Susceptibility of *Agrilus planipennis* (Coleoptera: Buprestidae) to *Beauveria bassiana* and *Metarhizium anisopliae*

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ABSTRACT The susceptibility of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) to selected strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin was evaluated through bioassays with direct immersion or foliar exposure under laboratory conditions. Results showed that *A. planipennis* adults were susceptible to *B. bassiana* and *M. anisoplae*. Significant time–mortality response was found for each isolates. Isolate *B. bassiana* GHA killed *A. planipennis* adults at a faster rate compared with other isolates tested, with the lowest average time-to-death values. The LC_{50} values estimated under direct immersion method ranged from 1.7×10^5 to 1.9×10^7, 3.5×10^4 to 5.3×10^5, and 4.1×10^3 to 2.9×10^5 conidia/ml for *B. basissiana* and from 3.2×10^6 to 1.1×10^7, 4.5×10^3 to 4.5×10^5, and 1.4×10^2 to 1.2×10^5 conidia/ml for *M. anisopliae* at 4, 5, and 6 d after treatment, respectively. By days 5 and 6, *B. bassiana* GHA outperformed all other isolates tested except ARSEF 7234, followed by ARSEF 7152, 6393, and 7180. Significant concentration–mortality response was also observed for two *B. bassiana* GHA formulations, BotaniGard ES and Mycotrol O, and *M. anisopliae* F52 when insects were treated through foliar exposure. The LC_{50} values ranged from 114.5 to 309.6, 18.4 to 797.3, and 345.3 to 362.0 conidia/cm^2 for BotaniGard, Mycotrol, and *M. anisopliae* F52, respectively. Based on the results of these bioassays, the efficacy of both *B. bassiana* GHA formulations and *M. anisopliae* F52 were similar against adult *A. planipennis*. The potential use of entomopathogenic fungi for management of *A. planipennis* in North America is discussed.

KEY WORDS *Agrilus planipennis*, susceptibility, entomopathogenic fungi, microbial control, bioassay

Agrilus planipennis Fairmaire (Coleoptera: Buprestidae), indigenous to parts of Asia, was first discovered in 2002 in Michigan (Haack et al. 2002). According to recent surveys, infestations are now present in Michigan, Ohio, Indiana, and Ontario, Canada (USDA–FS NCRS 2006). In most of its native range, *A. planipennis* is considered a minor and periodic pest of ash trees (*Fraxinus* spp.) (CAS 1986, Yu 1992, Hou 1993, Xu 2003, Gao et al. 2004). In North America, however, ash trees rapidly die because high *A. planipennis* larval populations convert host phloem into beetle frass. In 2000, >800 million ash trees were estimated on Michigan timberlands (USDA–FS FIA 2006), and *A. planipennis* have killed ≈15 million trees, highlighting its potential impacts on forest biodiversity, ash resources, and urban areas (MacFarlane and Meyer 2005, MDA 2006). In an effort to protect ash resources in North America, regulatory agencies imposed quarantines to limit *A. planipennis* spread, and eradication has been attempted by cutting and chipping ash trees in and around isolated infestations (USDA–APHIS 2006, CFIA 2006). Because of the lack of knowledge on *A. planipennis* biology and inadequate eradication methods, managers are now considering other management options such as containment, suppression, and biological control (Liu et al. 2003, Cappaert et al. 2005).

We initiated research on the natural enemies of *A. planipennis* in a Michigan woodlot in 2002. Less than 1% of larvae were parasitized and ≈2% were infected with fungal isolates of *Beauveria bassiana* (Balsamo) Vuillemin, *Paecilomyces farinosus* (Holm ex SF Gray) Brown & Smith, *Paecilomyces fumosoroseus* (Wize) Brown & Smith, *Lecanicillium lecanii* (Zimmerman) Viegas, and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Bauer et al. 2004, 2005). Successful use of entomopathogenic fungi as insect management tools (Feng et al. 1994, Jaronski and Goettel 1997, Lomer et al. 1997, Poprawski et al. 1997, Vandenberg et al. 1998a, Inglis et al. 2001) led to our investigations reported here.

