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# Virulence of entomopathogenic hypocrealean fungi infecting *Anoplophora glabripennis*

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**Abstract** Twenty isolates of four species of entomopathogenic hypocrealean fungi (*Beauveria bassiana*, *Beauveria brongniartii*, *Isaria farinosa*, and *Metarhizium anisopliae*) were found to be pathogenic to adults of the Asian longhorned beetle, *Anoplophora glabripennis*. Survival times for 50% of the beetles tested ($ST_{50}$) ranged from 5.0 (*M. anisopliae* ARSEF 7234 and *B. brongniartii* ARSEF 6827) to 24.5 (*I. farinosa* ARSEF 8411) days. Screening studies initially included strains of *B. brongniartii*, which is registered as a microbial control agent in Europe, Asia and South America but not in North America. At that time, we could not confirm that this fungal species is native to North America which added uncertainty regarding future registration of this species for pest control in the USA. Therefore, subsequent bioassays documented median survival times for three *M. anisopliae* isolates (5–6 days to death) and two of these isolates are suggested for further development because they are already registered for pest control in the USA.

**Keywords** *Anoplophora glabripennis* · Asian longhorned beetle · *Beauveria bassiana* · *Beauveria brongniartii* · *Metarhizium anisopliae* · Entomopathogenic fungi · Biological control · Bioassay

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## Introduction

Fungi are commonly encountered pathogens infecting cerambycids (Linsley 1959). Hypocrealean anamorphs in the genera *Beauveria*, *Isaria* (=*Paecilomyces*), and *Metarhizium* have been isolated from larval and adult stages of the Asian longhorned beetle, *Anoplophora glabripennis*, in China (Wang et al. 1990; Liu and Yamakazi 1996; Wang et al. 1997; Zhang et al. 1999; Shimazu et al. 2002; Haack et al. 2003) and North America (Poland et al. 2002; Dubois 2003).

*Anoplophora glabripennis* is an invasive species that has been introduced to the USA (Hajek 2007), Canada (CFS 2007), and Europe (Hérard et al. 2005). This species is a major tree-killer in China, and it is estimated that potential value loss of urban trees could exceed 660 billions US$ if *A. glabripennis* becomes established in North America (Nowak et al. 2001). Quarantine and eradication programs, therefore, have been undertaken in all areas where *A. glabripennis* has been detected in the USA (APHIS 2007). To date, the primary methods used in the *A. glabripennis* eradication program include survey, detection, quarantine, removal of infested trees, and trunk or soil injections of imidacloprid in remaining host trees around detected infestations. In the USA and Canada, respectively, approximately 21,866 and 25,000 infested, at risk and potential host trees have been removed during eradication programs (APHIS 2006; Gasman 2006). As a preventive measure, trees have been treated by trunk and soil injection with imidacloprid in the USA since 2000, with >89,000 trees treated in 2005 alone (APHIS 2006). Injecting trees with imidacloprid is thought to be only partially effective as this insecticide is not evenly distributed throughout trees after injection (Poland et al. 2006a). It also acts as an anti-feedant and may lead to increased dispersal of feeding adults (Poland et al. 2006b). Moreover, the use of imidacloprid was stopped in some areas of New York due to groundwater contamination. To date, there are no biological control methods available for this destructive pest of hardwood trees.

