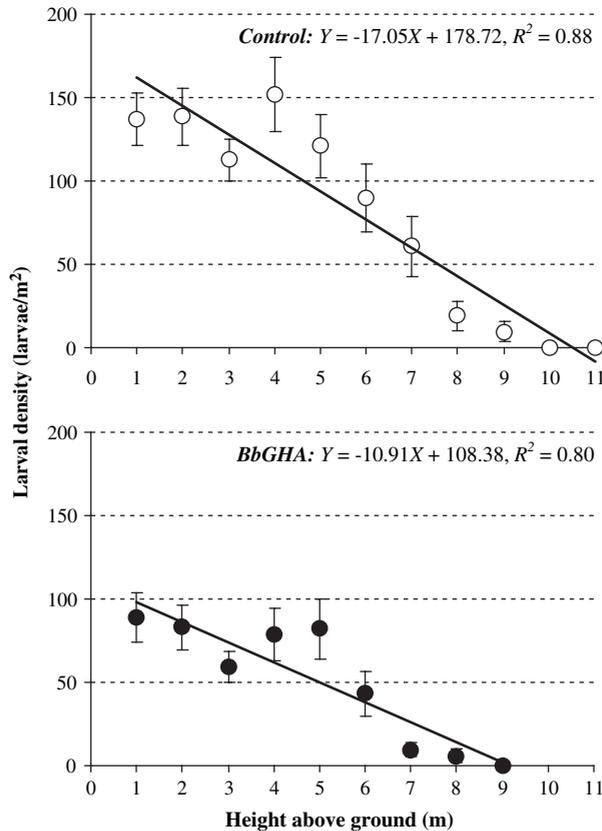


Figure 2. Correlation between post-treatment larval density for *A. planipennis* (Y) (Mean \pm SEM) and the trunk height above the ground (X) in green ash trees following applications of *B. bassiana* strain GHA (BotaniGard ES) at the Fox Hills site, Plymouth, MI, 2004–2005. Control = untreated. BbGHA = four applications of *B. bassiana* strain GHA at 30×10^{13} conidia/ha. Larval density was estimated based on a 30-cm long log section for each replicate. Each data point represents the average of 1–25 replicates.

of $Y = -17.05X + 178.7$ ($R^2 = 0.88$) and $Y = -10.91X + 108.38$ ($R^2 = 0.80$) for the control and the treatment, respectively (Figure 2).

A significant positive correlation between log diameter and *A. planipennis* larval density was also observed at this site (Control: $F_{1,9} = 37.29$, $P = 0.0002$; Treatment: $F_{1,9} = 12.97$, $P = 0.0087$), thus higher larval density was correlated with larger log sections. The linear regression equations were $Y = 13.18X - 18.42$ ($R^2 = 0.81$) and $Y = 7.75X + 6.54$ ($R^2 = 0.65$) for the control and the treatment trees, respectively (Figure 3).

No background fungal infections following 14 days of laboratory incubation were detected in *A. planipennis* larvae sampled before the BotaniGard treatments. The fungal exposure level based on 14 days of laboratory incubation was significantly higher in larvae from treated trees compared with that in larvae from control trees ($F_{1,28} = 55.97$, $P < 0.01$) (Table 3). A total of 21% of *A. planipennis* larvae from treated ash trees were later found infected by *B. bassiana*, compared to 0.4% exposure level observed in control trees. For *A. planipennis* larvae removed from treatment trees, the fungal infection rate ranged from 0 to 43% after 14 days of laboratory incubation, with no significant differences among trees