For this study, we screened *A. planipennis* adults against five isolates of *B. bassiana* and *M. anisopliae* at two concentrations. We then determined the concentration–mortality responses of adult *A. planipennis* to

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Table 1. Origin of test fungi

Accession no.[a]	Host	Origin
B. bassiana		
ARSEF 7152	*Anoplophora glabripennis* (Coleoptera: Cerambycidae)	Chicago, IL
ARSEF 6393	*A. glabripennis*	Ansonia, CT
GHA	*Melanoplus sanguinipes* (F.) (Orthoptera: Acrididae)	Montana, USA
M. anisopliae		
ARSEF 7234	*A. glabripennis*	Chicago, IL
F52	*Cydia pomonella* (Lepidoptera: Tortricidae)	Vienna, Austria
Metarhizium anisopliae var. *anisopliae*		
ARSEF 7180	*A. glabripennis*	Chicago, IL

[a] ARSEF, Agriculture Research Service Entomopathogenic Fungi Cultures, USDA, Ithaca, NY, 2005; GHA, technical powder, BotaniGard ES; and Mycotrol O, Emerald BioAgriculture Corp.; *M. anisopliae* F52, Earth BioSciences, New Haven, CT, and provided by A. E. Hajek.

fungal-based microbial insecticides registered in the United States: 1) *B. bassiana* strain GHA, registered since 1995, formulated as BotaniGard ES (petroleum-based formulation) and Mycotrol O (organic vegetable oil-based formulation) and 2) *M. anisopliae* strain F52, registered in 2005, formulated as TAE-001 Granular Bioinsecticide. Our goal was to identify a product for possible use in an aerial spray program targeting adult *A. planipennis.*

Materials and Methods

Insects. *A. planipennis*-infested green ash trees, *Fraxinus pennsylvanica* Marsh., with a diameter at breast height of 10–20 cm, were felled by chainsaw at Bicentennial Park, Livonia, MI (42° 25′36″ N, 83° 23′30″ W) from December 2002 to May 2003. The trees were cut into 60-cm logs and stored at 4°C. Adult *A. planipennis* were obtained for bioassay by incubating the infested logs in cardboard tubes (20–30 cm in diameter, 70 cm in length) (Caraustar Industries, Saginaw, MI) in a greenhouse at 20–26°C and 20–40% RH under natural lighting. The logs were elevated 3 to 4 cm off the tube bottoms with small wooden blocks to allow adults egress beneath the logs. Each tube was capped on one end with a solid plastic lid darkened with aluminum foil to exclude light, and on the other end with a plastic lid modified by the addition of plastic screening and a translucent collection cup. *A. planipennis* adults are highly phototropic and after emergence come to and stay in the collecting cup. They began emerging from the infested logs after ≈4 wk under these conditions. Beetles were collected daily into 120-ml plastic cups and fed fresh leaves from greenhouse-raised evergreen ash, *Fraxinus uhdei* (Wenzig) Lingelsh, every 2 d. Two- to 8-d-old adults were used in the bioassays.

Fungi. Six isolates of the fungal species *B. bassiana*, and *M. anisopliae* were evaluated for virulence against *A. planipennis* adults (Table 1). Conidial suspensions of *B. bassiana* GHA were made from technical powder (lot no. 02-05-02), BotaniGard ES (lot no. ESO021004), or Mycotrol O (lot no. Myco021102) (Emerald BioAgriculture Corp., Lansing, MI). A 5-ml stock suspension at $\approx 1 \times 10^9$ conidia/ml was made by adding 0.1 g of technical powder or 0.25 ml of products (BotaniGard and Mycotrol) to 5 ml of sterile distilled water (SDW), based on estimated conidia content from product labels. A laboratory subculture of TAE 001 granular was used for *M. anisopliae* F52. *M. anisopliae* F52, ARSEF 6393, 7152, 7180, and 7234 were cultured on quarter-strength Sabouraud dextrose agar with 0.25% (wt:vol) yeast extract (SDAY) (Goettel and Inglis 1997) and held at 24°C in the dark for 14 d. Fungal isolates received no more than four passages on artificial media. Conidia were harvested in 0.05% Tween 80 and suspended in 5 ml of SDW as stock for later use. Conidia of all isolates were counted under the compound microscope (400×) with a Petroff–Hausser hemocytometer on 1×10^{-2} serial dilution of the stock. Conidia with germination tubes longer than their width after 24-h incubation at 24 ± 1°C were considered germinated (Hywell-Jones and Gillespie 1990); only stock with >90% viable conidia was used for bioassay.