Non-woven fiber bands impregnated with cultures of *Beauveria brongniartii* (=*B. tenella*) are sold by Nitto Denko (Osaka, Japan) for control of adult cerambycids in Japanese orchards (product name = Biolisa Kamikiri) (Higuchi et al. 1997). One species targeted by these bands is *Anoplophora malasiaca*, a sister species to *A. glabripennis* (Lingafelter and Hoebeke 2002). Our laboratory began evaluating use of *B. brongniartii* and fungal band technology for control of *A. glabripennis* in North America in 1999 (Dubois 2003; Dubois et al. 2004a, b). When we began our studies, we focused bioassays on *B. brongniartii* based on promising results with this species against cerambycids in China and Japan (e.g., Higuchi et al. 1997; Zhang et al. 1999). Unfortunately, no products containing *B. brongniartii* for insect control are registered in the USA. In North America, *B. brongniartii* has been cited as infecting *Apis mellifera* (Hymenoptera: Apidae) (Prest et al. 1974), *Megachile rotundata* (Hymenoptera: Megachilidae) (Khachatourians 1992), *Plecia nearctica* (Diptera: Bibionidae) (Kish et al. 1977), *Aedes sierrensis* (Diptera: Culicidae) (Sanders 1972; Pinnock et al. 1973), *Otiorhynchus ligustici* (Coleoptera: Curculionidae) (David 1993), and *Atypoides riversi* (Araneae: Antrodiaetidae) (G. M. Thomas, personal communication). However, the sole culture available is not *B. brongniartii*, and the isolate from *A. sierrensis* was later considered a misidentification (Soares 1980). *B. brongniartii* was never isolated during extensive studies of *O. ligustici* in areas of New York State near locations where this fungal species was reported from Canada (G. Neumann, personal communication). Moreover, North American isolates of *B. brongniartii* in culture collections (ARSEF, ATCC, CCFC, UAMH) were all found to be incorrect identifications (Hajek, unpublished data). Interestingly, a recent molecular

phylogeny determined that isolates of *B. brongniartii* belong to a unique clade within the genus *Beauveria*. *B. brongniartii* is considered a complex of at least several cryptic species infecting Coleoptera that is distributed across Eurasia (Rehner and Buckley 2005). Differences between groups of Japanese *B. brongniartii* isolates infecting cerambycids versus scarabs have been documented previously using RFLP (Wada et al. 2003) and host specificity (Shimazu 1994). However, Rehner and Buckley (2005) did not identify *B. brongniartii* from North America. Thus, we could not confirm that *B. brongniartii* is native to North America. Because *B. brongniartii* has never been registered for pest control with the U.S. Environmental Protection Agency and we could not confirm endemicity, we continued bioassays using species of entomopathogenic fungi known to be native to North America, with greatest emphasis on fungal isolates that are already registered in the USA for pest control.

We report the results of bioassays using adult *A. glabripennis* in which we compared the virulence of strains of four species of hypocrealean anamorphs isolated from adult *A. glabripennis* or other cerambycids. We also tested the commercial strains *B. brongniartii* NBL 851, the isolate used in Biolisa Kamikiri; *B. bassiana* GHA (ARSEF 6444), currently available from BioWorks, Inc. (Fairport, New York); *M. anisopliae* ESC 1 (ATCC 62176), for which registration in the USA has lapsed; and *M. anisopliae* F 52 (ARSEF 7711), currently available from Novozymes (Salem, Virginia). Our goal was to test pathogenicity and identify isolates virulent to *A. glabripennis* adults.

## Materials and methods

### Fungal isolates and test insects

Six bioassays (A–F) were conducted using 20 fungal strains either isolated from cerambycids or commercialized strains (Table 1). The first five bioassays (A–E) were non-replicated screening bioassays testing 19 isolates, while bioassay F compared three *M. anisopliae* isolates. *B. bassiana* ARSEF 6444 (GHA) conidia were obtained as a non-formulated spore powder from Emerald BioAgriculture Corp. (Lansing, Michigan) and were used directly for Bioassay A. *B. brongniartii* NBL 851 conidia were scraped directly off commercial fiber bands obtained from Nitto Denko for Bioassays A and B. In all other cases, conidia were obtained from cultures from the USDA, ARSEF (Agricultural Research Service Collection of Entomopathogenic Fungal Cultures) or from our laboratory collection maintained at –80°C. Fungal cultures were grown on SDAY (Goettel and Inglis 1997) in 90 mm Petri dishes at 25°C for 2 weeks and cultures were air-dried for 24 h prior to conidial harvest. Conidia were scraped off mycelium, passed through a 250 μm sieve, dried for 2–5 days in sealed bags containing silica gel and stored at 5°C until use.

*A. glabripennis* are not available in the field in North America and can only be collected in large numbers from infested trees in limited areas in China during late June, July and August (Smith et al. 2004; Hajek et al. 2006). Therefore, we used laboratory-reared *A. glabripennis* for our bioassays. However, *A. glabripennis* develop slowly, requiring at least four months from oviposition to adult, and are costly to rear (ca. 21 US$ per adult beetle without overhead costs) (Keena 2005). As a consequence, sufficient beetles for replication were not always available and large, even-aged cohorts were never available.

