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Microbial control of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) with *Beauveria bassiana* strain GHA: Greenhouse and field trials ☆

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Abstract

In 2003–2004, the lethal and sublethal effects of *Beauveria bassiana* strain GHA on emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) adults and larvae were evaluated using topical spray and fungal band treatments in the greenhouse and field. *B. bassiana* strain GHA was moderately effective against *A. planipennis* adults in greenhouse studies. However, efficacy was improved in the field when *B. bassiana* was sprayed directly on trunk surfaces prior to adult emergence. In the greenhouse, adult infection rates ranged from 27.7% to 33.5% depending on the application rates that ranged from 25 to 75 $\times$ 10^{13} conidia/ha, whereas in the field, adult infection rates ranged from 58.5% and 83% at two application rates of 10 and 100 $\times$ 10^{13} conidia/ha. The sublethal effects of *B. bassiana* strain GHA was observed on *A. planipennis* adults and larvae surviving exposure to sprayed ash trunks. The adult longevity of females and males was significantly reduced by ca. 9 and 13 d, respectively; females also laid fewer eggs and larval development was prolonged. The use of *B. bassiana* strain GHA-fungal bands resulted in ca. 32% mortality of *A. planipennis* adults compared to ca. 1% for control adults. In addition, *B. bassiana* strain GHA trunk sprays in the fall resulted in ca. 8% mortality of *A. planipennis* under the bark of infested ash trees, compared to 1.6% mortality in the controls. Larval infection rate was positively correlated with larval density in the field. The development of *B. bassiana* strain GHA as a management tool for *A. planipennis* in North America was discussed.

Keywords: Emerald ash borer; *Agrilus planipennis*; *Beauveria bassiana* strain GHA; Lethal and sublethal effects; Greenhouse and field trials; Microbial control

1. Introduction

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an exotic wood-boring pest of ash trees (*Fraxinus* spp.) from northeastern Asia (CAS, 1986; Yu, 1992; Xu, 2003), was first discovered in Michigan and Ontario in 2002 (Haack et al., 2002). The spread of this destructive beetle throughout Michigan, Ohio, and Indiana has been facilitated by the transport of infested ash firewood, logs, and nursery stock; infestations of *A. planipennis* are now present in areas of Maryland, Illinois, Pennsylvania, and West Virginia (USDA APHIS, 2007). As of 2006, over 25 million of Michigan's 700 million ash trees have succumbed to this pest since its arrival in solid-wood packing materials during the 1990s (MDA, 2006). In North America, there are ca. 8 billion ash trees (USDA FS-NERS, 2004) and 16 different ash species (USGS, 2006). The adverse effects of *A. planipennis* on forest biodiversity, ash resources, riparian and urban areas will be substantial since ash trees are widely distributed and planted throughout North America (MacFarlane and Meyer, 2005; USDA FS-NERS, 2004).

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Damage to ash trees from *A. planipennis* is caused primarily by the large numbers of larvae feeding within the phloem, resulting in tree girdling. Crown dieback and epicormic branching are two early symptoms commonly observed among infested ash trees in the field. Total tree mortality occurs within a few years as the *A. planipennis* population in an area reaches a lethal density threshold. In Michigan, *A. planipennis* has a one- or two-year life cycle. For those completing development in one year, neonates hatching in early summer complete larval development during the fall, overwinter in a pupation cell in the outer sapwood or bark, pupate in the spring, and begin to emerge as adults in May, with peak emergence during June. *Agrilus planipennis* with a two-year life cycle overwinter under the bark as immature larvae, complete larval development the next summer, overwinter as mature larvae, and pupate and emerge as adults the following spring. Adults feed on ash foliage throughout their lives, and oviposit in bark crevices or between bark layers of ash trees from mid June to early August. Newly hatched larvae bore directly into the bark until reaching the phloem and sapwood, where they feed until maturation during the current (one-year generation) or following (two-year generation) fall.

Attempts to eradicate or confine this pest in North America were proven to be challenging due to the magnitude of the infestation, the lack of detection and control methods, and the difficulties involved in educating the public and enforcing the quarantines (USDA APHIS, 2006). Effective control measures are clearly needed to slow the spread of *A. planipennis*, contain isolated infestations, and control its populations at or below a tolerance threshold for survival of ash trees in North America.

Entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin have been used in the management of several agricultural defoliators such as grasshoppers and locusts (Jaronski and Goettel, 1997; Lomer et al., 1997), Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Poprawski et al., 1997), diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Vandenberg et al., 1998), and other species (Feng et al., 1994; Inglis et al., 2001). With the increasing importance of woodborers as invasive species worldwide, there is more interest in the use of fungi for sustainable management of these important tree pests (Hajek and Bauer, 2007). Encouraging results have been reported from leopard moth, *Zeuzera multistrigata* Moore (Lepidoptera: Cossidae) (Huang et al., 1990), Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) (Shimazu et al., 1995; Shimazu and Sato, 2003), Asian longhorned beetle, *Anoplophora glabripennis* (Motsch.) (Coleoptera: Cerambycidae) (Zhang et al., 1999a,b; Shimazu et al., 2002; Dubois et al., 2004a,b; Hajek et al., 2006), and *Agrilus auriventris* Saunders (Coleoptera: Buprestidae) (Fan et al., 1990). To evaluate the potential of fungal pathogens for management of *A. planipennis* in North America, we initiated research in Michigan in 2002 to survey for prevalence of fungal infections in the field and to screen for virulent isolates in the laboratory. Results showed that all life stages except eggs of *A. planipennis* were subject to fungal infection under field conditions (Bauer et al., 2004). Comparative laboratory bioassays comparing the activity of entomopathogenic fungi against *A. planipennis* demonstrated that *B. bassiana* strain GHA was highly virulent (Liu and Bauer, 2006). For the present study, we evaluated the lethal and sublethal effects of *B. bassiana* strain GHA on *A. planipennis* adults and larvae using greenhouse and field trials.

