Biological Control 50 (2009) 94–102

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Evaluating the virulence and longevity of non-woven fiber bands impregnated with *Metarhizium anisopliae* against the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae)

Ryan P. Shanley [a], Melody Keena [b], Micheal M. Wheeler [a,1], Jarrod Leland [c], Ann E. Hajek [a,*]

[a] Department of Entomology, Cornell University, Ithaca, NY 14853-2601, USA
[b] USDA-Forest Service, Northern Research Station, 51 Mill Pond Road, Hamden, CT 06514, USA
[c] Novozymes Biologicals, 5400 Corporate Circle, Salem, VA 24153, USA

ARTICLE INFO

Article history:
Received 28 July 2008
Accepted 25 March 2009
Available online 2 April 2009

Keywords:
Anoplophora glabripennis
Metarhizium anisopliae
Beauveria bassiana
Beauveria brongniartii
LC_{50}
Fungal bands
Biological control
Entomopathogenic fungi

ABSTRACT

Fiber bands impregnated with entomopathogenic fungi (=fungal bands) provide an effective method for controlling the invasive Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae). In this study we investigated the effective longevity of fungal bands for use against *A. glabripennis*, using several fungal strains. In the laboratory, adult beetles were immobilized on squares of fungal band impregnated with *Metarhizium anisopliae* (Metsch.) Sorokin isolate F 52 (ARSEF 7711) cultures to establish the median lethal concentration of 3.08×10^6 conidia/cm^2. During the summers of 2001–2004, bands impregnated with strains of *M. anisopliae* (2002–2004), *Beauveria bassiana* (2002), and *Beauveria brongniartii* (2001) were attached to trunks of trees in Queens, New York City to evaluate changes in densities of viable conidia over intervals of up to 119 days. The viability of conidia from bands was not associated with time in the field although total conidial density and density of viable conidia decreased with increasing time in the field. Bioassays conducted using samples of *M. anisopliae* F 52 bands in field sites in 2004 demonstrated that adult *A. glabripennis* died more quickly when exposed to higher concentrations of viable conidia. Although densities of viable conidia on bands decreased over time for all trials, they never decreased below the LC_{50} estimates calculated in this study for *M. anisopliae* F 52. Our results demonstrate the pathogenicity of *M. anisopliae* F 52 fungal bands against *A. glabripennis* adults and show that the conidia from fungal bands can retain virulence in the field for at least 112 days.

1. Introduction

Non-woven fiber bands impregnated with cultures of fungal pathogens and placed around tree trunks or branches were first developed as a biological control method to control adults of longhorned beetles boring in orchard trees in Japan. The efficacy of fungal bands has been evaluated for use against several tree-boring cerambycids with much success (e.g., Shibata et al., 1991; Higuchi et al., 1997; Shimazu, 2004; Hajek et al., 2006). A commercial fungal band product named BiolisaKamikiri (Nitto Denko, Japan), based on *Beauveria brongniartii* (Sacc.) Petch, has been developed for control of several cerambycid pests in Japanese orchards (Higuchi et al., 1997). Recently, BiolisaMadura is now available in Japan for control of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), the vector of pinewood nematode, *Bursaphelenchus xylophilis* (Steiner and Buhrer) Nickle (Shimazu, 2009). While tree-boring pests are often difficult to target with chemical insecticides, fungal bands take advantage of the tendency of many tree-boring cerambycids to walk on tree trunks and branches, especially during what is known as the prematuration or preoviposition feeding period (Iba, 1993).

The Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), was first discovered in North America in Brooklyn, New York City in 1996, followed by subsequent North American discoveries between 1998 and 2009 in Chicago, Illinois, New Jersey, Toronto, Ontario and Worcester, Massachusetts (Hu et al., in press). Moreover, infestations of this beetle have been found since 2001 in Austria, France, Germany, Italy and Japan. *A. glabripennis* has a wide host range, and attacks both stressed and seemingly healthy trees. This pest poses a serious economic and ecological threat to urban and forest trees (Nowak et al., 2001). Therefore, major programs to eradicate *A. glabripennis* have been undertaken. Infested trees are removed, and uninfested host trees are often treated with imidacloprid through trunk and soil injections (USDA, APHIS, 2007). This systemic insecticide, however, is not distributed evenly within trees and has exhibited strong antifeedant effects for *A. glabripennis* (Poland et al., 2001, 2006). Thus, it is questionable whether beetles are

* Corresponding author. Fax: +1 607 255 0939.
E-mail address: aeh4@cornell.edu (A.E. Hajek).
[1] Present address: USDA, ARS, Biological Integrated Pest Management Research Unit, Robert W. Holley Center for Agriculture and Health, Tower Road, Ithaca, NY 14853-2901, USA.

1049-9644/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2009.03.013

likely to receive a lethal dose of imidacloprid and whether feeding on inoculated trees will ultimately increase dispersal.

As with other cerambycids, reductions in both adult longevity and female oviposition have been found when *A. glabripennis* adults are exposed to fungal bands (e.g., Hajek et al., 2006; Shanley, 2007). Fungal infection can be horizontally transmitted during mating for many cerambycids (Shibata and Higuchi, 1988; Tsutsumi and Yamanaka, 1995), including *A. glabripennis* (A.E.H., unpublished data). Horizontal transmission from female *A. glabripennis*, resulting in egg and larval mortality, also occurs (Hajek et al., 2008). The longevity of activity of fungal bands makes them an especially appropriate control method for *A. glabripennis*, because adults are long-lived. Laboratory-reared *A. glabripennis* adults lived 78.9 ± 3.3 (mean ± SD) days for females and 98.1 ± 9.0 days for males (Keena, 2006). Ninomiya and Higuchi (1998) reported that *B. brongniartii* fungal bands retain conidial densities causing > 50% mortality of *Psacothea hilaris* (Pascoe) and *Anoplophora chinensis* (=*A. malasiaca* (Thomson)) (Coleoptera: Cerambycidae) for more than 30 days in the field in Japan. Determining the longevity of activity of fungal bands under field conditions is contingent on establishing the critical density of conidia (the number of conidia per square centimeter of band material) required to kill the target pest. Critical conidial densities for 50% mortality have been quantified for *B. brongniartii* against the yellow-spotted longicorn beetle, *P. hilaris* (Higuchi et al., 1997; Tsutsumi, 1998), and for *Beauveria bassiana* (Balsamo) Vuillemin against *M. alternatus* (Shimazu, 2004). Studies investigating critical lethal concentrations of entomopathogenic fungi against *A. glabripennis* adults have utilized dipping assays with suspensions of *B. bassiana*, *B. brongniartii*, and *Paecilomyces* spp. conidia (Zhang and Liu, 1996; Shimazu et al., 2002; Dubois et al., 2008).