*Anoplophora glabripennis* adults were reared in the USDA-ARS quarantine facility (Ithaca, NY) according to the protocol of Dubois et al. (2002). As part of our normal rearing procedure, freshly eclosed adults were checked once every 4–7 days for

**Table 1** Fungal isolates tested against *Anoplophora glabripennis* adults

| Species | Isolate[a] | Origin | Host | Host stage |
|---|---|---|---|---|
| *Beauveria bassiana* | ARSEF 6391 (ST 1) | Syracuse, New York[b] | *Anoplophora glabripennis* | Adult |
| *Beauveria bassiana* | ARSEF 8413 (ST 2) | Syracuse, New York[b] | *Anoplophora glabripennis* | Adult |
| *Beauveria bassiana* | ARSEF 6393 (VD 12) | Ansonia, Connecticut[b] | *Anoplophora glabripennis* | Adult |
| *Beauveria bassiana* | ARSEF 6444 (GHA)[d] | Lethbridge, Alberta, Canada | *Locusta migratoria*[e] | Adult |
| *Beauveria bassiana* | ARSEF 8412 (Q2K393) | Ithaca, New York[c] | *Anoplophora glabripennis* | Adult |
| *Beauveria bassiana* | ARSEF 8414 (WU 50) | Wuhe, Anhui, China | *Anoplophora glabripennis* | Adult |
| *Beauveria brongniartii* | NBL 851[c] | Gunma, Japan | *Psacothea hilaris* | Adult |
| *Beauveria brongniartii* | ARSEF 6412 (F 1101) | Kukisaki, Ibaraki, Japan | *Anoplophora malasiaca* | Adult |
| *Beauveria brongniartii* | ARSEF 6827 (WU 20) | Wuhe, Anhui, China | *Anoplophora glabripennis* | Adult |
| *Isaria farinosa* | ARSEF 8411 (02-NY5-6-1) | Ithaca, New York[c] | *Anoplophora glabripennis* | Adult |
| *Metarhizium anisopliae* | ATCC 62176 (ESC 1)[d] | Illinois | Soybean nematode cyst | – |
| *Metarhizium anisopliae* | ARSEF 7234 (VD 1) | Ansonia, Connecticut[b] | *Anoplophora glabripennis* | Adult |
| *Metarhizium anisopliae* | ARSEF 8416 (VD 3) | Ansonia, Connecticut[b] | *Anoplophora glabripennis* | Adult |
| *Metarhizium anisopliae* | ARSEF 6392 (VD 5) | Ansonia, Connecticut[b] | *Anoplophora glabripennis* | Adult |
| *Metarhizium anisopliae* | ARSEF 8417 (VD 7) | Ansonia, Connecticut[b] | *Anoplophora glabripennis* | Adult |
| *Metarhizium anisopliae* | ARSEF 8418 (VD 8) | Ansonia, Connecticut[b] | *Anoplophora glabripennis* | Adult |
| *Metarhizium anisopliae* | ARSEF 8419 (VD 9) | Ansonia, Connecticut[b] | *Anoplophora glabripennis* | Adult |
| *Metarhizium anisopliae* | ARSEF 7711 (F 52)[d] | Vienna, Austria | *Cydia pomonella* | Larva |
| *Metarhizium anisopliae* | ARSEF 8420 (WU 19) | Wuhe, Anhui, China | *Anoplophora glabripennis* | Adult |
| *Metarhizium anisopliae* | ARSEF 8415 (FS 6) | Ansonia, Connecticut[b] | *Anoplophora glabripennis* | Larva |

[a] ARSEF = Agricultural Research Service Collection of Entomopathogenic Fungal Cultures; ATCC = American Type Culture Collection; NBL 851 is the isolate of *Beauveria brongniartii* used by Nitto Denko, Osaka, Japan in Biolisa Kamikiri. Numbers in parentheses relate to Hajek lab designation and were used in Dubois (2003)