2. Materials and methods

2.1. Fungus

Beauveria bassiana strain GHA was tested against *A. planipennis* using two application methods: topical spray and fungal band treatment. Topical spray was performed with BotaniGard ES (Lot# ESO021004, Emerald BioAgriculture, Lansing, MI) on infested ash logs, targeting emerging adults in the greenhouse and on infested ash trunks targeting emerging adults or active larvae under the bark in the field. The fungal band treatment was applied to uninfested ash tree trunks targeting *A. planipennis* adults caged in the field. Fungal bands were made by Dr. Ann Hajek, Cornell University, from non-woven polyester fiber bands impregnated with a sporulating fungal culture of *B. bassiana* strain GHA, originating from Mycontrol technical powder (Lot# 940919, Mycotech Corp., Butte, MT) (Higuchi et al., 1997; Dubois et al., 2004a,b).

Prior to use, conidial content and viability of *B. bassiana* strain GHA was evaluated. For BotaniGard ES, a stock suspension of ca. 10^9 conidia/ml was made by adding 0.25 ml BotaniGard ES to 4.75 ml sterile distilled water (SDW) based on estimated conidia content from product label. A 10^{-2} serial dilution from the stock was then used to check viability by plating 100 μl suspension on quarter-strength Sabouraud dextrose agar with 0.25% w/v yeast extract (SDAY) (Goettel and Inglis, 1997). Two plates were incubated at 24 °C in the dark; conidial viability was estimated 24 h later by examining 400 conidia from each of the two plates using a compound microscope (magnification 400×). Conidia with germination tubes longer than their width were considered germinated (Hywell-Jones and Gillespie, 1990). It is generally accepted that a good batch contains >90% viable conidia (Ekesi et al., 1999; Poprawski et al., 2000; Fernandez et al., 2001; Liu et al., 2002; Tefera and Pringle, 2003). For the fungal bands, a 25 cm^2 (5 × 5 cm) piece of band was cut into smaller pieces (ca. 2 mm^2), placed into a 250 ml Pyrex beaker, stirred with a magnet stirrer for 5 min; spore counts were made from a 10^{-2} serial dilution from the stock. Conidial viability was determined as described above.

2.2. Greenhouse cage trial for emerging adults

We evaluated the effects of *B. bassiana* strain GHA on *A. planipennis* adults emerging from infested logs by spray-

ing their surfaces with BotaniGard ES suspension prior to emergence. *A. planipennis*-infested green ash trees (*F. pennsylvanica* Marsh.), with a diameter at breast height (DBH) of 10–20 cm, were felled by chainsaw at Bicentennial Park, Livonia, Michigan in early March 2003. Trees were cut into 60-cm logs and stored at 4 °C until use. The infested logs were removed from chill during the summer and held in an incubator at 24 °C, 50–60% ambient RH with a photoperiod of 16:8 (L:D) h for 28 d. Previous observations showed that adult emergence from logs began after 30 d under these conditions. After removal from the incubator and prior to adult emergence, the logs were sprayed with BotaniGard ES suspension. This was done in a swinging boom spray cabinet (Allen Machine Works, Midland, MI) with a no. 8001E nozzle (Spray Systems Co. Wheaton, IL) set 35 cm above the log surface. The upper and lower surfaces of each log were sprayed at the rate of 187.1 L/ha (20 gallons/acre) at 25 p.s.i. We used three fungal concentrations and a control consisting of 0.02% BotaniGard ES blank formulation (Emerald BioAgriculture Corp., Lansing, MI). Each treatment was repeated three times. Based on the conidial content of BotaniGard ES (2×10^{13} conidia/qt), an application of 2.34 l/ha (1 qt/acre) provided coverage of 5×10^5 conidia/cm^2. To achieve the three application rates of 25, 50, and 75×10^{13} conidia/ha in our assays, we used 1.25, 2.5, and 3.75 ml of BotaniGard ES to make a 20-ml suspension, which was sprayed in the cabinet. The concentration of each application was 1.25×10^9, 2.5×10^9, and 3.75×10^9 conidia/ml, respectively. After treatment, logs were held in the greenhouse at 20–26 °C, 20–40% RH under natural lighting individually in 28 × 28 × 51 cm aluminum cages (Bioquip, Rancho Dominguez, CA) with a greenhouse-raised evergreen ash tree (*F. uhdei* (Wenzig) Lingelsh) as food for emerging *A. planipennis* adults; the ash trees were watered daily. A freshly cut uninfested green ash log (7–9 cm in diameter, 25 cm long) with paraffin-sealed ends was also placed in each cage as *A. planipennis* oviposition sites. Each day, adult emergence was recorded by marking the exit holes on each log with a pen, and dead adults were removed from the cage. Dead adults were then incubated at 24 °C individually in a moist chamber consisting of a 60-mm plastic Petri dish lined with moist sterile filter paper. Fungal infection was confirmed by the presence of mycosis on cadavers 7 d after death. At the end of the assay, we counted the number of *A. planipennis* eggs on each oviposition log.