In the current study, the LD_{50} for *Metarhizium anisopliae* (Metsch.) Sorokin, isolate F 52 (ARSEF 7711) fungal bands was determined in the laboratory by exposing adults to conidial lawns; the LC_{50} is important to estimate because it has been used as a threshold for determining fungal band efficacy. Conidial densities on fungal bands over time in the field were determined for this isolate, along with four additional isolates. Bands of five fungal isolates (three isolates of *M. anisopliae*, one isolate of *B. bassiana* and one isolate of *B. brongniartii*) were placed on trees in Queens, New York, during four consecutive summers (2001–2004) for up to 119 days. Samples of fungal bands were evaluated periodically to quantify densities of conidia capable of germinating and to determine pathogenicity and virulence.

2. Materials and methods

2.1. Fungal isolates and experimental insects

Five fungal isolates were used in these studies (Table 1). *M. anisopliae* ARSEF 7711 (F 52) was used in the laboratory for concentration/response trials as well as for the fungal band field activity trial in 2004. This isolate is presently registered with the US Environmental Protection Agency and is marketed by Novozymes Biologicals, Inc. (Salem, VA). Bands of all other isolates were used only for fungal band field activity trials. In 2001, the *B. brongniartii* isolate that is commercialized for BiolisaKamikiri was used in fungal bands; although in 2001, *B. brongniartii* was not known to be native to North America, permission was granted by federal authorities for localized studies with these fungal bands. In 2002, two isolates originating from *A. glabripennis* adults in the United States were used: *B. bassiana* ARSEF 6393 and *M. anisopliae* ARSEF 7234. In 2003, *M. anisopliae* ATCC 62176 was used for fungal bands. This isolate had previously been produced by EcoScience as ESC-1, but registration with the US Environmental Protection Agency had lapsed.

Anoplophora glabripennis is a quarantined insect in North America and field populations are extremely low so bioassays were conducted in quarantine facilities. The majority of bioassays were conducted using adult *A. glabripennis* reared in the US Department of Agriculture, Agricultural Research Service quarantine on the Cornell University campus using artificial diet and following rearing procedures described by Dubois et al. (2002). Because these beetles take a long time to develop and are expensive to rear (estimated at US$21 per adult without overhead costs; Keena, 2005), ample numbers of beetles for replication were not always available and even-aged cohorts were never available. For the concentration response bioassay, adequate numbers of adult *A. glabripennis* were not available in time at Cornell so two concentrations and controls were conducted at the US Department of Agriculture, Forest Service quarantine at Ansonia, Connecticut, using the same experimental procedures and environmental conditions as at Cornell. Rearing methods for these latter beetles have been described by Keena (2005).

2.2. Laboratory LC_{50} bioassay

Fungal bands were produced using 50 × 500 mm strips of non-woven polyester fabric (uncompressed material used for stuffing quilts = quilt batting; 203.4 g/m^2; Soft and Bright; The Warm Company, Lynnwood, WA) as the substrates in and on which fungal cultures grew. Strips were dipped into 7 l of warm (~45 °C) full strength Sabouraud dextrose agar (SDA premix at 65 g/l; Neogen) plus 10 g/l yeast extract (Sigma) (=SDAY) inoculated with 4-day-old liquid cultures of *M. anisopliae* F 52 grown in 2 l of Sabouraud dextrose yeast broth (SDY: 40 g dextrose, 10 g peptone, 10 g yeast, 1 l water) in 2.8 l Fernbach flasks at 175 RPM and 27 °C. The fungal inoculum was combined at a ratio of 1 l of the fungal cell suspension to 3.5 l of warm SDAY under a biosafety hood and mixed with a sterile spatula immediately prior to dipping fabric strips. After inoculating the fiber strips, they were maintained on racks at high humidity (~100%) to allow growth and sporulation on band sur-

Table 1
Fungal isolates grown in non-woven fiber bands for this study.

Species	Isolate[a]	Origin	Host
Metarhizium anisopliae	ARSEF 7711 (F 52)[b]	Vienna, Austria	*Cydia pomonella* larva
Metarhizium anisopliae	ATCC 62176 (ESC 1)[b]	Illinois	Soybean nematode cyst
Metarhizium anisopliae	ARSEF 7234 (VD 1)	Ansonia, Connecticut[c]	*Anoplophora glabripennis* adult
Beauveria bassiana	ARSEF 6393 (VD 12)	Ansonia, Connecticut[c]	*Anoplophora glabripennis* adult
Beauveria brongniartii	NBL 851[b]	Gunma, Japan	*Psacothea hilaris* adult

[a] ARSEF, Agricultural Research Service collection of Entomopathogenic Fungal cultures; ATCC, American Type Culture Collection; NBL 851 is the isolate of *B. brongniartii* used for fungal band production by Nitto Denko, Osaka, Japan during the time of this study (presently produced by Idemitsu Kosan Co., Tokyo, Japan). Isolate numbers VD 1 and VD 12 relate to the isolate identification system in the Hajek lab.

[b] Commercialized isolates: ARSEF 7711 available from Novozymes Biologicals Inc., Salem, Virginia as F 52; Registration presently lapsed for ESC 1; NBL 851 presently produced by Idemitsu Kosan Co., Tokyo, Japan.

[c] Isolated from cadavers of *A. glabripennis* from naturally infested wood cut in Chicago, Illinois (ARSEF 7234) and Bayside, New York (ARSEF 6393) with beetles emerging in the USDA, Forest Service quarantine in Ansonia, Connecticut.

faces for 2–3 weeks, as described by Shanley (2007). After the surfaces of bands were covered with conidia, bands were dried for 2 days at 80% RH and then placed in dry plastic bags for storage at 4 °C until use (i.e., up to 10 months storage).