Bioassays. Assays were carried out at 24°C, 50–60% ambient relative humidity with a photoperiod of 16:8 (L:D) h unless otherwise stated. To verify mycosis, dead insects were removed and placed individually in a moist chamber consisting of a 60-mm plastic petri dish lined with moist sterile filter paper. Fungal infection was confirmed on all cadavers based on mycosis observed 7 d after death.

Time–Mortality Response. *A. planipennis* adults were screened against three *B. bassiana* isolates (ARSEF 6393, 7152, and GHA) and two *M. anisopliae* isolates (ARSEF 7180 and 7234). Each fungal isolate was tested at two concentrations (10^6 and 10^7 conidia/ml) with 0.02% Tween 80 in SDW as the control. For bioassay of each isolate and concentration, five *A. planipennis* adults were immersed in 5 ml of fungal suspension in a 10-ml plastic vial and inverted for 10 s. Each isolate–concentration combination was replicated eight times. After immersion, adults were removed from the fungal suspension, placed briefly on a sterile paper towel to remove excess liquid, and held individually in 60-mm plastic petri dishes with fresh ash foliage, which was replaced every 2 d. Mortality was monitored daily for 7 d.

Concentration–Mortality Response. The concentration–mortality responses of *A. planipennis* adults to two *B. bassiana* GHA formulations, BotaniGard and Mycotrol, and *M. anisopliae* F52 were tested by exposing adults to sprayed ash foliage. A swinging boom spray cabinet (Allen Machine Works, Midland, MI) with a no. 8001E nozzle (Spray Systems Co., Wheaton, IL) was used to apply fungal suspensions to the upper surface of leaf rectangles (2 by 4 cm) cut from fresh evergreen ash leaves at the rate of 187.1 liter/ha (20 gal/acre) under 25 psi. The nozzle was set 35 cm above the sprayed surface of the leaf rectangles. Based on the conidial content of 2×10^{10} conidia/ml for both *B. bassiana* GHA formulations, coverage was 5×10^5 conidia/cm^2 with the application rate of 2338 ml/ha. For our assays, five application rates (0.023, 0.234, 2.338, 23.38, and 233.8 ml/ha or 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} qt/acre) were used to provide coverage of 5, 50, 500, 5,000, and 50,000 conidia/cm^2. To achieve the application rate of 233.8 ml/ha, 25 μl of BotaniGard or Mycotrol was needed to make a 20-ml suspension at the concentration of 2.5×10^7 conidia/ml (an equivalent of 94.6 ml suspended in 75.7 liters of water) and sprayed through the cabinet. Ten-fold serial dilutions were made by adding 2 ml of stock to 18 ml of SDW, resulting a concentration of 2.5×10^6, 2.5×10^5, 2.5×10^4, 2.5×10^3 conidia/ml for the application rate of 23.38, 2.338, 0.234, 0.023 ml/ha, respectively. For *M. anisopliae* F52, the same five serial application rates were used, where conidia stock from laboratory subculture was used to make the 20-ml suspension of 2.5×10^7 conidia/ml. Controls were 0.02% BotaniGard blank and 0.02% Mycotrol blank (both provided by Emerald BioAgriculture Corp., and 0.02% Tween 80 for *M. anisopliae* F52.

To estimate the actual coverage of fungal conidia on the surface of the treated leaf rectangles, five SDAY media plates (100 mm in diameter) were treated along with the leaf rectangles at each application rate inside the spray cabinet. Microscopic examination of the plates after spray found no significant clumps of conidia at each application rate. All media plates were sealed with parafilm and cultured at 24°C in dark for 72 h. Numbers of colony-forming units (CFUs) on the five media plates treated at the rates of 0.023 ml/ha were then counted; this rate was chosen to facilitate CFU enumeration. A sample of media plates at the rate of 0.234 ml/ha also was examined to compare spray consistency between rates within each assay. CFUs ranged from 21 to 628 at the rate of 0.023 ml/ha for a 10-cm media plate for all assays. The average number of CFUs per square centimeter from 0.023 ml/ha rate was used to calculate the actual conidial coverage on leaf rectangles for all application rates.