2.3. Summer field cage trial for emerging adults

We evaluated the efficacy of *B. bassiana* strain GHA on emerging *A. planipennis* adults using moderately infested green ash trees in an even-aged plantation at Fox Hill Golf Club (Plymouth, MI) in June 2003. These trees were about 20-yr old ranging in DBH between 9–14 cm and height between 8 and 10 m. On 26 June, we selected a 180-cm long trunk section, 50 cm above the ground, from each tree. The old adult exit holes (where *A. planipennis* adults had emerged the year before) on each trunk section were counted and marked with a pen; the trunk was then sprayed with BotaniGard ES a few days before predicted emergence of *A. planipennis* adults. Treatments included two application rates of BotaniGard ES and an untreated control; each treatment was repeated five times. A 11.4-L (3 gallon) Roundup professional sprayer (The Fountainhead Group, Inc, New York Mills, NY) equipped with a flat fan nozzle was used in this trial. BotaniGard ES suspension was applied on the north and south side of the trunk under 35 p.s.i. at the concentration of 5×10^7 and 5×10^8 conidia/ml, corresponding to application rates of 10×10^{13} and 100×10^{13} conidia/ha, respectively. The volume of fungal suspension each tree received was calculated on the basis of trunk-section surface area. The volume was then transformed to spray duration (sec) after sprayer calibration. After treatment, each treated trunk section was caged to contain *A. planipennis* adults after emergence. Cages were constructed of Brite-Kote™ aluminum screen (18 × 16 mesh) (76.2 cm × 30.5 m roll; Phifer Wire Products, Inc., Tuscaloosa, AL). A rectangular piece of screen (76.2 × 190 cm) was cut from the roll and wrapped around the 180-cm long trunk section to make a cylindrical cage. A staple gun (Duo-Fast CS-5000, Duo-Fast Corp., Elgin, IL) filled with 1.3 cm (½ inch) staples were used to secure and seal the cage at both ends as well as along the trunk where the two long sides of the screen met. In earlier tests, staples spaced 0.5 cm apart prevented escape of *A. planipennis* adults. Duct tape was used on both ends to further seal the cage. The cages were so constructed that >3 cm of space was left between the screening and the tree trunk. In addition, an ash branch with 4–5 healthy leaves was enclosed inside each cage to provide food for emerging adults. See Hajek and Bauer (2007) for a photo of the cages in the field. After 6 weeks, the cages were disassembled and adult mortality was determined. Dead adults were cultured for fungal infection individually in 24-well plastic plates under saturated conditions, and mycosis confirmed 7 d later. The trees used in this study were felled in September 2003, and the treated trunk sections were returned to laboratory for dissection. The number of *A. planipennis* larvae from each section was recorded.

2.4. Fall field trial for active larvae under the bark

To explore the possible effects of *B. bassiana* strain GHA on *A. planipennis* larvae under the bark, infested white ash trees (*F. americana* L.) (7–10 cm in diameter, 5–6 m in height) with significant longitudinal bark splits were selected in a large parking lot in Okemos, MI on 11 October 2003. The lower 180 cm trunk section above the ground from each tree was treated with *B. bassiana* strain GHA, formulated as BotaniGard ES, at 5×10^8 conidia/ml. The fungal suspension was applied to the selected trunk section from both the north and the south side of the tree using a hand atomizer. The amount of fungal suspension applied to each tree was calculated based on the surface

area of the selected trunk section at the rate of 70×10^{13} conidia/ha. The immediate upper 180 cm trunk section for each tree was used as the untreated control. A total of 13 trees were used in this treatment. Treated trees were felled and separated into 60 cm log sections on February 2004 and brought back to the laboratory for dissection. *A. planipennis* larvae, prepupae, and pupae dissected from the logs and screened for fungal infection as described above; mycosis was confirmed after 14 d.

2.5. Summer fungal band application for introduced adults

We evaluated the efficacy of fungal bands with *B. bassiana* strain GHA against adult *A. planipennis* in the field. Green ash trees with no external symptoms of infestation on the lower trunks were selected from the ash plantation in Fox Hill Golf Club on 09 July 2003. For the treatment, a fungal band (5 cm wide × 50 cm long) was stapled around the circumference of the tree trunk at 100 cm above the ground; no fungal band was applied to the trunk of control trees. A rectangular aluminum screen (76.2 × 110 cm) was used to construct the cage on the trunk of each tree as described above, including the enclosure of an ash branch with 4–5 healthy leaves as food for the adults. The bottom of each cage was placed at the height of 50 cm above the ground. Toward the top end of each cage, a 5 cm diameter hole was left open for the introduction of *A. planipennis* adults. A total of 10 adults were then transferred into each cage before it was sealed with staples and duct tape. The *A. planipennis* adults used for this experiment were collected from infested green ash trees in Livonia, MI one day prior to treatment. There were 40 replicates for the treatment and 10 replicates for the control. Four weeks later, the cages were disassembled, and *A. planipennis* adult mortality examined. Dead adults were removed and individually evaluated for fungal infection as described above; mycosis was confirmed 7 d later.

2.6. Data analysis

Percent mortality and fungal infection rate were first subjected to angular (arcsin square root) transformation before analysis. Analysis of variance (ANOVA) ($\alpha = 0.05$) was used to analyze the data for larval and adult density and infection rate in greenhouse and field cage trials; adult longevity and fecundity in the greenhouse trial; life-stage composition of larvae in the field cage trial and fungal band treatment; and fungal infection rate in the fungal band treatment. Student-Neuman-Keul's test ($\alpha = 0.05$) was used if significant differences were detected between different treatments (PROC GLM) (SAS Institute, 2004). A paired *t*-test was used to examine the treatment effects of *B. bassiana* strain GHA on *A. planipennis* larvae after trunk sprays (SAS PROC MEANS) (SAS Institute, 2004). Regression analysis (PROC REG) (SAS Institute, 2004) was used to analyze the correlation between larval density and fungal infection rate for the fall field trial for active larvae under the bark.