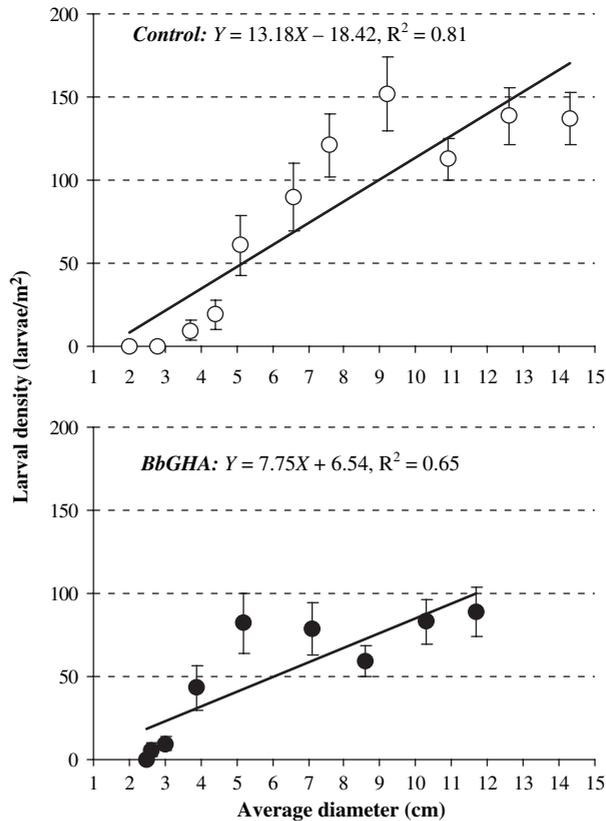


Figure 3. Correlation between post-treatment larval density for *A. planipennis* (Y) (Mean ± SEM) and average log diameter (X) in green ash trees following applications of *B. bassiana* strain GHA (BotaniGard ES) at the Fox Hills site, Plymouth, MI, 2004–2005. Control = untreated. BbGHA = four applications of *B. bassiana* strain GHA at 30×10^{13} conidia/ha. Larval density was estimated based on a 30-cm long log section for each replicate. Each data point represents the average of 1–25 replicates.

($F_{24, 155} = 1.42$, $P = 0.105$). A positive correlation was found between fungal exposure level and larval density ($F_{1,178} = 53.89$, $P < 0.001$, $R^2 = 0.23$). Analysis on spatial distribution within the trees showed that more *A. planipennis* larvae from the lower trunk (0–6 m above the ground) were infected with the fungus than were larvae from the upper trunk (Figure 4), corresponding to the spatial distribution of host larval density (Figure 2 – BbGHA).

Significantly fewer *A. planipennis* adults emerged from trees with light crown dieback the year following treatment with *B. bassiana* compared to similar control trees ($F_{1,18} = 23.71$, $P < 0.0001$). The average adult density was 57 adults/m² for control trees, whereas only 21 adults emerged from the same surface area of fungal-treated trees (Table 4). In these trees, *A. planipennis* adult density generally decreased with increasing height along the tree trunk. The highest density was found at 2 and 3 m above the ground for the control and fungal-treated trees, respectively (Figure 5). Trees treated with *B. bassiana* produced fewer *A. planipennis* adults at every height compared to the controls (Figure 5). No emerged *A. planipennis* adults except one from the treatment was confirmed with fungal infection after 14 days of laboratory incubation, resulting in no significant difference

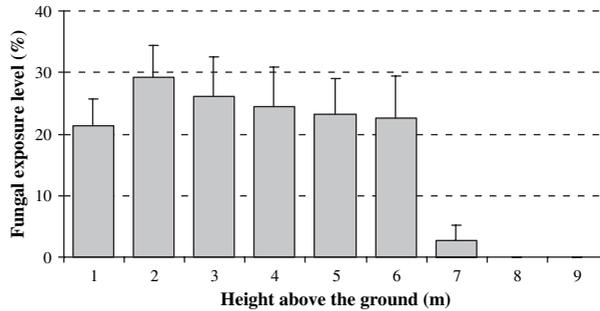


Figure 4. Spatial distribution of *A. planipennis* larvae infected with *B. bassiana* (Mean \pm SEM) on green ash trees following foliar and trunk applications of *B. bassiana* strain GHA (BotaniGard ES) at the Fox Hills site, Plymouth, MI, 2004–2005. Fungal exposure level equals the number of larvae with mycosis divided by the total number of larvae incubated. Larvae were incubated individually in 24-well plastic plates under saturated conditions in the laboratory for 14 days after removal from a 30-cm long log section for each replicate. Each data point represents the average of 2–25 replicates.

in fungal exposure level between treatment and control trees ($F_{1,18} = 1.33$, $P = 0.251$) (Table 4).

Conidial persistence on leaves in the field

Beauveria bassiana conidia persisted well in the field as indicated by the results of 2-day leaf exposure bioassays of adult *A. planipennis*; these bioassays were performed 2, 96, 168, and 264 h after field applications of *B. bassiana* on white ash trees. Overall, adult mortality caused by fungal infection based on 7 days of incubation during the bioassays was significantly higher for adults exposed to foliage treated with fungus when compared with that for adults exposed to control leaves ($F_{1,74} = 140.17$, $P < 0.0001$) (Table 5). After the 2-day exposure period, adult mortality ranged from 78% for those exposed to foliage collected 264 h following fungal application to 100% on foliage collected 2 h later, with no significant difference in adult mortality among leaves collected on different days post-application ($F_{3,25} = 2.46$, $P = 0.08$) (Table 5). Similarly, control mortality ranged from 20 to 46%, with no significant difference among leaves collected at different hours after application ($F_{3,35} = 1.96$, $P = 0.14$) (Table 5). White ash foliage collected from the north side of the tree did not differ significantly from foliage collected from the south side of the

Table 4. Adult density and fungal exposure level of *A. planipennis* after treatment of *B. bassiana* strain GHA (BotaniGard ES) at 30×10^{13} conidia/ha through foliar and trunk application on green ash trees at the Fox Hills site, Plymouth, MI, 2004–2005.