[b] Isolated from cadavers of *A. glabripennis* from naturally infested wood cut in New York, New York and Chicago, Illinois and transported to quarantines. Fungus isolated from cadavers with fungal outgrowth obtained from quarantine laboratories in Syracuse, New York and Ansonia, Connecticut

[c] Isolated from laboratory colony

[d] Commercialized isolates

[e] Originally isolated as *B. bassiana* isolate ARSEF 201 from *Diabrotica undecimpunctata* in Corvallis, Oregon, but reisolated from *Locusta migratoria*

maturation. Insects were considered adults when sclerotized and active, at which time they were weighed and fed twigs of sugar maple (*Acer saccharum*).

## Bioassay procedures

Conidial viability was assessed for the fungal strains (Table 1) prior to each bioassay (Table 2) by inoculating two 90 mm diameter Petri dishes containing SDAY with 1 ml of a conidial suspension of $\sim 10^7$ conidia/ml in 0.05% Tween 80. The Petri dishes were incubated for 12–16 h at 23°C in darkness, and 100 spores were randomly scored for germination on each Petri dish to determine percent germination. Percent germination was used to adjust conidial suspensions based on viable conidia.

For the initial screening bioassays testing 19 fungal strains against *A. glabripennis* adults, a 50 ml suspension of $10^7$ viable conidia/ml was prepared in sterilize deionized water with 0.05% Tween 80. Adults were submerged individually in conidial suspensions for 15 s while constantly agitating the suspension throughout the immersion. Control *A. glabripennis* were submerged for an equivalent time in 0.05% Tween 80 and maintained in the same manner as treated insects.

After treatment, beetles were allowed to drain on paper towels for 15 s and then placed in sealed clean 650 ml (170 × 80 mm) polypropylene containers containing a saturated cotton ball. A duration of 24 h later, freshly cut sugar maple twigs were provided as food and three 1–2 mm diameter holes were then punched in container lids. Fresh twigs were provided every 2–7 days. Beetles were maintained at 22.5 ± 2.5°C and were monitored daily for death. Dead adults were transferred individually to 60 ml plastic cups containing a saturated cotton ball. The cups were sealed, and the cadavers were monitored for fungal outgrowth after 2 weeks.

Adults can be long-lived (Keena 2006; e.g., one laboratory-reared *A. glabripennis* male lived 288 days (R. P. Shanley, personal communication)) and can vary in weight, with females generally larger and heavier than males (Hajek et al. 2004). For all bioassays, insects of similar ages and weights were distributed approximately equally among treatments. We also maintained an equal sex ratio among treatments within each bioassay. Sex ratios and average ages and weights for insects used in these bioassays are summarized in Table 2.

We estimated the virulence of 19 isolates (Table 1) during five screening bioassays, with sample sizes from 10 to 20 *A. glabripennis* adults during each bioassay, without replication (Bioassays A–E). Treated insects and controls were monitored daily until death or for 30 days. Subsequently, three isolates of *M. anisopliae* (ARSEF 7234 [VD 1], ARSEF 7711 [F 52], and ATCC 62176 [ESC 1]) were tested at sample sizes from 11 to 13 *A. glabripennis* adults for each of five replicates initiated on different days using freshly prepared conidial suspensions (Bioassay F). Both treated and control insects were monitored daily until death or for 15 days.

## Data analysis

For each bioassay, age and weight were compared across treatments within each bioassay; age was analyzed using Kruskal–Wallis tests because data were not normally distributed and log-transformed weight was analyzed using general linear models (Proc GLM; SAS Institute 2006). Median survival times ($ST_{50}$) and 95% confidence intervals for adults receiving each treatment were calculated based on Kaplan–Meier survival distribution