To conduct bioassays, adult *A. glabripennis* were exposed to a range of conidial densities on surfaces of bands. To prepare the different conidial concentrations following the methods of Higuchi et al. (1997), 25 cm^2 squares cut from fungal bands were placed in a drying oven at 42 °C for ten intervals, ranging from 0–20 h; exposure to heat was used to obtain a range of conidial densities because with increasing periods at 42 °C, increasing proportions of conidia were killed. For each heating interval, squares cut from the same batch of fungal bands were used. The density of viable conidia per square centimeter was quantified both before and after exposing band squares to heat. For each quantification, three randomly chosen squares cut from fungal bands were used to quantify conidial density and percent germination (see below). The control treatment consisted of squares of fungus-free band material placed into the oven at 42 °C for 6.3 h (i.e., the mean time of heat exposure over all conidial concentrations).

To quantify the total number of conidia per square centimeter of a piece of band material, a slightly modified technique from that used by Higuchi et al. (1997) was employed. From a fungal band, a 25 cm^2 square was cut and homogenized in 300 ml of 0.2% Tween 20 using a 2 l blender (Hamilton Beach) for 1 min. The resulting suspension was filtered through a 150 μm mesh filter into a 500 ml plastic bottle. An additional 200 ml of deionized water was used to wash any contents remaining in the blender into the plastic bottle. The conidial suspension was sonicated for 5 min to suspend and separate conidia. A hemocytometer was used to calculate the number of conidia per square centimeter of fungal band material.

From each conidial suspension prepared above, 0.75 ml was spread onto each of 3 plates of 2% water agar in 100 mm plastic petri dishes. Petri dishes were incubated at 20 °C for 48 h, and then transferred to 4 °C for a maximum of 48 h until percent germination could be quantified. Germination percentages were recorded for 20 conidia at each of 10 separate locations on each petri dish at 400× for a total of 200 conidia. Conidia were considered germinating if the germ tube was longer than half the major axis of the conidium. The mean proportion germination for the three petri dishes was multiplied by the conidial density to determine the number of viable conidia per square centimeter for that piece of fungal band. Concentrations of viable conidia on samples used for these bioassays ranged from 1.32×10^5 to 3.95×10^7 conidia/cm^2.

Adult *A. glabripennis* used for bioassays were between 10 and 30 days old, and were randomly assigned to each of the 10 conidial concentrations and one group of controls, with four males and three females in each group. Each fungal-treated beetle was exposed ventrally by holding it on an individual 5 cm^2 square of fungal band for 30 s. Control beetles were exposed to fungal-free band material in the same manner. Before and after being exposed to a band square, each beetle was housed in an opaque 473 ml plastic cup with transparent lid at 24 °C and 16:8 (L:D). Each beetle was provided with four fresh 10 cm long *Acer pensylvanicum* L. twigs every 7 days. Beetles were checked daily for death, and *A. glabripennis* surviving ⩾40 days were considered uninfected. For randomly chosen replicates, beetles not dying were checked daily until death, while for other replicates, beetles were monitored daily for only 40 days. Cadavers were placed in plastic 59.1 ml cups containing moist cotton balls. Cups were sealed with parafilm and cadavers were observed for fungal outgrowth for at least 2 weeks.

2.3. Fungal bands in the field

2.3.1. Fungal bands for field activity trials

In 2002 and 2003, bands for use in the field were grown as described by Shanley (2007) except the 2 l of fungal inoculum grown in SDY was added to 4 l SDAY (Goettel and Inglis, 1997) instead of 7 l SDAY and bands were incubated at 25 °C instead of 27 °C. Once the fungus had fully grown throughout bands, produced conidia on the surface, and bands had been dried for 2 days at 80% RH, bands were stored at 4 °C for <2 weeks before use. Fungal bands for 2002 and 2003 were produced using the same 203.4 g/m^2 quilt batting material used for LC_{50} bioassays (see above). In 2004, manufacture of the quilt batting material that had been used previously for field studies was going to be discontinued. We could not find a replacement that was a similar weight so we included a similar, but thinner, material (135.6 g/m^2; Soft and Bright; The Warm Company, Lynnwood, WA) in trials for comparison.

Beauveria brongniartii bands used in the field in 2001 were produced by Nitto Denko, (Osaka, Japan) following published procedures (Higuchi et al., 1997). The major differences in methods for producing fungal bands included use of 4% corn steep liquor plus 2% glucose, pH 4 at 25 °C for growing fungal cells. Large-scale liquid cultures were grown in 30 or 100 l fermenters with air inflow rates of 0.5 vvm and an agitation speed of 400 or 180 rpm. To prevent foaming, 0.3% silicon oil was added. Liquid fungal cultures were next diluted with 4% corn steep liquor and 6% glucose and the resulting suspension was used to inoculate band material made of virgin wood pulp (density: 306 g/m^2). The fungus was grown in bands for 4–5 days at RH>97% and bands were then dried for 1–2 days before use (Higuchi et al., 1997).

2.3.2. Densities of viable conidia in bands from the field

For all 4 years, densities of viable conidia were calculated following the methods described above. For each treatment each year, three representative bands were randomly selected for quantification of initial viable conidial density before bands were placed in the field. To evaluate the activity of fungal bands over time in the field, studies were conducted in the Calvary Cemeteries and Lutheran All Faiths Cemetery, Queens, New York City. Mature ur-

Table 2
Bioassays conducted in Queens, New York from 2001–2004.

Fungal species and isolate	Starting date	Tree diameters (mean cm ± SE)	Height of bands (mean m ± SE)	# sample dates	# beetles exposed per treatment
Metarhizium anisopliae ARSEF 7711	10 August 2004	53.0 ± 3.2	5.6 ± 0.3	5, 10[a]	9
Metarhizium anisopliae ATCC 62176	30 June 2003	51.0 ± 1.8	4.2 ± 0.1	13	9
Beauveria bassiana ARSEF 6393	18–19 July 2002	75.3 ± 3.1	4.0 ± 0.1	10	6–9
Metarhizium anisopliae ARSEF 7234	18–19 July 2002	75.9 ± 3.9	4.0 ± 0.1	9	9–12
Beauveria brongniartii NBL 851	27 July 2001	103.3 ± 7.7	5.0 ± 0.2	5[b]	6–15
Beauveria brongniartii NBL 851	18 August 2001	78.3 ± 4.5	4.6 ± 0.3	3	6–8

[a] Thick bands were sampled 10 times and bands made with thinner material were sampled five times.
[b] Due to insufficient adult *A. glabripennis*, bioassays were only conducted for four of the five times that viable conidia were quantified for field-exposed bands.