Each application rate was replicated four times for the two *B. bassiana* GHA formulations and *M. anisopliae* F52. Five ash leaf rectangles were sprayed together in a 100-mm plastic petri dish and allowed to air dry for ≈30 min before transfer to 60-cm petri dishes (one leaf rectangle/dish). Adult *A. planipennis* were introduced individually into each petri dish for a 24-h exposure period, at which time the sprayed leaf rectangle was replaced with a fresh, untreated evergreen ash leaflet. Adult mortality was monitored daily for 14 d with fresh leaflets provided every other day. We included five adults per replicate, four replicates per application rate, six application rates including the control per bioassay, and two bioassays per isolate and formulation over time.

Statistical Analysis. For time–mortality responses, adult survival (average time to death) was first analyzed using analysis of variance (ANOVA) ($\alpha = 0.05$). Fisher least significant difference (LSD) multiple comparison ($\alpha = 0.05$) followed if a significant difference was detected (SAS Institute 2004). Time–mortality data were then used to fit a complementary log-log model (CLL model) (Preisler and Robertson 1989, Robertson and Preisler 1992, Nowierski et al. 1996, Feng et al. 1998) and analyzed using SAS PROC GENMOD (SAS Institute 2004). Conditional response parameters obtained were used to estimate cumulative mortality probabilities (Preisler and Robertson 1989, Robertson and Preisler 1992). The logarithm time-dependent median lethal concentration values (LC_{50}) were estimated by the formulas of Robertson and Preisler (1992). Goodness-of-fit for the binomial variable was tested by adopting Hosmer–Lemeshow test (Nowierski et al. 1996, Feng et al. 1998) in SAS PROC LOGISTIC (SAS Institute 2004). For concentration–mortality responses, percentage of mortality data were corrected with Abbott's formula (Abbott 1925) and subjected to angular transformation before analysis. LC_{50} values were estimated with Probit analysis (SAS Institute 2004). Variation between the two assays performed for each isolate was tested using PROC GLM (SAS Institute 2004). A $P > 0.05$ for assay effect indicates nonsignificant variability. In this case, data from two assays were combined for each isolate to estimate the relative potency index. The relative mean potency was calculated by dividing the LC_{50} of each test isolate by that of the designated standard. Significant differences between the test isolates and the standard were identified when the 95% confidence interval (CI) of the potency index did not include 1.0 (Robertson and Preisler 1992).

Results

Time–Mortality Response. Adult mortality was mostly observed between 4–6 d after exposure through direct immersion, with no mortality for the first 3 d. The cumulative mortality at 6 d after treatment ranged from 80 to 97.5 and from 97.5 to 100% for the concentration of 10^6 and 10^7 conidia/ml, respectively. Only 12.5% adults died in the control within the same period.

Significant treatment effect was observed for fungal isolate ($F = 5.46$; df = 4, 390; $P < 0.01$) as well as concentration ($F = 47.64$; df = 1, 390; $P < 0.01$) when adult survival after treatment was considered. Higher concentrations produced significantly lower time-to-death values for all isolates except ARSEF 7234 (Table

Table 2. Time-to-death for *A. planipennis* adults exposed to different fungal isolates at two concentrations through direct immersion

Fungal isolate	Average time-to-death (d)	
	10^6 (conidia/ml)	10^7 (conidia/ml)
B. bassiana		
ARSEF 7152	5.6 ± 2.0Aa (4–14)	4.2 ± 0.5Ab (4–6)
ARSEF 6393	5.6 ± 1.8Aa (4–12)	4.7 ± 0.6Bb (4–6)
GHA	4.6 ± 2.0Ca (4–8)	4.2 ± 2.0Ab (4–5)
M. anisopliae		
ARSEF 7234	4.8 ± 0.9BCa (4–7)	4.6 ± 0.8Ba (4–7)
M. anisopliae var. *anisopliae*		
ARSEF 7180	5.4 ± 1.1ABa (4–8)	4.6 ± 0.6Bb (4–6)

Means ± SD (range) followed by the same lowercase letter within a row and the same uppercase letter within a column are not significantly different (Fisher LSD multiple comparison; $\alpha = 0.05$). Each mean represents the average days of time-to-death for 40 adults within eight replicates.