**Table 2** Overview of bioassays screening a diversity of Hypocrealean fungi against *Anoplophora glabripennis* adults

| Bioassay | Numbers of insects/treatment | Sex ratio (female:male) | Weight (g) Mean ± SE[a] | Age (d) Mean ± SE[a] | Control mortality (%)[b] | Control fungal outgrowth (%)[b] |
|---|---|---|---|---|---|---|
| A | 10 | 3:7 | 1.167 ± 0.045 | 21.0 ± 2.6 | 20.0 | 0.0 |
| B | 12 | 5:7 | 0.812 ± 0.033 | 53.4 ± 3.7 | 45.5 | 0.0 |
| C | 20 | 11: 9 | 0.764 ± 0.014 | 16.6 ± 0.6 | 15.0 | 0.0 |
| D | 12 | 6:6 | 0.832 ± 0.024 | 10.0 ± 0.6 | 8.0 | 5.0 |
| E | 13 | 7:6 | 0.851 ± 0.034 | 13.8 ± 1.1 | 0.0 | 0.0 |
| F | 63 | 29:34 | 0.515 ± 0.010 | 15.5 ± 0.9 | 36.1 | 3.3 |

[a] Mean ± standard error

[b] Bioassays A–E: controls were censored at 30 days. Bioassay F: controls were censored at 15 days

functions (Proc LIFETEST; SAS Institute 2006). For the screening bioassays (Bioassays A–E), $ST_{50}$s and percentage of cadavers with fungal outgrowth were not compared statistically due to the low sample sizes for each bioassay and lack of replication by isolate. To evaluate whether days to death differed by sex in each bioassay, general linear models were used for each bioassay. For the replicated bioassay comparing three *M. anisopliae* isolates (Bioassay F), the Cox proportional hazards model was used to evaluate treatment, sex and replicate (Proc PHREG; SAS Institute 2006). Subsequent contrasts between treatments for one of the replicates were conducted using Proc PHREG, adjusted with the Bonferroni correction. Percentage fungal outgrowth was compared using chi-squared tests (SAS Institute 2006), and alpha-levels in pairwise comparisons were adjusted using the Bonferroni correction.

## Results

*Anoplophora glabripennis* adults in treatment and control groups did not differ significantly by age ($\chi^2$ tests; $P > 0.05$) or weight ($F$-tests; $P > 0.05$). For the screening bioassays (Bioassays A–E), among the 355 fungus-treated beetles, all but four died in <30 days. While control mortality ranged from 0 to 45.5%, the percentage of control cadavers with fungal outgrowth remained at <5% for all bioassays.

In screening bioassays, $ST_{50}$s varied from 5.0 (*B. brongniartii* ARSEF 6827) to 24.5 (*I. farinosa* ARSEF 8411) days between inoculation of adults and death (Table 3). Although only one isolate of *Isaria farinosa* (=*Paecilomyces farinosus*) was tested, the median survival time for this isolate was 3–4 times longer than many other isolates. The six *B. bassiana* isolates tested tended to take longer to kill than the three *B. brongniartii* or nine *M. anisopliae* isolates. For each of the five screening bioassays, there were no significant differences in days to death between males and females ($\chi^2$ tests; $P > 0.05$). There was a trend for fungal outgrowth more consistently occurring from cadavers of adults treated with *M. anisopliae* than the other fungal species (Table 3).

Interestingly, adult *A. glabripennis* stiffened, spread their elytra, and clung to twigs when dying from infection with *B. brongniartii* isolate NBL 851. This behavior did not occur with other isolates tested, including other isolates of *B. brongniartii*. Adults that died from the other isolates fell to the bottom of the container and did not spread their elytra before death.

For the bioassay comparing the three *M. anisopliae* isolates (Bioassay F), by 15 days after treatment, all beetles treated with ARSEF 7234 (VD 1) and ARSEF 7711 (F 52) had died, and all but two of the 63 adults (3.2%) inoculated with ATCC 62176 (ESC 1) had died (Table 4). While control mortality by 15 days was 36.1% ($n = 61$), very few (3.3%) of the resulting cadavers yielded fungal outgrowth, although outgrowth was characteristic of treated insects (Table 4). There was a significant three-way interaction for fungal isolate, sex, and replicate (Wald $\chi^2 = 17.17$, df = 8, $P = 0.0283$), so we explored this further. Testing sex, isolate, and isolate × sex separately for each replicate, these effects were not significant for four out of five replicates ($P > 0.05$). While sex and the interaction between sex and fungal isolate were never significant, in one replicate, fungal isolate differed as a main effect (Wald $\chi^2 = 10.87$; $P = 0.0044$). For this replicate, ATCC 62176 (ESC 1) (11.8 ± 1.3 days to death) killed beetles more slowly than either ARSEF 7234 (VD 1) (7.6 ± 0.7) or ARSEF 7711 (F 52) (7.1 ± 0.6) (Wald $\chi^2$ tests; total $\alpha = 0.05$ with Bonferroni correction). *M. anisopliae* isolates differed in the percentages of cadavers yielding outgrowth ($\chi^2$ tests; $P < 0.05$); cadavers of adults killed by ARSEF 7234 (VD 1) yielded