3. Results

3.1. Greenhouse cage trial for emerging adults

The emergence of *A. planipennis* adults from infested logs in the greenhouse began one day after the logs were sprayed and continued for about a week. The density of *A. planipennis* adults was similar for control and treatment logs, with a range of 54.4–300.9 adults/m^2 (Table 1). Adults died of fungal infection among the treatment logs was significantly greater than from control logs ($F = 5.15$, $df = 3$, 8, $P = 0.03$) (Table 1). Adult infection rate ranged from 0 to 46.3, 22.6 to 47.1, and 20.0 to 48.9 for the application rate of 25, 50, and 75×10^{13} conidia/ha, respectively, with no significant difference between the three fungal application rates (Table 1); no fungal infection was observed in adults from the control logs (Table 1).

The adult longevity of both female and male *A. planipennis* was reduced significantly by pre-emergent fungal treatments ($F = 24.52$, $df = 4$, 353, $P < 0.01$) (Table 2). The average survival time of *A. planipennis* females was almost twice as long for adults emerging from control logs (22.3 d) than for adults emerging from the three fungal treatments (ranging from 13.0 to 14.3 d); no significant differences were found between fungal concentrations.

Table 1
Adult density and infection rate of *A. planipennis* after emerging from infested logs sprayed with *B. bassiana* strain GHA and held in cages in the greenhouse, East Lansing, MI 2003

Treatment (conidia/ha)[a]	Reps	No. of adults emerged	Adult density (/m^2)[b] (Mean ± SEM)	No. of adults infected[c]	Infection rate[b,d] (Mean ± SEM)
0	3	65	129.2 ± 46.8A	0	0A
25×10^{13}	3	114	189.4 ± 59.3A	43	27.7 ± 14.1B
50×10^{13}	3	78	142.4 ± 25.1A	28	37.7 ± 7.6B
75×10^{13}	3	101	179.4 ± 44.0A	36	33.5 ± 8.4B

[a] BotaniGard ES with a label recommended application rate of 5–85 × 10^{13} conidia/ha.
[b] Means followed by the same upper case letter within a column are not significantly different (Student-Neuman-Keul test, $\alpha = 0.05$).
[c] Mycosis was examined for all cadavers.
[d] Infection rate = (number of adults died of fungal infection/total number of adults emerged) × 100%.

Table 2
Effects of *B. bassiana* strain GHA on adult longevity and fecundity of *A. planipennis* when applied through pre-emergent trunk spray in the greenhouse, East Lansing, MI 2003

Treatment (conidia/ha)	Reps	No. of adults emerged	Longevity (days) (Mean ± SEM) (*n*)[a]		No. of eggs produced	Fecundity (eggs/female)[a] (Mean ± SE)
			Male	Female		
0	3	65	27.8 ± 2.2 (27)Ab	22.3 ± 1.7 (38)Aa	91	4.2 ± 3.3A
25×10^{13}	3	114	12.9 ± 1.0 (46)Ba	13.0 ± 0.8 (67)Ba	29	0.7 ± 0.4A
50×10^{13}	3	78	15.7 ± 1.2 (39)Ba	13.3 ± 1.3 (39)Ba	55	1.2 ± 1.0A
75×10^{13}	3	101	15.9 ± 1.1 (50)Ba	14.3 ± 1.1 (51)Ba	23	0.6 ± 0.2A

[a] Means followed by the same lower case letter within a row or same upper case letter within a column are not significantly different (Student-Neuman-Keul test, $\alpha = 0.05$).

Although there was higher fecundity in females from control logs, this difference was not significant (Table 2). No significant difference was observed between sexes except for the control, where *A. planipennis* males survived significantly longer than the females (Table 2).

3.2. Summer field cage trial for emerging adults

The number of old exit holes and larval densities were similar for treatment and control trunk sections from the ash trees used in this study (Table 3). The surface area of treatment trunk sections ranged from 0.47 to 0.80 m^2, resulting in the application of 120–200 ml fungal suspension to treatment trunk surfaces on each tree. Adult emergence into the screen cages began a few days after the trunk sections were sprayed. Densities of emerging adults ranged from 2.7 to 41.8 adults/m^2 and were similar for the treatment and control trees (Table 3). Adult fungal infection rates were significantly higher on treated trunk sections compared to controls ($F = 21.97$, $df = 2, 12$, $P < 0.01$). The infection rate for *A. planipennis* adults emerging from the treated trunk sections ranged from 33.3% to 100% and 62.5% to 100% for the rate of 10 and 100×10^{13} conidia/ha, respectively, with no significant differences between the two application rates; no adults died of fungal infection from the control trunk sections (Table 3). No significant difference in post-treatment larval density was found between fungal treated and control trees (Table 3), however, analysis of the larval stages revealed more prepupae and fewer second, third and forth instars were found in control trees than in the fungal-treated trees, suggesting sublethal effects of fungal exposure in the developmental rate of larvae (Fig. 1II).

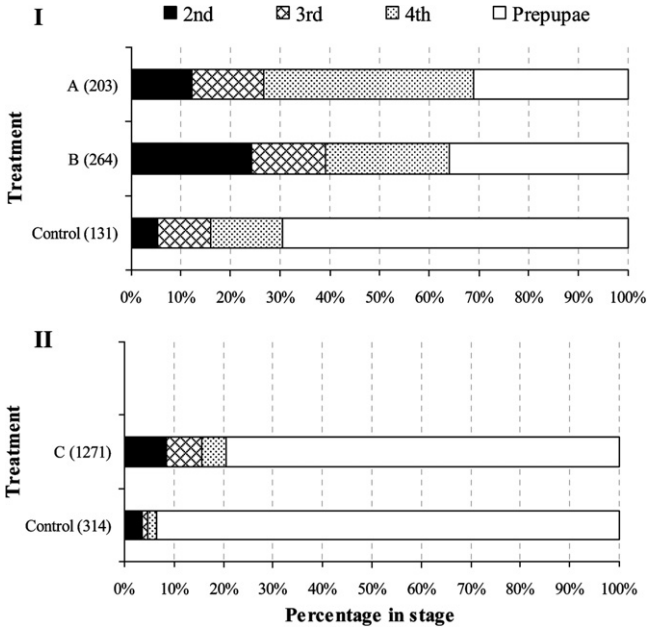

Fig. 1. Stage composition of *A. planipennis* larvae after exposure to *B. bassiana* strain GHA through trunk spray in the field at Plymouth (I) and Okemos (II), MI 2003-04. Control = Untreated, A = 10×10^{13} conidia/ha, B = 100×10^{13} conidia/ha, C = 70×10^{13} conidia/ha). Numbers following each treatment represent the total number of *A. planipennis* larvae dissected from the treatment trees at the end of the trial.