ban trees (Table 2) used for studies predominantly included maples (*Acer* spp.) (>65%). Each year, beginning in summer, bands were placed around trunks of trees at approximately 4–6 m height, always completely in the shade. Thereafter, at varying successive intervals of from 1–4 weeks, three randomly chosen bands of each treatment were removed from tree trunks for evaluation. In 2001, *B. brongniartii* band exposures were begun twice, with each trial only evaluated for 33–47 days due to the 'September 11' tragedy in nearby Manhattan. With each successive year of this study from 2001 to 2003, we realized that bands should be evaluated for longer than we had planned. Thus, in 2002 bands were on trees for 63–70 days while in 2003 and 2004, bands were on trees in the field for a total of 119 and 112 days, respectively. In 2004, thicker bands were evaluated 10 times (weeks 0, 4, 8, 10 and subsequently weekly until 16) and thinner bands were evaluated five times (weeks 0, 4, 8 12 and 16). Bands removed from trees were placed at 4 °C for up to 3 days before being evaluated to determine the density of viable conidia as well as pathogenicity and virulence.

2.3.3. Bioassays with bands from the field

From each of the bands from trees in the field, three 5 × 5 cm pieces were cut. Each piece was placed in a 60 mm petri dish and one adult beetle was placed in each Petri dish, with the lid closed, for 30 s; with the lid closed, all but the smallest beetles were immobilized on the band square during the exposure. Each beetle was then placed individually in a polypropylene container (650 ml, 170 × 80 mm) with a water-saturated cotton ball and 5–10 twigs of sugar maple (*Acer saccharum* Marsh.). After treatment, beetles were reared at 25 °C in the same manner as the concentration response bioassay beetles. For each interval that bands were removed from the field, 6–15 beetles were used per treatment, including equal numbers of males and females whenever possible, based on availability of beetles from the colony. An equal number of controls were included for each interval, exposing them to unaltered band material in the same way. Beetle age (mean ± SE) at time of fungal exposure was 25.1 ± 1.6 days for 2001 bioassays, 35.7 ± 0.8 for 2002 bioassays, 16.7 ± 1.3 for 2003 bioassays and 20.2 ± 1.0 for 2004 bioassays.

The method for quantifying the densities of viable conidia involves blending pieces of the entire band although, in reality, only the part of the band facing away from the tree trunk would be exposed to beetles. In bioassays conducted in 2001–2003, beetles were always exposed to the sides of bands facing outward from tree trunks. To test whether viable conidial densities on inner sides of bands (i.e., against tree trunks) differed from densities of the outer sides of bands, in 2004, beetles were randomly exposed to either the outside of the band or the inside of the band for comparison of days to death. This comparison was begun with the second bioassay date (at 4 weeks after bands had been hung in the field) and continued through the remaining bioassay dates.

2.4. Data analysis

The density of viable conidia on bands was calculated as the product of proportion germination and total conidia/cm^2 of band. Laboratory LC_{50} bioassay data with fungal-exposed beetles dying in <40 days were analyzed by probit analysis (Starr, 1997). For LC_{50} bioassays, chi-squared tests were used to compare the probability of mortality among beetles with regard to sex, weight, and age.

As with analyses of laboratory assays, for field trials longevity was calculated only using adults dying within 40 days of exposure to bands. For bands exposed in the field, a mixed model was used to test for an association between proportion conidial germination and days in the field, across all trials except the 2004 thin bands, with year * band as a random effect (SAS Institute, 2004). Mixed models were also used to test whether total conidial density on bands or density of viable conidia on bands were associated with days in the field, with year * band as a random effect. Total conidial density on bands and density of viable conidia on bands were log-transformed for these analyses. The only valid comparison of changes in density of viable conidia among fungal strains across time was possible for the 2002 isolates, because these bands were placed in the field at the same time during the same year. A slope test (Sokal and Rohlf, 1981) was used to compare the change in density of viable conidia across time for the two isolates tested in 2002. Student's *t*-tests (SAS Institute, 2004) were used to compare densities of viable conidia when the study was begun with the last sample date for that year * isolate combination. A Wilcoxon two-sample test was used to compare days to death for adult *A. glabripennis* exposed to fungal band samples before they were placed in the field with samples from after retrieval from the field. Chi-squared tests with an overall $\alpha = 0.05$, adjusted with the Bonferroni inequality, were used to compare percentages of sporulating cadavers among isolate * year combinations.

To evaluate associations between rainfall and temperature, weather data collected at La Guardia Airport (ca. 6–7.5 km from study sites) were used (National Climatic Data Center, National Oceanic and Atmospheric Administration (NOAA), Asheville, NC). Due to the limited numbers of samples for individual trials, the two trials for 2001 were merged. To analyze the change in density of viable conidia from the start to end of each sampling interval, we used the following calculation to estimate the growth rate in conidial density: $r_t = \log_e(N_t/N_{t-1})$. Pearson correlation coefficients were used to evaluate correlation between average temperature/day, average rainfall/day and proportion of days with rain across sampling intervals for each isolate * year combination (PROC CORR; SAS Institute, 2004).

3. Results

3.1. Laboratory bioassays

A strong correlation was found between conidial concentration, measured as the density of viable conidia/cm^2 of band, and the percentage of beetles dying within 40 days (percentage of beetles dying = $-1.47703 \pm 0.998235 * \log$ (conidial density/cm^2); SE of slope = 0.4001; heterogeneity $\chi^2 = 4.07$, df = 4, $P = 0.3970$). The median lethal concentration for *M. anisopliae* F 52 fungal bands against adult *A. glabripennis* was determined to be 3.08×10^6 viable conidia/cm^2 ($\alpha = 0.05$; 95% confidence interval = 1.06×10^6, 3.59×10^7). There were no differences in the probability of mortality among beetles with regard to sex ($\chi^2 = 1.65$, $P = 0.1991$), weight ($\chi^2 = 2.72$, $P = 0.0990$), or age ($\chi^2 = 3.50$, $P = 0.0613$).

Cadavers of most beetles exhibiting fungal outgrowth died within 40 days (32 out of 34, 94.1%), while most beetles not showing outward signs of infection survived the 40 day study period (45 out of 46, 97.8%). These data suggest that 40 days was a reasonable upper limit for evaluating whether beetles were infected. For beetles dying within 40 days and exhibiting post-mortem fungal outgrowth, the mean number of days to death was 19.7 ± 1.4 days (range: 8–37; $n = 32$). The mean longevity for beetles not showing outward signs of infection was 85.0 ± 8.6 ($n = 14$). Only one beetle died within the 40-day study period without exhibiting fungal outgrowth, dying 20 days after being exposed to a fungal band with 6.08×10^5 conidia/cm^2. All of the beetles in the control treatment survived for over 40 days.