**Table 3** Median survival time in days (and 95% confidence intervals) and % fungal outgrowth of laboratory-reared *A. glabripennis* inoculated by dipping adults in suspensions of $10^7$ conidia/ml of four entomopathogenic species of hypocrealean anamorphs (Screening Bioassays A–E)

| Species | Isolate | Bioassay | No. of insects | Median days survival time ($ST_{50}$ ± CI)[a] | Fungal outgrowth |
|---|---|---|---|---|---|
| *Beauveria bassiana* | ARSEF 6391 (ST1) | A | 10 | 13.5[a] (10.0–21.0) | 50.0 |
| *Beauveria bassiana* | ARSEF 6444 (GHA) | A | 10 | 10.5 (9.0–14.0) | 100.0 |
| *Beauveria bassiana* | ARSEF 8414 (WU 50) | B | 12 | 9.0 (7.0–14.0) | 25.0 |
| *Beauveria bassiana* | ARSEF 6393 (VD 12) | C | 20 | 17.0 (16.0–18.0) | 100.0 |
| | | D | 12 | 13.0 (7.0–16.0) | 100.0 |
| | | E | 13 | 15.0 (11.0–18.0) | 100.0 |
| *Beauveria bassiana* | ARSEF 8412 (Q2K953) | C | 20 | 17.0[a] (10.0–20.0) | 90.0 |
| *Beauveria bassiana* | ARSEF 8413 (ST 2) | C | 20 | 18.0 (11.0–23.0) | 100.0 |
| *Beauveria brongniartii* | NBL 851 | A | 10 | 9.0 (–) | 80.0 |
| | | B | 12 | 7.5 (7.0–8.0) | 50.0 |
| *Beauveria brongniartii* | ARSEF 6412 (F 1101) | A | 10 | 9.0 (9.0–10.0) | 100.0 |
| *Beauveria brongniartii* | ARSEF 6827 (WU 20) | B | 12 | 5.0 (4.0–6.0) | 66.7 |
| *Isaria farinosa* | ARSEF 8411 (02NY5-6-1) | D | 12 | 24.5[a] (16.0–27.0) | 91.7 |
| *Metarhizium anisopliae* | ARSEF 8420 (WU19) | B | 12 | 9.5 (6.0–11.0) | 66.7 |
| *Metarhizium anisopliae* | ARSEF 7234 (VD 1) | C | 20 | 9.0 (-) | 95.0 |
| | | D | 12 | 8.0 (6.0–9.0) | 100.0 |
| | | E | 13 | 9.0 (8.0–9.0) | 100.0 |
| *Metarhizium anisopliae* | ARSEF 8416 (VD 3) | D | 12 | 7.0 (6.0–8.0) | 100.0 |
| *Metarhizium anisopliae* | ARSEF 6392 (VD 5) | C | 20 | 8.0 (7.0–9.0) | 100.0 |
| *Metarhizium anisopliae* | ARSEF 8417 (VD 7) | C | 20 | 6.5 (6.0–8.0) | 100.0 |
| *Metarhizium anisopliae* | ARSEF 8418 (VD 8) | C | 20 | 8.0 (7.0–9.0) | 100.0 |
| *Metarhizium anisopliae* | ARSEF 8419 (VD 9) | C | 20 | 6.0 (6.0–7.0) | 95.0 |
| *Metarhizium anisopliae* | ARSEF 8415 (FS 6) | C | 20 | 9.0 (6.0–10.0) | 100.0 |
| *Metarhizium anisopliae* | ATCC 62176 (ESC 1) | E | 13 | 8.0 (7.0–8.0) | 100.0 |