Treatment (conidia/ha)	Reps	Sub-samples ^a	No. of adults emerged	Density (/m ²) (Mean \pm SEM) ^b	No. of adults infected ^c	Infection rate (Mean \pm SEM) ^b
0	10	93	1152	57.2 \pm 4.5A	0	0A
30×10^{13}	10	74	286	21.0 \pm 5.0B	1	0.2 \pm 0.2A

^aEach replicate (tree) contained 7–11 subsamples. ^bMeans followed by the same upper case letter within a column are not significantly different (Least Squares Means, $\alpha = 0.05$). ^cAdults were incubated individually in 24-well plastic plates under saturated conditions in the laboratory for 14 days after being dissected out from the logs; mycosis was inspected for all cadavers.

Table 5. Adult mortality and time-to-death for *A. planipennis* when exposed to white ash foliage following application of *B. bassiana* strain GHA (BotaniGard ES) at the Airport site, Ann Arbor, MI, 2004.

Hours after Application ^a	No. of adults ^b	Mortality (%) (Mean ± SEM) ^{c, e}		Time-to-death (d) (Mean ± SEM) ^{d, e}	
		Control	<i>BbGHA</i>	Control	<i>BbGHA</i>
2	50	46.0 ± 10.4Aa	100.0 ± 0Ab	7.8 ± 0.3Aa	4.5 ± 0.1Ab
96	50	18.0 ± 5.5Aa	96.0 ± 2.7Ab	9.5 ± 0.5BCa	5.4 ± 0.2Bb
168	50	26.0 ± 4.3Aa	88.0 ± 5.3Ab	9.0 ± 0.4ABa	6.1 ± 0.2Cb
264	50	20.0 ± 7.9Aa	78.0 ± 12.1Ab	10.8 ± 0.6Ca	6.9 ± 0.3Db

^aThe crowns of white ash trees were sprayed with *B. bassiana* strain GHA at the rate of 30×10^{13} conidia/ha. Treated leaves were collected from the field at different hours after application. ^b*Agrilus planipennis* adults were exposed to sprayed foliage in groups of 5 for 48 h, with 5 adults per replicate and 10 replicates per treatment. ^cCumulative mortality measured at 7-days post-exposure. ^dAdult mortality was determined daily for 14 days after exposure. Those still moving beyond that were recorded as alive but counted as 14 days in the calculation of time-to-death. No adults lived longer than 13 days in fungal treatment while 29 adults were still alive by 14 days for the control. ^eMeans followed by the same lower case letter within a row and same upper case letter within a column are not significantly different (Student–Neuman–Keul test, $\alpha = 0.05$).

trees in terms of adult mortality for treated ($F_{1,35} = 0.99, P = 0.33$) or control trees ($F_{1,35} = 2.65, P = 0.11$).

The mortality of *A. planipennis* adults occurred mainly between 4 and 7 days after exposure to *B. bassiana* applications to the foliage, with minimal mortality for the first 3 days. Adults exposed to the treated foliage had a significantly shorter life span compared with those exposed to control foliage ($F_{1,394} = 209.92, P < 0.0001$). A significant difference in time-to-death was observed for adults exposed to foliage collected at different hours after application ($F_{3,195} = 34.22, P < 0.0001$), with the lowest time-to-death found for adults exposed to fungal-treated foliage collected at 2 h after application (Table 5). A significant difference in time-to-death was also found for adults exposed to leaves collected at different hours from the control trees ($F_{3,195} = 7.22, P < 0.0001$), with the lowest time-to-death for adults exposed to foliage collected at 2 h after application, followed by 168, 96,