3.2. Field trials of fungal bands

For trials with fungal bands using the five different fungal isolates from 2001 to 2004, densities of viable conidia on bands de-

clined over time in the field ($F_{1,163}$ = 111.78; P < 0.05) (Table 3, Fig. 1). To examine this decline further, we analyzed whether both the percent germination and conidial density declined over time in the field. Percent conidial germination from band samples did not decline with the time that samples were in the field ($F_{1,163}$ = 1.66; P = 0.1997). However, the total conidial densities decreased over time in the field ($F_{1,163}$ = 214.58, P < 0.0001). The slopes of decline in conidial density across days in the field for *M. anisopliae* ARSEF 7234 ($-6.81 \times 10^5 \pm 3.49 \times 10^5$, slope ± SE) was less steep than *B. bassiana* ARSEF 6393 ($-3.13 \times 10^6 \pm 6.77 \times 10^5$) in 2002 ($t_{16}$ = −3.21; P < 0.05). In 2004, the densities of viable conidia on thicker bands were not significantly different from densities of viable conidia on thinner bands, either at the beginning of the study, before bands were placed on tree trunks (t = 0.39; P = 0.7194), or after 112 days in the field (t = 2.25; P = 0.0876).

In bioassays, percent mortality of fungal band-exposed beetles remained at 100% from start to end of trials in 2001–2003. Throughout the 2001–2003 trials, while the density of viable conidia always decreased by the end of the trial, mean lethal time for bioassay beetles only increased between start and end for the 27 July 2001 trial for *B. brongniartii* NBL 851 and the 2003 trial using *M. anisopliae* ATCC 62176 (Table 4). Percentages of cadavers sporulating for trials conducted 2001–2003 varied from 41.8% to 78.4% by isolate (Table 4). The isolate with the fewest sporulating cadavers was *B. bassiana* ARSEF 6393, used in the field in 2002.

In 2004, mortality dropped below 100% before the end of the trial (day 112 after bands were placed in the field) although still remaining at >50%, so estimation of an LC_{50} was not considered valid. For the 2004 bioassays, the concentration of viable conidia on bands was negatively associated with log-transformed days to death (r^2 = 0.5755; days to death = −9.2303 * log (viable conidia/cm^2)+185.16; P = 0.0010), with beetles exposed to higher concentrations of viable conidia on bands dying more quickly (Fig. 2). Days to death did not differ between beetles exposed to the sides of fungal bands oriented against the tree trunk versus the sides of fungal bands oriented away from the tree trunk ($F_{1,104}$ = 1.72; P = 0.1927). Merging data from beetles exposed to thick and thin bands, the vast majority of cadavers from beetles exposed to *M. anisopliae* F 52 produced conidia (92.0%). For the thick bands alone, the percentage of cadavers that sporulated was equivalent to percentages of sporulating cadavers from *B. brongniartii* NBL 851 (2001) and *M. anisopliae* ATCC 62176 (2003) (Table 4).

Change in conidial density was negative the majority of the sampling intervals for each isolate, although each isolate * year combination experienced from 1–4 sampling intervals when conidial densities increased. Analyses of correlations between weather data and changes in conidial densities did not demonstrate an overall trend. Both 2001 *B. brongniartii* and 2002 *B. bassiana* bands showed a negative correlation between conidial growth rates and the proportion of days of rain (Table 5). In both of these years, some sampling intervals received no rain at all but when rain was more frequent, conidial densities declined more. The sampling period in 2003 was not very rainy compared with other years (Table 5) or rainfall was positively correlated with r_t; thus, in this instance there was a general trend for conidial density to increase when there was more rain (Fig. 1; Table 5). For 2004 samples, the temperature from start to end of the trial was generally lower than other years of this study. The r_t was marginally negatively associated with daily temperature, suggesting that conidial densities decreased with increasing temperatures.

4. Discussion

The LC_{50} is important to determine because it is used as a threshold for fungal band activity; Higuchi et al. (1997) considered that fungal bands for use in the field must contain the LC_{50} density to be considered effective. Findings from our study confirmed that *A. glabripennis* mortality due to fungal infection acquired from exposure to *M. anisopliae* F 52 bands is concentration-dependent, and that beetle longevity decreases as viable conidial density on the bands increases. Cadavers from most beetles dying within 40 days after exposure to *M. anisopliae* F 52 bands in the lab and field produced conidia under optimal conditions in the laboratory. Two isolates tested had equivalent percentages of sporulating cadavers as *M. anisopliae* F 52, although two isolates had lower percentages Table 4. Not all cadavers of cerambycids killed by fungal pathogens produce fungal outgrowth; cadavers of beetles that were assumed to have died from *M. anisopliae* F 52 infections but failed to exhibit fungal outgrowth have been previously reported for *A. glabripennis* exposed to *M. anisopliae* F 52 (Shanley, 2007) and *B. brongniartii* (Dubois et al., 2004b), as well as for *M. alternatus* exposed to *B. bassiana* (Shimazu and Takatsuka, 2006).

Researchers determining LC_{50}s of fungal bands have used different techniques for exposing different beetle species to different fungal strains. In our study, we estimated an LC_{50} of 3.08×10^6 conidia/cm^2 for *M. anisopliae* F 52 fungal bands by immobilizing *A. glabripennis* adults on top of pieces of fungal band for 30 s. Higuchi et al. (1997) used a walking bioassay to determine the critical concentration required for *B. brongniartii* fungal bands against *P. hilaris*; these tree-boring cerambycids were exposed to fungus by walking on bands for "more than 5 s," which resulted in an LC_{50} of 1×10^7 conidia/cm^2 of fungal band. Shimazu (2004) used a paintbrush to apply dried conidia from *B. bassiana* fungal bands to the tarsi of Japanese pine sawyer, *M. alternatus* adults. In 4-day-old *M. alternatus*, median lethal concentrations of 1.9×10^6 and 2.4×10^4 conidia/individual were found after 14 and 30 days, respectively. In "aged adults" (i.e., >10 days since emergence) LC_{50}s of 5.5×10^6 and 2.8×10^5 conidia/individual were found after 14 and 30 days, respectively, (Shimazu, 2004).