[a] Trials were censored at 30 days by which time all insects had died except two adults challenged with ARSEF 8411 and one adult each challenged with ARSEF 8412 and ARSEF 6391

**Table 4** Median survival time (and 95% confidence intervals) and percent of cadavers with fungal outgrowth for *A. glabripennis* adults treated with suspensions of $10^7$ conidia/ml of three *Metarhizium anisopliae* isolates (Bioassay F)

| Fungal isolate | Number of insects | Days to death ($ST_{50}$ ± CI)[A,B] | % Fungal outgrowth[2] |
|---|---|---|---|
| ARSEF 7234 (VD 1) | 63 | 5.0 (4.0–7.0) | 90.5 a |
| ARSEF 7711 (F 52) | 62 | 6.0 (5.0–6.0) | 77.4 ab |
| ATCC 62176 (ESC 1) | 63 | 6.0 (5.0–7.0) | 61.9 b |

[A] Bioassays censored at 15 days

[B] Values followed by the same letter are not significantly different at $\alpha = 0.05$. See text for results of comparison of days to death

the highest percentage outgrowth and cadavers killed by ATCC 62176 (ESC 1) the lowest, although all isolates yielded fungal outgrowth in >50% of cadavers.

## Discussion

All isolates tested were pathogenic to *A. glabripennis* adults. Screening bioassays estimated virulence against *A. glabripennis* adults; some of the isolates (*B. bassiana* (1 out of 6), *B. brongniartii* (3 out of 3), and *M. anisopliae* (9 out of 9)) killed 50% of adults exposed to $10^7$ conidia/ml in <10 days. In screening bioassays, the lowest $ST_{50}$s (<8 days) were seen for two isolates of *B. brongniartii* (ARSEF 6827 [WU 20] and NBL 851) and three isolates of *M. anisopliae* (ARSEF 8416 [VD 3], ARSEF 8417 [VD 7] and ARSEF 8419 [VD 9]). The majority of these most virulent strains were originally isolated from *A. glabripennis* and all were originally isolated from cerambycids (see Table 1). Shimazu et al. (2002) previously reported that *B. brongniartii* isolate F 1101 (ARSEF 6412) was highly virulent to *A. glabripennis* adults and suggested its potential for biopesticide development against this pest. We found that the three *B. brongniartii* isolates tested tended to kill *A. glabripennis* adults more quickly (range: 5.0–9.0 days) than most of the *B. bassiana* isolates tested (9.0–18.0 days) or the *I. farinosa* isolate (24.5 days) although $ST_{50}$s for *M. anisopliae* isolates (5.0–9.5 days) were similar to those for *B. brongniarti* isolates (Tables 3 and 4). Products based on *B. brongniartii* are produced in Europe and Africa for control of scarabs, in Asia for control of cerambycids, and in South America for control of a diversity of insect pests but this fungal species is not used for pest control in North America (Faria and Wraight, submitted). In fact, at the time these bioassays were conducted, we could not confirm that *B. brongniartii* is native to North America. Therefore, our subsequent, replicated bioassays emphasized *M. anisopliae* isolates because this latter species is native, and strains are registered with the U.S. Environmental Protection Agency for pest control. However, very recently, we learned of several *B. brongniartii* isolates from British Columbia, which were confirmed using molecular methods (B. D. King and S. A. Rehner, personal communication). Whether closer observation followed by molecular confirmation will identify additional *B. brongniartii* isolates from North America in the future remains to be seen.

During screening bioassays, we included an isolate of *I. farinosa* because this species was repeatedly isolated from *A. glabripennis* cadavers in the Ithaca quarantine. We hypothesized that this pathogen was transported into this arthropod quarantine as an external contaminant of freshly cut wood brought in for *A. glabripennis* oviposition. The single *I. farinosa* isolate tested was pathogenic but killed *A. glabripennis* more slowly than any of the other isolates. Strains of *I. farinosa* are also known to attack *A. glabripennis* in China (Wang et al. 1997), and were subsequently studied in the field using parasitoids to vector fungus to larvae for biological control (Wang et al. 1999). However, Shimazu et al. (2002) investigated the virulence of Chinese strains of *Isaria* isolated from an *A. glabripennis* adult and larva, and reported weak pathogenicity and virulence, only for the strain from an adult.