3.3. Fall field trial for active larvae under the bark

Dissection of the white ash trunk sections in February 2004 revealed that 2nd, 3rd, 4th larvae and prepupae of *A. planipennis* were present; prepupae were the predominant stage (Fig. 1II). The treatment surface area ranged

Table 3
Effects of *B. bassiana* strain GHA on *A. planipennis* adults when applied through pre-emergent trunk spray in the field, Plymouth, MI 2003

Treatment (conidia/ha)	Reps	No. of old Exit holes[a]	No. of adults emerged	Adult density (/m^2)[a] (Mean ± SEM)	No. of adults infected[b]	Infection rate[a,c] (Mean ± SEM)	Larval density (/m^2)[a] (Mean ± SEM)
0	5	2.6 ± 1.2A	26	8.8 ± 2.3A	0	0A	40.0 ± 18.3A
10×10^{13}	5	4.4 ± 1.8A	42	14.0 ± 7.0A	20	58.5 ± 14.3B	62.6 ± 25.4A
100×10^{13}	5	5.8 ± 4.8A	55	18.1 ± 6.0A	43	83.0 ± 7.8B	84.3 ± 17.6A

[a] Means followed by the same upper case letter within a column are not significantly different (Student-Neuman-Keul test, $\alpha = 0.05$).
[b] Mycosis was examined for all cadavers.
[c] Infection rate = (number of larvae died of fungal infection/total number of larvae dissected out) × 100.

Table 4
Larval density and infection rate of *A. planipennis* when exposed to *B. bassiana* strain GHA through trunk spray in the field, Okemos, MI 2003-04

Treatment (conidia/ha)	Reps	No. of larvae found	Larval density (/m^2)[a] (Mean ± SEM)	No. of larvae infected[b]	Infection rate[c] (Mean ± SEM)
0	13	314	82.6 ± 23.9A	2	1.6 ± 1.3A
70×10^{13}	13	1271	187.9 ± 46.1B	131	7.9 ± 2.2B

[a] Means followed by the same upper case letter within a column are not significantly different (Paired *t*-test, $\alpha = 0.05$).
[b] Mycosis was examined for all cadavers.
[c] Infection rate = (number of larvae died of fungal infection/total number of larvae dissected out) × 100%. Means followed by the same upper case letter within a column are not significantly different (Student-Neuman-Keul test, $\alpha = 0.05$).

from 0.38 to 0.61 m^2, resulting in an application range of 54–86 ml fungal suspension for the treated trunk sections on each tree. Larval densities were highly variable between trees. Fungal-treated trunk sections contained significantly more larvae than did the control trunk sections when they were treated ($t = 3.48$, $n = 13$, $P < 0.01$) (Table 4). Fungal infections occurred in the *A. planipennis* 4th-instar larvae and prepupae exclusively. The overall infection rate of the *A. planipennis* larvae and prepupae was 7.9% for the treatments trees and 1.6% for the controls, with significant differences between them ($t = 3.30$, $n = 13$, $P < 0.01$) (Table 4). Marginal positive density dependence was observed for the infection rate of *A. planipennis* larvae ($F = 3.60$, $df = 1, 11$, $P = 0.08$) (Fig. 2). This correlation was improved significantly ($Y = -0.37 + 0.28\ X$, $R^2 = 0.47$) when logarithm (log 10) transformed larval density and angular transformed percent infection rate was used in the analysis ($F = 9.8$, $df = 1, 11$, $P < 0.01$). Larval development was also delayed in the fungal-treated ash trunks compared to the controls, as evidenced by significantly fewer *A. planipennis* prepupae ($F = 11.42$, $df = 1, 24$, $P < 0.01$), and significantly more 2nd ($F = 6.73$, $df = 1, 24$, $P = 0.02$), 3rd ($F = 6.59$, $df = 1, 24$, $P = 0.02$) and 4th ($F = 5.02$, $df = 1, 24$, $P = 0.03$) instars (Fig. 1II).

3.4. Summer fungal band application for introduced adults

Conidial density of *B. bassiana* strain GHA on the fungal band was 6.4×10^8 conidia/cm^2, with conidia variability ranging from 81.4 to 87.9%. A significant treatment effect was observed for *B. bassiana* strain GHA ($F = 37.19$, $df = 1, 48$, $P < 0.01$) with 31.6% *A. planipennis* adult mortality resulting from fungal infection on treated trees vs. 1.1% on control trees (Fig. 3).

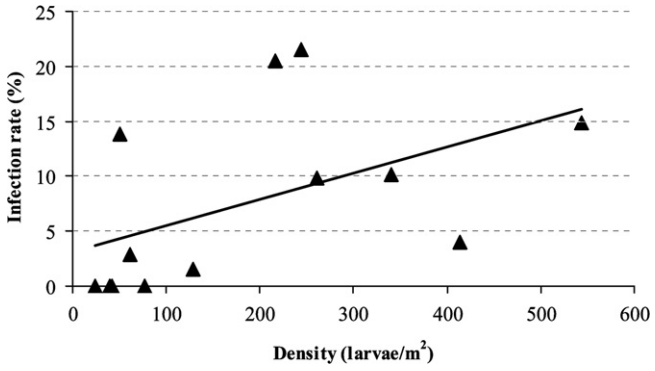

Fig. 2. Density dependent infection rate of *B. bassiana* strain GHA on *A. planipennis* larvae after exposure through trunk spray in the field, Okemos, MI 2003-04.