LC_{50} values have been used in studies of fungal band longevity to determine activity retention time in different host/pathogen systems. For *B. brongniartii* fungal bands against various tree-bor-

Table 3
Densities of viable conidia/cm^2 on non-woven fiber bands when placed in the field and when retrieved from the field.

Fungal species and strain	Starting date	Starting density of viable conidia (mean ± SE)	Days in field	Ending density of viable conidia (mean ± SE)	P values[a]	% of original concentration remaining
Metarhizium anisopliae ARSEF 7711[b]	10 August 2004	$1.55 \times 10^8 \pm 1.62 \times 10^7$ a	112	$1.98 \times 10^7 \pm 2.26 \times 10^6$ b	0.0128	12.7
Metarhizium anisopliae ATCC 62176	30 June 2003	$8.58 \times 10^7 \pm 7.74 \times 10^6$ a	119	$2.50 \times 10^7 \pm 3.50 \times 10^6$ b	0.0020	29.1
Metarhizium anisopliae ARSEF 7234	18–19 July 2002	$9.79 \times 10^7 \pm 6.26 \times 10^6$ a	63	$4.12 \times 10^7 \pm 1.54 \times 10^7$ b	0.0183	42.1
Beauveria bassiana ARSEF 6393	18–19 July 2002	$3.06 \times 10^8 \pm 2.67 \times 10^7$ a	70	$9.48 \times 10^7 \pm 3.01 \times 10^7$ b	0.0064	31.0
Beauveria brongniartii NBL 851	27 July 2001	$4.16 \times 10^8 \pm 4.39 \times 10^7$ a	33	$1.00 \times 10^8 \pm 9.40 \times 10^6$ b	0.0021	24.0
Beauveria brongniartii NBL 851	18 August 2001	$3.64 \times 10^8 \pm 1.64 \times 10^7$ a	47	$5.73 \times 10^7 \pm 9.14 \times 10^6$ b	<0.0001	15.7

[a] Student's t-test (SAS Institute, 2004) used to compare densities of viable conidia within rows.
[b] Based only on thicker band material.

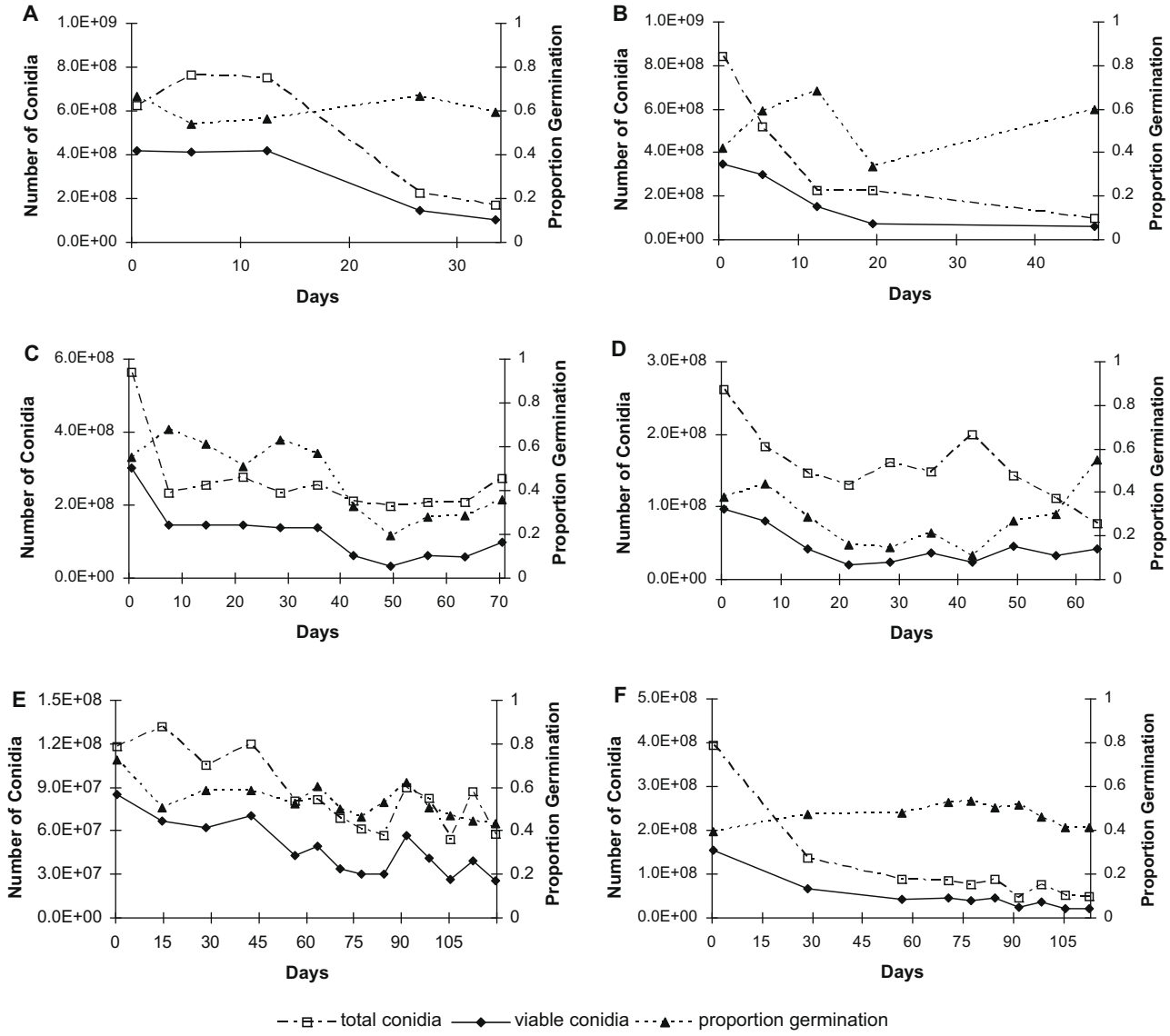

Fig. 1. Total conidia/cm^2 (open squares with dashes), proportion germination (filled triangles with small dashes) and viable conidia/cm^2 (filled diamonds with solid lines) for fungal bands on trees in Queens, New York City from 2001 to 2004. (A) *Beauveria brongniartii* NBL 851 on trees beginning 27 July 2001, (B) *B. brongniartii* NBL 851 on trees beginning 18 August 2001, (C) *Beauveria bassiana* ARSEF 6393 in 2002, D. *Metarhizium anisopliae* ARSEF 7234 in 2002, (E) *M. anisopliae* ATCC 62176 in 2003, (F) *M. anisopliae* ARSEF 7711 in 2004. On the *y*-axis, values should be interpreted as follows: 2.0E+08 = 2.0×10^8.