Bioassays in this study focused on adults after preliminary studies demonstrated lower virulence of strains of *B. brongniartii* and *B. bassiana* against larvae (Dubois 2003). Shimazu et al. (2002) also found variable and often low virulence against *A. glabripennis* larvae. Another reason that we focused on adults is that methods for ensuring that fungal conidia reach larvae within infested trees have not been developed while application techniques for adults have been developed (Higuchi et al. 1997). Several of the isolates

included in the present study (*B. bassiana* isolates ARSEF 6393 [VD 12] and ARSEF 6444 [GHA], *B. brongniartii* isolates ARSEF 6827 [WU 20] and NBL 851, and *M. anisopliae* isolate ARSEF 7234 [VD 1]) have been tested against *A. glabripennis* adults using fungal bands in the field (Dubois et al. 2004a, b; Hajek et al. 2006). These studies demonstrated that *B. bassiana* isolate ARSEF 6393 (VD 12), *B. brongniartii* isolates ARSEF 6827 (WU 20) and NBL 851, and *M. anisopliae* isolate ARSEF 7234 (VD 1) reduced *A. glabripennis* longevity and fecundity in caged and open field tests. However, field results are less successful at clarifying differences in virulence among isolates compared with the bioassay results reported in the present study.

In both the screening bioassays and replicated bioassays comparing the three *M. anisopliae* strains days to death did not differ between sexes. Female *A. glabripennis* are usually larger and heavier than males (Hajek et al. 2004) so one could hypothesize that it would take longer for entomopathogenic fungi to kill females versus males. However, uninfected females normally have shorter longevity compared with uninfected males (Keena 2006). In agreement with the lack of difference in days to death between sexes seen in this study, Shimane and Kawakami (1994) also found that pathogenicity of *B. brongniartii* and *M. anisopliae* did not differ between sexes of the cerambycid *Psacothea hilaris*.

For some bioassays, control mortality was quite high, ranging to 45.5% (Table 2). *A. glabripennis* are slow to develop and expensive to rear and large numbers of young adults were never available at the same time for bioassays. In particular, for the bioassay with the highest control mortality (B; 45.5%), the average age of adults at the initiation of the bioassay was 53.4 ± 3.7 days, and the study was censored 30 days later, by which time many control beetles could be dying of natural causes (Keena 2006). However, 50% of fungal-treated beetles in bioassay B died in ≤5–9.5 days, >1.5 weeks before they would die naturally (Table 3). We cannot determine whether the advanced age of adults when they were treated with fungus in bioassay B affected time to death but we suggest that studies comparing time to death after fungal inoculation for different ages of *A. glabripennis* adults should be conducted.

In other studies with cerambycids, fungus-induced mortality did not always yield fungal outgrowth from cadavers. In bioassays using *A. glabripennis* adults collected in the field, the percent of cadavers yielding outgrowth can be low (Dubois 2003). Shimazu (1994) determined that the lack of mycelial outgrowth from the cadavers of *Monochamus alternatus* killed by *B. brongniartii* resulted from contaminants such as enteric bacteria interfering with fungal sporulation. However, in our results from bioassays conducted in a quarantine with insects reared on artificial diet, percent fungal outgrowth rarely dropped below 50% of cadavers. The characteristic stiffening and clinging on the branches of *A. glabripennis* adults dying from infections by *B. brongniartii* isolate NBL 851 was also reported by Higuchi (1999) for this same fungal strain infecting *A. malasiaca* and *P. hilaris*. However, the spreading of the elytra before death due to NBL851 seen during this study was not previously reported.

In conclusion, the present study demonstrated high virulence of selected isolates of *B. brongniartii* and *M. anisopliae* against *A. glabripennis* adults. During this study we could not confirm that *B. brongniartii* is native to North America although recent results suggest otherwise. Until this question is resolved, we will emphasize development and use of *M. anisopliae* for control of *A. glabripennis* because this fungal species is native and strains are already registered for pest control in the USA.

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