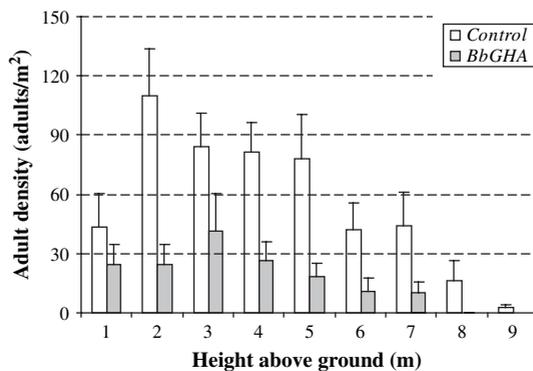


Figure 5. Spatial distribution of post-treatment adult density for *A. planipennis* from green ash trees with light (0–24%) crown dieback following applications of *B. bassiana* strain GHA (BotaniGard ES) at the Fox Hills site, Plymouth, MI, 2004–2005. Control = untreated. *BbGHA* = four applications of *B. bassiana* strain GHA at 30×10^{13} conidia/ha. Adult density (Mean ± SEM) was determined by rearing adults from a 70-cm long log section for each replicate. Each data point represents 2–10 replicates.

and 264 h (Table 5). The time-to-death values ranged from 2 to >14 days for adults in the controls, whereas the life span for adults exposed to fungal-treated foliage ranged from 1 to 13 days. A total of 29 adults were still alive by the end of the 14-day observation period from those exposed to control foliage, compared with none surviving from those exposed to *B. bassiana*-treated foliage.

Beauveria bassiana was the primary cause of *A. planipennis* adult mortality in the treated area. For those exposed to fungal-treated foliage sampled at different hours after treatment, mycosis was confirmed from 74 to 80% cadavers after 7 days of laboratory incubation, whereas for the controls, fungal outgrowth was only recorded on cadavers from foliages collected at 2 h (36%) and 168 h (5%). Due to the close spacing (2×2 m) of the white ash trees at this field site and the high infectivity of *B. bassiana* strain GHA in *A. planipennis* (Liu and Bauer 2006), the fungal infections from control foliage likely resulted from drift during application.

Discussion

The results from this study indicate that *B. bassiana* strain GHA, formulated as BotaniGard ES, may be useful for managing *A. planipennis* in the field. Through foliar and trunk applications of this fungus, new colonisation by *A. planipennis* was reduced by 41% on fungal-treated trees compared to untreated controls. We also found 20% of field-collected larvae infected with fungus following 14 days of laboratory incubation. At a different field site with well-established populations of *A. planipennis*, applications of *B. bassiana* strain GHA led to lower *A. planipennis* densities, resulting in some alleviation of tree damage caused by this pest. Overall, we found the fungal-treated trees contained 47% fewer larvae and produced 63% fewer adults for the next generation when compared to the controls. As a result, the treatment trees sustained 42% less crown dieback than did the control trees. The level of fungal exposure based on 14 days of laboratory incubation, was positively correlated with larval densities. This is likely a result of the overlapping larval galleries found throughout the phloem and suggests horizontal transmission of the fungus might be occurring under the bark of these trees. Horizontal transmission of *B. bassiana* was recorded among spruce bark beetle, *Ips typographus* L. (Coleoptera: Scolytidae) in the laboratory and under field conditions (Kreutz, Zimmermann, and Vaupel 2004).

We tested the persistence of *B. bassiana* strain GHA conidia on ash leaves following field application using adult-infectivity bioassays. Conidial persistence remained high even after 264 h, at which time beetle mortality gradually declined by 22%. High persistence of conidia increases the probability of exposure from leaf surfaces to adult *A. planipennis*, which feed on ash leaves in the tree canopy throughout their lives. Conidial contamination of adults from leaf surfaces may result in direct mortality or indirect mortality through horizontal transmission to other individuals and vertical transmission to neonates. To reduce the application frequency and area sprayed for cost-benefit reasons, however, we suggest future research determine the contribution of sprayed leaves vs. sprayed trunks in the observed *A. planipennis* mortality and reduced crown dieback.

The effects of *B. bassiana* strain GHA on *A. planipennis* field populations can be evaluated directly by comparing pest population levels, or indirectly by examining tree conditions, before and after the treatment. The accuracy of the evaluation depends on the adoption of a precise and reliable sampling protocol for the former, and a strong correlation between pest population and crown dieback for the latter. As a cryptic wood-boring insect that spends most of its life cycle as larvae under the bark, we now know the

distribution of *A. planipennis* is highly unpredictable and variable within and between ash trees in a stand for several years following initial colonisation. This, combined with the lack of information on temporal and spatial patterns, continues to make accurate sampling of *A. planipennis* larval populations in the field extremely difficult. In addition, each sample tree must be felled and carefully debarked to determine the number of *A. planipennis* infested the phloem. This is a destructive and labor-intensive process since number of trees need to be sampled to stabilise the high variation in larval density between sites and trees.