2). Time-to-death values were lowest for *B. bassiana* GHA at both concentrations, followed by 7234, 7180, 6393, and 7152 at 10^6 conidia/ml and 7152, 7180, 7234, and 6393 at 10^7 conidia/ml (Table 2). Isolate *B. bassiana* GHA killed *A. planipennis* adults faster compared with all other isolates tested.

Mortality at 6 d after exposure was used in the modeling analysis to minimize potential bias to the estimated deviance and Pearson's chi-square statistics due to zero mortality. Treatment mortality was used directly without correction because no control mortality was caused by fungal infection, whereas 97% mortality in the treatments was attributed to fungal infection during the observation period. The results of time–mortality data fitting the CLL model were presented in Table 3. The Hosmer–Lemeshow's goodness-of-fit statistic C (H-L $\hat{C}$) and nonsignificant *P* values showed our results fit the CLL model. In addition, significant correlations between mortality and log concentration were found for each isolate, with the steepest slope for *B. bassiana* ARSEF 7152, followed by *M. a.* variety *anisopliae* ARSEF 7180, *B. bassiana* ARSEF 6393, GHA, and *M. anisopliae* ARSEF 7234. The steeper the slopes, the stronger the correlation between mortality and concentration.

The lethal concentration effects of different fungal isolates against *A. planipennis* adults, represented by the logarithms of LC_{50}, was inversely correlate with time after exposure (Fig. 1). *B. bassiana* GHA had the lowest log(LC_{50}) value for the time period 4 (5.22), whereas isolate 7234 had the lowest log(LC_{50}) for both time periods 5 (3.65) and 6 (2.14). Isolates 7152, 6393, and 7180 showed similar and lower levels of virulence in all time periods.

Concentration–Mortality Response. Adult mortality at 10 d was used in the analysis as most mortality occurred between 6 and 10 d after the 24-h exposure period. Mortality ranged from 0 to 35% for the lowest concentration and 95–100% for the highest concentration. The average control mortality was 15, 10, and 20% for the same period for BotaniGard, Mycotrol, and *M. anisopliae* F52, respectively.

The Probit model fits the concentration–mortality data well as no significant departure from the model was found for all assays (Pearson's chi-square goodness-of-fit test: $\alpha = 0.05$, df = 3) (Table 4). Significant concentration-mortality responses were observed for BotaniGard, Mycotrol, and *M. anisopliae* F52, as indicated by the positive slope values (Table 4). Steeper slope indicates greater concentration–mortality response. The LC_{50} values ranged from 114.5 to 309.6, 18.4 to 797.3, and 345.3 to 362.0 conidia/cm^2 for BotanicGard, Mycontrol, and *M. anisopliae* F52, respectively (Table 4).

No significant variability was found between assays for BotaniGard ($F = 1.77$; df = 1, 4; $P = 0.25$), Mycotrol ($F = 7.06$; df = 1, 4; $P = 0.06$), and *M. anisopliae* F52 ($F = 2.72$; df = 1, 4; $P = 0.17$). Therefore, the average value of all parameters of the two assays was used to estimate potency index. When designating BotaniGard as the standard isolate, the mean potency index was 0.9 and 1.7 for Mycotrol and *M. anisopliae* F52, respectively (Table 4). No significant difference was found between these three materials, as indicated by the inclusion of 1.0 in the 95% CIs of both indices (Table 4).

Discussion

The results of our assays demonstrated that *A. planipennis* adults were susceptible to *B. bassiana* and *M. anisopliae* when treated by direct immersion as well as foliar exposure. Judging by the time–mortality response, *B. bassiana* GHA consistently outperformed all other test isolates except 7234 at 5 and 6 d post-

Table 3. Parameters used to fit CLL model of time-mortality for *A. plainpennis* adults

Fungal isolate	df	Scaled deviance	Pearson's χ^2	Mean deviance	H-L $\hat{C}$	*P* value (H-L $\hat{C}$)	Slope β (SE)	*P* value[a]
B. bassiana								
ARSEF 7152	2	1.58	3.10	0.79	0.50	0.9182	1.5156 (0.3561)	0.0001
ARSEF 6393	2	2.27	1.69	1.14	3.22	0.3595	0.9030 (0.2458)	0.0002
GHA	2	2.51	0.73	1.25	0.33	0.9545	0.6451 (0.1586)	0.0001
M. anisopliae								
ARSEF 7234	2	1.99	0.36	1.00	0.15	0.9854	0.3424 (0.1012)	0.0007
M. a. var. *anisopliae*								
ARSEF 7180	2	2.78	0.84	1.29	0.19	0.9792	1.0288 (0.1720)	0.0001

[a] *P* value for the test of slope (H_0: $\beta = 0$) from the regression of adult mortality against log fungal dose.