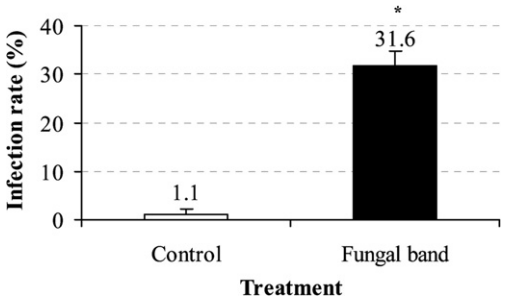

Fig. 3. Infection rate of *A. planipennis* adults when exposed to *B. bassiana* strain GHA through fungal band application in the field, Plymouth, MI 2003. There were 10 and 40 replicates for the control and fungal band treatment, respectively. Asterisk(*) indicates significant difference between the two treatments (Student-Neuman-Keul test, $\alpha = 0.05$).

4. Discussion

The results from our greenhouse and field studies demonstrated that *B. bassiana* strain GHA has good potential as a management tool for suppressing *A. planipennis* population densities. We found early summer pre-emergent trunk sprays of *B. bassiana* strain GHA infected and killed *A. planipennis* adults, which became infected under the bark before emergence possibly through entry of fungal conidia into bark splits, during emergence when chewing through fungal treated bark, or after emergence by walking and ovipositing on the treated bark. Fall trunk sprays resulted in larval mortality under the bark, though less than occurred following early summer applications, both showing positive correlations between larval densities and fungal infection rates. In addition, *B. bassiana* strain GHA applications resulted in sublethal effects to *A. planipennis* on both adults and larvae that survived fungal exposure. The use of fungal bands, impregnated with *B. bassiana* strain GHA cultures was less effective than the pre-emergence trunk applications.

Of the approaches we tested in this study, topical spray of *B. bassiana* strain GHA to *A. planipennis*-infested tree trunks prior to summer emergence was the most effective method for suppressing *A. planipennis* populations. The

higher adult infection rates observed in the field cage trial compared to that of greenhouse trial may have resulted from differences in log conditions, cage setups, and other abiotic factors. A similar approach of topical spray of *B. bassiana* for emerging woodboring insects were also tested for the longhorned beetle *M. alternatus* on pine trees in Japan (Shimazu et al., 1982).

Sublethal effects observed for *A. planipennis* adults surviving *B. bassiana* strain GHA pre-emergence trunk sprays included shortened adult longevity. In the field, this would result in lower fecundity, thus lower larval densities in ash trunks. Further research on the sublethal effects of fungal exposure on *A. planipennis* fecundity would be facilitated by observations of mated pairs in individual container after treatment as well as more replication due to the level of high variability in *A. planipennis* fertility and fecundity in the laboratory (Bauer and Liu, unpublished data). Other sublethal effects of fungal treatments on *A. planipennis* included prolonged larval development period. Similar results have been reported for other fungi and insects including *B. bassiana*, *B. brongniartii* (Saccardo) Petch, and *M. anisopliae* against Asian longhorned beetle (Dubois et al., 2004a,b); *B. bassiana* against western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae) (Noma and Strickler, 2000) and the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) (Torrada-Leon et al., 2006); and *B. bassiana* and *M. anisopliae* against *Ceratitis capitata* (Diptera: Tephritidae) (Quesada-Moraga et al., 2006). A combination of behavioral and physiological responses is believed responsible for reduced performance of insects surviving exposure of insect pathogenic fungi (Sharma et al., 1994; Hajek and St. Leger, 1994).

Fungal band, a live fungal culture growing on a fiber band, were initially developed with *B. brongniartii* for management of Japanese pine sawyer (Higuchi et al., 1997; Higuchi, 1999). Fabrication techniques have since been used with other fungi for management of other woodboring insect pests, including the Asian longhorned beetle (Zhang et al., 1999b; Dubois et al., 2004a). The advantage of the fungal band conidial production is its >1 month persistence in the field, thus prolonging efficacy (Higuchi, 1999; Dubois et al., 2004a). We found this application method was relatively ineffective for control of *A. planipennis* because adults are more likely to fly than walk on tree trunks and across the fungal band, as do longhorned beetles, for which the fungal band method was originally designed.

Agrilus planipennis larvae appear well protected under the bark of ash trees, however, in an earlier natural enemy study, we found entomopathogenic fungi resulting in ca. 2% larval mortality in southeastern Michigan (Bauer et al., 2004). The successful infection of *A. planipennis* larvae by *B. bassiana* strain GHA following trunk sprays suggests the longitudinal bark splits found on infested ash trunks may facilitate the fungal infection process. To improve infection rates, we suggest further research on the timing of fungal trunk sprays, perhaps during the summer when the bark splits first begin appear because of callous formation around *A. planipennis* galleries.

Wood-boring insects are considered difficult to control because the most damaging stage, usually the larva, live in cryptic and inaccessible habitats (Hajek and Bauer, 2007). *A. planipennis* adults, however, are exposed and vulnerable as they emerge through the ash bark, feed on ash foliage throughout the tree crown, and mate and lay eggs on ash branches and trunks. Based on the results of these studies, we believe entomopathogenic fungi such as *B. bassiana* strain GHA could be useful in the containment and management of *A. planipennis* in North America. However, more research is needed to optimize application rates and delivery methods, determine the efficacy of foliar and trunk applications, and assess the effects on non-target insects. If successful, the use of fungi will provide an environmentally-friendly alternative to conventional systemic insecticides, which are being evaluated for management of *A. planipennis* in urban trees.