The homogeneous plantation of large green ash trees at Fox Hills site was unique in southeastern Michigan, as ash trees are generally of different ages and species growing in mixed hardwood stands, planted singly along streets, or as small stock growing in rows at nurseries. We felt the use of this green ash plantation at the Fox Hills site and our even-aged white ash plantings at the Airport site would limit the variability introduced by differences in tree age, species, site, and population densities of *A. planipennis* in and around these sites. Due to the close proximity of trees and our concerns for cross contamination to control trees during the fungal sprays, however, we decided not to implement complete randomised or randomised complete block designs at these sites. To minimise spray drift, the treatment trees were sprayed individually with *B. bassiana* in one area and the control trees were left unsprayed in the other area. The trees were then sampled randomly from both the treatment and control groups for *A. planipennis* to evaluate BotaniGard efficacy using this spray regime.

At the Airport site, we dissected entire trees for *A. planipennis* larvae because the trees were small and newly infested with relative low population densities. We found that all trees except one in the control area were infested. *A. planipennis* larvae were distributed along the trunks, with a tendency for higher densities toward the base; the level of fungal exposure was similar throughout. These results demonstrate that sampling only from the upper smaller diameter sections of trees or small trees will underestimate *A. planipennis* density in the field. For the Fox Hills site, where the trees were larger and in the late stage of infestation, we used multiple subsamples from each selected tree to estimate *A. planipennis* larval and adult populations. In these heavily infested trees, *A. planipennis* density was negatively correlated with trunk height above the ground and positively correlated with log diameter. Similar trends were found for adult emergence of the small white marmorated longhorned beetle, *Monochamus sutor* L. (Coleoptera: Cerambycidae) on Dahurian larch (*Larix dahurica* (Turcz. ex Trautv.)) and Mongolian Scots pine (*Pinus sylvestris* var. *mongolica* Litvinov) in China; adult emergence decreased with the height along the trunk and increased with trunk diameter (Zhang, Byers, and Zhang 1993).

After full leaf flush in ash, crown dieback is one of the more conspicuous external symptoms of advanced *A. planipennis* infestation in the field. Using crown dieback to estimate *A. planipennis* field population density, however, necessitated understanding how these factors were correlated. This was done by dissecting ash trees with different levels of crown dieback for *A. planipennis* larvae after leaf flush and before adult emergence. *Agriilus planipennis* population density was then analysed against crown defoliation to establish the correlation. At the Fox Hills site, we found crown dieback was generally inversely correlated with larval density; i.e. trees with lower dieback had more larvae than trees with higher crown dieback. This occurred because the larval damage and adult emergence took place before crown dieback reached the highest levels. Thus, this symptom is a delayed, rather than an instant response of ash trees to attack by *A. planipennis*. A similar method was used to estimate attack rates in oaks (*Quercus* spp.) by the two-lined chestnut borer, *A. bilineatus* (Weber), although the damage criteria was the presence of branches with yellowing foliage (Haack and Benjamin 1982).

During the course of this study, we found overlapping generations of *A. planipennis* larvae within individual trees, creating unexpected challenges for evaluating the efficacy of fungal treatments on larval populations. In general, larvae hatching from eggs in the current year were smaller and younger instars than those that hatched the year before. However, over the years of working with *A. planipennis*, we have learned that larval developmental rate is highly dependent on its host tree, temperature, and possibly genetics. In addition, larvae can overwinter at any stage and diapause is facultative. Some larvae may require 2 years to complete development, whereas others catch up and become synchronised with the older larvae that emerge as adults in the spring. Before developing experimental treatments for management of *A. planipennis*, we recommend evaluating the infestation history and synchrony of the populations as this will confound results and possibly affect the outcome of the study.

When *A. planipennis* was discovered in North America, we had only limited knowledge of its biology, interaction with ash trees, and population dynamics. Although our research during the last 5 years has been focused on developing management tools for *A. planipennis*, we have also gained valuable knowledge of its biology, developed laboratory and field methods for its study, and provided possible solutions for its management in North America. In this study, we found ground-based foliar and trunk applications reduced the number of *A. planipennis* feeding in and emerging from infested ash trees, reduced crown dieback in infested ash trees, and reduced new infestation in healthy ash trees. These findings, as well as those from previous studies (Liu and Bauer 2006, 2008), suggest *B. bassiana* strain GHA has potential for managing *A. planipennis* in the field. The use of insect pathogenic fungi for controlling destructive wood-boring insects is not without precedent, with their success resulting from their high infectivity, virulence, diversity, and a moist environment inside trees (Hajek and Bauer 2007). We suggest continued and expanded research on *B. bassiana* strain GHA for control of *A. planipennis*, by evaluating its efficacy in larger field trials, such as treatment of large ash trees in parks and forests, as well as ash trees beyond the ash-removal zones during eradication efforts at outlier infestations.

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