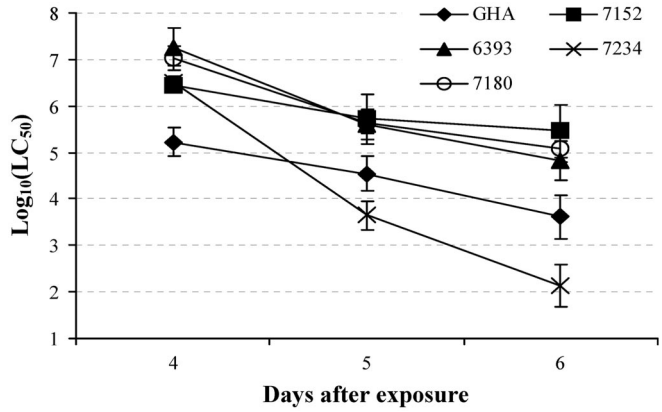

Fig. 1. Logarithm of the time-dependent LC_{50} (conidia per milliliter) values with standard errors for *B. bassiana* GHA, ARSEF 7152, 6393, *M. anisopliae* ARSEF 7234, and *M. a.* variety *anisopliae* ARSEF 7180 against *A. planipennis* adults.

treatment, as indicated by the lower time-to-death and LC_{50} values. Ideally, more concentrations in the adult screening bioassays would be beneficial to the estimation of model parameters. However, availability of *A. planipennis* adults during the assays limited the number of test concentrations. Compared with direct immersion method, the foliar exposure bioassays better simulated field applications. Results from our concentration–mortality response showed no significant differences between *M. anisopliae* F52 and *B. bassiana* GHA against *A. planipennis* adults. Furthermore, no significant difference was detected between the two *B. bassiana* GHA formulations: BotaniGard and Mycotrol. The similarity in efficacy for those two formulations could have potential implications on future pest management plans when economic factors were considered as Mycotrol, formulated with organic vegetable oil, is more expensive but may be more acceptable to the public.

Beauveria bassiana GHA was registered in 1995 as a biopesticide (EPA 2006a) and has been bioassayed against a variety of insects. The LC_{50} for adult *A. planipennis* from our study ranged from 18.4 to 797.3 conidia/cm^2. Although direct comparison of virulence between different insect hosts using different methods is not possible, a literature review of published LC_{50} values suggested comparable virulence of *B. bassiana* GHA against *A. planipennis.* For example, Vandenberg (1996) reported an LC_{50} of 282 conidia/cm^2 for *B. bassiana* Mycotech 726 (the *Bemisia*-passaged GHA strain) against adult Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), by using a Burgerjon spray tower to spray the aphids on the plants. In another study, an LD_{50} of 614 conidia/cm^2 is reported for second instars of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), with topical sprays of *B. bassiana* GHA suspensions (Vandenberg et al. 1998b). Poprawski et al. (1999) reports an LD_{50} value of 1.42×10^4 conidia/cm^2 for adult brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphidiade), after treatment of infested seedlings with *B. bassiana* GHA by using a Potter spray tower.