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References

Bauer, L.S., Liu, H.-P., Haack, R.A., Petrice, T.R., Miller, D.L., 2004. Natural enemies of emerald ash borer in southeastern Michigan. In: Mastro, V., Reardon, R. (Eds.), Proceedings of Emerald Ash Borer Research and Technology Meeting, Port Huron, MI. USDA FS FHTET-2004-02, p. 33.

CAS (Chinese Academy of Science, Institute of Zoology), 1986. 1061. *Agrilus marcopoli* Obenberger. Agriculture Insects of China (part I), China Agriculture Press, Beijing, p. 445.

Dubois, T., Hajek, A.E., Hu, J.-F., Li, Z.-Z., 2004a. Evaluating the efficacy of entomopathogenic fungi against the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae), by using cages in the field. Environmental Entomology 33, 62–74.

Dubois, T., Li, Z-Z., Hu, J-F., Hajek, A.E., 2004b. Efficacy of fiber bands impregnated with *Beauveria brongniartii* cultures against Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Biological Control 31, 320–328.

Ekesi, S., Maniania, N.K., Ampong-Nyarko, K., Onu, I., 1999. Effect of intercropping cowpea with maize on the performance of *Metarhizium anisopliae* against *Megalurothrips sjostedti* (Thysanoptera: Thripidae) and predators. Environmental Entomology 28, 1154–1161.

Fan, M-Z., Li, L-C., Guo, C., Wu, X-M., Sun, Z-B., 1990. Control of *Agrilus auriventris* by the strain Ma83 of *Metarhizium anisopliae*. Disinsectional Microorganism 3, 278–279.

Feng, M., Poprawski, T.J., Khachatourians, G.G., 1994. Production, formulation, and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. Biocontrol Science and Technology 4, 3–34.

Fernandez, S., Groden, E., Vandenberg, J.D., Furlong, M.J., 2001. The effect of mode of exposure to *Beauveria bassiana* on conidia acquisition and host mortality of Colorado potato beetle *Leptinotarsa decsemilineata*. Journal of Invertebrate Pathology 77, 217–226.

Goettel, M.S., Inglis, G.D., 1997. Chapter V-3. Fungi: hyphomycetes. In: Lacey, L.A. (Ed.), Manual of Techniques in Insect Pathology. Academic Press, San Diego, CA, pp. 213–249.

Haack, R.A., Jendek, E., Liu, H-P., Marchant, K.R., Petrice, T.R., Poland, T.M., Ye, H., 2002. The emerald ash borer: a new exotic pest in North America. Newsletter of the Michigan Entomological Society 47 (3&4), 1–5.

Hajek, A.E., Bauer, L.S., 2007. Microbial control of wood-boring insects attacking forest and shade trees. In: Lacey, L.A., Kaya, H.K. (Eds.), Field Manual of Techniques in Invertebrate Pathology. Springer, Secaucus, NJ, pp. 505–533.

Hajek, A.E., Huang, B., Dubois, T., Smith, M.T., Li, Z-Z., 2006. Field studies of control of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) using fiber bands containing cultures of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria brongniartii*. Biocontrol Science and Technology 16, 329–343.

Hajek, A.E., St Leger, R.G., 1994. Interactions between fungal pathogens and insect hosts. Annual Review of Entomology 39, 293–322.

Higuchi, T., 1999. Biolisa® Kamikiri—a biorational agricultural pesticide contributing to environmental conservation. Nitto Technology Report 37, 49–51.

Higuchi, T., Saika, T., Senda, S., Mizobata, T., Kawata, Y., Nagai, J., 1997. Development of biorational pest control formulation against longicorn beetles using a fungus, *Beauveria brongniartii* (Sacc). Petch. Journal of Fermentation Bioengineering. 84, 236–243.

Huang, J-S., He, Y-L., Lin, Q-Y., 1990. Use of new paste preparation of *Beauveria bassiana* in the forest to control *Zeuzera multistrigata* (Lepidoptera: Cossidae). Chinese Journal of Biological Control 6, 121–123.

Hywell-Jones, N.L., Gillespie, A.T., 1990. Effect of temperature on spore germination in *Metarhizium anisopliae* and *Beauveria bassiana*. Mycological Research 94, 389–392.

Inglis, G.D., Goettel, M.S., Butt, T.M., Strasser, H., 2001. Use of Hyphomycetous fungi for managing insect pests. In: Butt, T.M., Jackson, C., Magan, M. (Eds.), Fungi as Biocontrol Agents: Progress, Problems and Potential. CABI Publishing., Wallingford, UK, pp. 23–69.

Jaronski, S.T., Goettel, M.S., 1997. Development of *Beauveria bassiana* for control grasshoppers and locusts. In: Goettel, M.S., Johnson, D.L. (Eds.), Microbial Control of Grasshoppers and Locusts. Memoirs of the Entomological Society of Canada, vol. 171, Ottawa, Canada, pp. 225–237.

Liu, H-P., Skinner, M., Parker, B.L., Brownbridge, M., 2002. Pathogenicity of *Beauveria bassiana, Metarhizium anisopliae*, (Deuteromycotina: Hyphomycetes), and other entomopathogenic fungi against *Lygus lineolaris* (Hemiptera: Miridae).. Journal of Economic Entomology 95, 675–681.

Liu, H-P., Bauer, L.S., 2006. Susceptibility of *Agrilus planipennis* (Coleoptera: Buprestidae) to *Beauveria bassiana* and *Metarhizium anisopliae*. Journal of Economic Entomology 99, 1096–1103.