Table 4
Percent mortality and days to death for adult *Anoplophora glabripennis* exposed to fungal bands before placement in the field and when retrieved from the field.

Fungal species and strain	Starting date	Cadavers sporulating[a] (%)	Before bands were placed in the field[b]			Band removal from the field[b]			
			# Beetles tested	Mortality (%)	Days to death (mean ± SE)	Total days in the field	# Beetles tested	Mortality (%)	Days to death (mean ± SE)
Metarhizium anisopliae ARSEF 7711[c]	10 August 2004	89.0 a	9	100.0	9.9 ± 0.9 a	112	9	55.6	15.1 ± 2.3 b
Metarhizium anisopliae ATCC 62176	30 June 2003	78.4 ab	9	100.0	9.9 ± 0.7 a	119	9	100.0	14.7 ± 1.2 b
Metarhizium anisopliae ARSEF 7234	18–19 July 2002	62.5 bc	9	100.0	13.0 ± 0.8 a	56	9	100.0	12.2 ± 1.1 a
Beauveria bassiana ARSEF 6393	18–19 July 2002	41.8 c	9	100.0	14.3 ± 2.5 a[d]	63	7	100.0	13.1 ± 2.9 a
Beauveria brongniartii NBL 851	27 July 2001	74.5 ab	15	100.0	5.8 ± 0.5 a	21	6	100.0	7.8 ± 0.9 b
Beauveria brongniartii NBL 851	18 August 2001	—[e]	6	100.0	7.7 ± 0.3 a	47	6	100.0	9.0 ± 0.7 a

[a] Sporulation from cadavers of beetles dying in <40 days after exposure. Differing letters in italics within this column denote significant differences between percentages of sporulating cadavers (chi-squared tests; overall $\alpha = 0.05$, adjusted with the Bonferroni inequality).

[b] % Mortality and days to death for adult beetles dying <40 days after fungal exposure. Differing letters following days to death indicate statistically significant differences in means from start to end of each trial (Wilcoxon two-sample test; $\alpha = 0.05$).

[c] Beetles exposed to thick bands.

[d] Data are for bands taken down 14 days after placement in the field. Bioassays from samples before placement in the field were compromised because only adult beetles >50 days old were available for use.

[e] Data not taken.

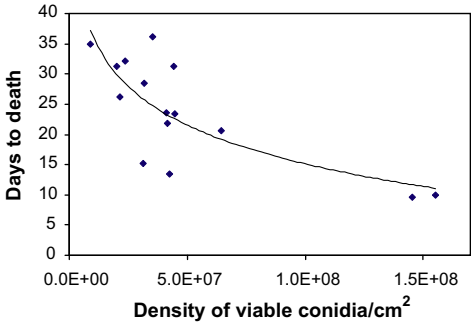

Fig. 2. Days to death for *Anoplophora glabripennis* adults exposed to densities of viable conidia on fungal bands of *Metarhizium anisopliae* ARSEF 7711 that had been on trees in Queens, New York City in 2004. Symbols represent means of groups of beetles exposed to different conidial densities on fungal band samples. On the *x*-axis, values should be interpreted as follows: 5.0E+07 = 5.0×10^7.

ing cerambycids, fungal bands have been found to remain active in the field for at least 20 days (Tsutsumi, 1998), about 30 days (Dubois et al., 2004b; Matsuura et al., 1997), or for up to 40 days (Higuchi et al., 1997). Our study demonstrated that *M. anisopliae* F 52 fungal bands attached to trees in New York City maintained densities greater than the LC_{50} for *A. glabripennis* for at least 112 days. In New York, *A. glabripennis* adults typically emerge from July to September (Nowak et al., 2001) and in Chicago, most adults were observed on trees from July to October (Haack et al., 2006). Thus, the extended longevity of fungal bands in the field suggests that hanging bands once per season would be sufficient for enabling exposure for the majority of adult beetles through one season.

Fungal bands of numerous species have now been shown to retain high concentrations of viable conidia on their exterior surfaces for several months. In our study, we found high densities of viable conidia resulting in >50% infection from exposure to bands in the field for up to 119 days. How do conidia remain viable for such extended periods? Conidia on band surfaces are produced from cultures of entomopathogenic fungi growing within the bands. Sporulated cadavers of fungus-killed insects also contain cultures of the entomopathogenic fungi that killed hosts and sporulated externally on cadavers. Sporulating cadavers have been shown to provide a persistent fungal reservoir in the field for numerous fungi (e.g., Sprenkel and Brooks, 1977; Daoust and Pereira, 1986; Thomas et al., 1996; Sawyer et al., 1997). Conidia of *B. bassiana* on cadavers of curculionid and chrysomelid pests of cowpeas remained viable for at least 16 weeks (=112 days), with only some loss by 24 weeks (=168 days) when stored outside in protected conditions (Daoust and Pereira, 1986). For sporulating cadavers unprotected from sun and rain, most *B. bassiana* conidia were lost within 2 weeks, although for conidia remaining on cadavers no decline in viability was seen (Daoust and Pereira, 1986). This trend of decreasing densities of conidia on cadavers across time but only limited decrease in viability of remaining conidia was also seen in our study. Field longevity of conidia that have been harvested from mycelium can also retain viability for extended periods if stored under specific conditions, e.g., dry and often at lower temperatures. Jaronski (1997) demonstrated that the fungal isolate can greatly influence longevity of conidia. While conidia from some fungal strains do not survive for very long, if maintained under specific temperatures and moisture levels, 50% of conidia of *B. bassiana* GHA sprayed on undersides of cantaloupe leaves in the fields can survive an average of 533 days (Meikle et al., 2003).