Metarhizium anisopliae F52 was recently registered in the United States as the active ingredient of the biopesticide for use in control of ticks and elaterid

Table 4. Concentration–mortality response of *A. planipennis* adults exposed to *B. bassiana* and *M. anisopliae* through foliar exposure

Fungal isolate	Concn[a] (conidia/cm^2)	No. of insects[b]	Slope ± SE	LC_{50} (95% CL) (conidia/cm^2)	χ^{2c}	Mean potency index[d] (95% CI)
B. bassiana GHA						
BotaniGard ES						S
Assay 1	1.85~18,500	120	1.21 ± 0.36	309.6 (0.4–45687)	8.60	
Assay 2	3.85~38,500	120	0.76 ± 0.13	114.5 (40.6–287.6)	3.24	
Mycotrol O						0.9 (0.1–42.6)
Assay 1	0.35~3,500	120	0.67 ± 0.12	18.4 (6.7–51.0)	2.41	
Assay 2	3.43~34,300	120	1.03 ± 0.17	797.3 (368.6–1795)	2.56	
M. anisopliae F52						1.7 (0.1–62.4)
Assay 1	1.40~14,000	120	0.82 ± 0.14	362.0 (142.8–875.4)	3.91	
Assay 2	7.20~72,000	120	1.04 ± 0.17	345.3 (161.2–779.8)	4.38	

[a] Each assay with five 10-fold serial dosages and a control.
[b] Five insects per replicate, four replicates per dosage, five dosages plus the control per assay.
[c] Pearson's chi-square goodness-of-fit test on the Probit model ($\alpha = 0.05$, df = 3) (SAS Institute 2004).
[d] Mean potency index and its confidence intervals were estimated based on formulas by Robertson and Preisler (1992). Parameters used in estimation were the average values of the two assays for each isolate. LC_{50} values are significantly different if their 95% CIs do not include 1.0 (Robertson and Preisler 1992). S, standard isolate with which other test isolates compared.

beetles (EPA 2006b). Although Bruck et al. (2005) reported a LC_{50} value of 2.7×10^6 spores/g dry soil against second instars of cabbage maggots, *Delia radicum* (L.) (Diptera: Anthomyiidae), literature on its host range is lacking. Results from our study could serve as a starting point for future investigations of this fungus for management of forest insect pests.

Wood-boring insects such as *A. planipennis* are difficult to manage in the field, because they are protected under tree bark during much of their life cycle. However, the adult stage is exposed and vulnerable to control measures while resting and feeding on ash foliage up in the tree canopy. For example, in a 1966 Chinese study, 100% mortality was achieved when adult *A. planipennis* were caged on white ash trees sprayed with conventional insecticides, including dimethoate (Liu 1966). Adults also were susceptible when insecticides were applied to the trunk shortly before emergence (Liu 1966). Entomopathogenic fungi such as *B. bassiana* and *M. anisopliae* are known from hundreds of insect species, including wood-boring insects (Humber 2005). The ability of fungi to actively invade live insects through the cuticle and proliferate in the field makes them unique tools for management of insect pests. *Agrilus auriventris* Saunders (Coleoptera: Buprestidae), for example, is managed by application of *M. anisopliae* spores to tree trunks, resulting in >70% mortality of larvae and adults in ≈2 wk (Fan et al. 1990). Our study is the first to evaluate the virulence of entomopathogenic fungi against *A. planipennis.* If entomopathogenic fungi were used in the field, *A. planipennis* adults would be exposed while feeding on leaves and ovipositing on tree bark. The high humidity under the bark provides ideal conditions for fungal spores to germinate and infect immature stages of *A. planipennis.* Cracks and crevices in tree bark caused by *A. planipennis* infestation provide opportunities for fungal conidia to reach the insects.

In general, entomopathogenic fungi are considered safer to the environment than conventional insecticides, and can be used in sensitive areas. Fungi also may persist and proliferate in the environment. Their ability to transmit horizontally and vertically in pest populations may improve efficacy. That both *B. bassiana* and *M. anisopliae* are considered safe to nontarget organisms and beneficial insects such as predators, parasitoids, and honey bees in the field (Brinkman and Fuller 1999, Ekesi et al. 1999, Cottrell and Shapiro-Ilan 2003, Dunkel and Jaronski 2003, Kanga et al. 2003) makes them even more attractive compared with conventional insecticides.

Based on the results of our studies, we think fungal entomopathogens may prove useful for management of *A. planipennis* populations in North America. To date, our research efforts have been focused on registered products formulated with *B. bassiana* GHA because of its 1) virulence against *A. planipennis,* 2) availability of product and registration label, and 3) good safety record. However, further research is needed before general recommendations can be made about the use of fungal-based microbial insecticides, including optimal site of application, delivery methods, and rates; persistence and environmental fate; effects on nontarget insects; and long-term efficacy data.

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