Lomer, C.J., Prior, C., Kooyman, C., 1997. Development of *Metarhizium* spp. for control grasshoppers and locusts. In: Goettel, M.S., Johnson, D.L. (Eds.), Microbial Control of Grasshoppers and Locusts. Memoirs of the Entomological Society, Canada, vol. 171. Ottawa, Canada, pp. 265–286.

MacFarlane, D.W., Meyer, S.P., 2005. Characteristics and distribution of potential ash tree hosts for emerald ash borer. Forest Ecology and Management 213, 15–24.

MDA (Michigan Department of Agriculture), 2006. Emerald ash borer. Available from: <http://www.michigan.gov/mda/0,1607,7-125-1568_2390_18298—,00.html>.

Noma, T., Strickler, K., 2000. Effects of *Beauveria bassiana* on *Lygus Hesperus* (Hemiptera: Miridae) feeding and oviposition. Environmental Entomology 29, 394–402.

Poprawski, T.J., Carruthers, R.I., Speese III, J., Vacek, D.C., Wendel, L.E., 1997. Early-season applications of the fungus *Beauveria bassiana* and introduction of the hemipteran predator *Perillus bioculatus* for control of the Colorado potato beetle. Biological Control 10, 48–57.

Poprawski, T.J., Greenberg, S.M., Ciomperlik, M.A., 2000. Effect of host plant on *Beauveria bassiana* and *Paecilomyces fumosoroseus*-induced mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Environmental Entomology 29, 1048–1053.

Quesada-Moraga, E., Ruiz-Garcis, A., Stantiago-Alvarez, C., 2006. Laboratory evaluation of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against puparia and adults of *Ceratitis capitata* (Diptera: Tephritidae). Journal of Economic Entomology 99, 1955–1966.

SAS Institute. 2004. SAS/STAT user's guide, Version 9.1. SAS Institute. Cary, NC.

Sharma, S., Agarwal, G.P., Rajak, R.C., 1994. Pathophysiological alterations caused in *Heliothis armigera* by toxin metabolites of *Beauveria bassiana*. Indian Journal of Experimental Biology 32, 168–171.

Shimazu, M., Kushida, T., Katagiri, K., 1982. Microbial control of *Monochamus alternatus*—spraying of pathogens onto the infested pine trees just before adult emergence. Transactions of the Japanese Forestry Society 93, 399–400.

Shimazu, M., Sato, H., 2003. Effects of larval age on mortality of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) after application of nonwoven fabric strips with *Beauveria bassiana*. Applied Entomology and Zoology 38, 1–5.

Shimazu, M., Tsuchiya, D., Sato, H., Kushida, T., 1995. Microbial control of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) by application of nonwoven fabric strips with *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) on infested tree trunks. Applied Entomology and Zoology 30, 207–213.

Shimazu, M., Zhang, B., Liu, Y-N., 2002. Fungal pathogens of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) and their virulences. Bulletin of Forestry and Forest Products Research Institute 1 (1), 123–130.

Tefera, T., Pringle, K.L., 2003. Effect of exposure method to *Beauveria bassiana* and conidia concentration on mortality, mycosis, and sporulation in cadavers of *Chilo partellus* (Lepidoptera: Pyralidae). Journal of Invertebrate Pathology 84, 90–95.

Torrada-Leon, E., Montoya-Lerma, J., Valencia-Piza, E., 2006. Sublethal effects of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) on the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) under laboratory conditions. Mycopathologia 162, 411–419.

USDA APHIS (Animal and Plant Health Inspection Service). 2006. Eradication of emerald ash borer in Michigan, Ohio, and Indiana: Implementation of the strategic plan. Available from: <http://www.aphis.usda.gov/plant_health/plant_pest_info/emerald_ash_b/downloads/strategicpla.pdf>.

USDA APHIS (Animal and Plant Health Inspection Service), 2007. Emerald ash borer. Available from: <http://www.aphis.usda.gov/ppq/ep/eab>.

USDA FS-NERS (Forest Service, Northeastern Research Station), 2004. Effects of urban forests and their management on human health and environmental quality: Emerald ash borer. Available from: <http://www.fs.fed.us/ne/syracuse/Data/Nation/data_list_eab.htm>.

USGS (US Geological Service), 2006. Digital representations of tree species range maps from "Atlas of United States Trees" by Elbert L. Little, Jr. (and other publications). Available from: <http://esp.cr.usgs.gov/data/atlas/little/>.

Vandenberg, J.D., Shelton, A.M., Wilsey, W.T., Ramos, M., 1998. Assessment of *Beauveria bassiana* sprays for control of diamondback

moth (Lepidoptera: Plutellidae) on crucifers. Journal of Economic Entomology 91, 624–630.

Xu, G-T., 2003. *Agrilus marcopoli* Obenberger. Atlas of Ornamental Pests and Diseases. China Agriculture Press, Beijing, pp. 321–322.

Yu, C-M., 1992. *Agrilus marcopoli* Obenberger. In: Xiao, G-R. (Ed.), Forest Insects of China, second ed. China Forestry Publishing House, Beijing, pp. 400–401.

Zhang, B., Liu, Y-N., Bai, Y., Shimazu, M., 1999a. Pathogenic fungi of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in Ningxia Hui Autonomous Region and their virulence. Journal of Beijing Forestry University 21 (4), 67–72.

Zhang, B., Bai, Y., Shimazu, M., Jiro, I., 1999b. Microbial control of *Anoplophora glabripennis* adults by application of non-woven fabric strips with *Beauveria bassiana* and B. *brongniartiii*. Journal of Northwest Forestry University 14 (1), 68–72.