Over time in the field during our study, conidial densities declined on bands but proportion germination did not decline. We hypothesize that conidial densities decrease as conidia are blown, washed and abraded from the surfaces of bands. Higuchi et al. (1997; Fig. 10b) found that for bands of *B. brongniartii*, periodic moistening, as with rainfall, resulted in resurgences in the densities of conidia on bands. In our study we documented increases in conidial densities across time, especially in 2003 when conidial increases were correlated with rainfall. A study of the persistence of activity of *B. brongniartii* fungal bands demonstrated a slight resurgence in conidial densities between days 6 and 9, which was hypothetically associated with rainfall (Xu et al., 2003). However, studies of *M. anisopliae* and *B. bassiana* fungal bands in China did not detect resurgence in numbers of conidia on bands over 119 days (Xu, 2002). The potential for resurgence of conidia on fungal bands in the field clearly merits further study.

The pathogenicity and virulence of *M. anisopliae* F 52 against adult *A. glabripennis* have been verified through both dipping and walking bioassays (Hajek et al., 2007; Shanley, 2007). Our study utilized a contact bioassay, designed to emulate the acquisition of infection from adults walking on fungal bands, to estimate the median lethal concentration for *M. anisopliae* F 52 fungal bands against *A. glabripennis*. The calculated median lethal concentration of 3.08×10^6 viable conidia per cm^2 for *M. anisopliae* F 52 fungal bands from lab bioassays with fungal bands is fairly consistent with the LC_{50}s calculated from other fungal pathogen/tree-boring cerambycid systems. In addition, Tsutsumi (1998) found no significant difference in *P. hilaris* longevity regardless of whether the beetles walked on the fungal bands for 5 or 60 s. Likewise, Dubois et al. (2004b) found no significant difference in *A. glabripennis* longevity when walking on *M. anisopliae* fungal bands for 5 versus 25 s (although a significant difference in longevity was found for one of the two strains of *B. brongniartii* tested). Tsutsumi and Yamanaka (1995) found that adult *P. hilaris* walking on *B. brongniartii* fungal bands containing 10^8, 10^6 and 10^5 conidia/cm^2 acquired around 10^7, 10^5 and 10^4 conidia/beetle, respectively. After allowing adult *M. alternatus* to walk on *B. brongniartii* fungal bands containing *B. bassiana* "for more than 5 cm," Shimazu (2004) found 8.5×10^5 conidia/individual. Thus, for numerous cerambycid/fungal pathogen combinations, adults walking on fungal bands for relatively brief periods can acquire large numbers of conidia.

Table 5
Associations of natural log-transformed changes in densities of viable conidia on fungal bands with average temperature and rainfall and the proportion of days with rain (mean ± SE) during sampling intervals.

Fungal species and strain	Starting date	Average daily temperature (°C) (min–max range)	Average daily rain (cm) (min–max range)	Proportion rainy days (min–max range)	Significant correlations with r_t
Metarhizium anisopliae ARSEF 7711	10 August 2004	13.6 ± 1.9 (5.9–23.8)	0.33 ± 0.09 (0.04–0.93)	0.23 + 0.03 (0.14–0.43)	Avg temp/d: $\rho = -0.65564$ $P = 0.0552$
Metarhizium anisopliae ATCC 62176	30 June 2003	20.5 ± 0.9 (11.0–26.5)	0.31 ± 0.10 (0.00–0.97)	0.37 ± 0.07 (0.07–0.93)	Avg rain/d: $\rho = 0.59244$ $P = 0.0329$
Metarhizium anisopliae ARSEF 7234	18–19 July 2002	25.0 ± 0.9 (20.4–28.1)	0.39 ± 0.22 (0.00–2.07)	0.21 ± 0.06 (0.00–0.43)	Not significant
Beauveria bassiana ARSEF 6393	18–19 July 2002	24.7 ± 0.8 (20.4–28.1)	0.36 ± 0.20 (0.00–2.07)	0.19 ± 0.06 (0.00–0.43)	Prop. days rain: $\rho = -0.81869$ $P = 0.0038$
Beauveria brongniartii NBL 851[a]	27 July 2001 and 18 August 2001[a]	24.6 ± 0.9 (20.0–28.3)	0.18 ± 0.06 (0.00–0.45)	0.26 ± 0.06 (0.00–0.50)	Prop. days rain: $\rho = -0.73502$ $P = 0.0378$

[a] Data for bands in the field during these two periods in 2001 are merged for analysis.

Our results suggest that different fungal isolates and species may differ in persistence of densities of viable conidia on fungal bands. In most cases, direct comparisons of the decline in conidial densities over time among isolate * year combinations were not possible. For the one instance where bands made with different fungal isolates were in the field at the same time (in 2002), densities of viable conidia on *B. bassiana* bands declined more quickly than *M. anisopliae* bands although *B. bassiana* bands also started with higher densities. Whether these different initial conidial densities are characteristic of fungal isolates deserves further study.

Across trials there was no general correlation of changes in conidial density with average temperature, average rainfall or rainfall frequency, although for individual isolate * year combinations significant associations were found (Table 5). Fungal bands in this study were not protected from rainfall, especially rain that flowed down tree trunks. For three of the five isolate * year combinations, rainfall frequency or rainfall amount were correlated with changes in density of conidia but correlations seem somewhat contradictory when comparing across years. For 2001 and 2002, rainfall frequency was negatively correlated with changes in conidial density while for 2003, rainfall amount was positively correlated with changes in conidial density. These results clearly indicate that more study is necessary to understand relationships between fungal bands and rainfall.

Non-woven bands impregnated with cultures of entomopathogenic fungi provide a potential alternative or addition to use of systemic insecticides for the control of *A. glabripennis*. The strategy of using fungal bands is based on the premise that, especially during the preoviposition feeding period, when *A. glabripennis* adults walk on tree trunks and branches, they could contact a fungal band and become infected. Adult females may also be exposed when they walk on branches and trunks to lay eggs, often with a male guarding them. Although the pathogenicity and virulence of conidia in fungal bands has been well documented for *A. glabripennis* (Dubois, 2003; Dubois et al., 2004a,b; Hajek et al., 2006; Shanley, 2007), questions remain regarding the required number of applications of fungal bands per year in infested areas. We now suggest that *M. anisopliae* F 52 bands probably only need to be hung on trees one time each year because they can remain active for at least 112 days.

Acknowledgments

Field studies in Queens would have been impossible without the assistance of Matt Saucerman and Frank Buccello as well as Ming Hua Huang, Branislav Simic, and Michael Singho, all from the New York State Department of Agriculture and Markets. Field samples were evaluated at Cornell by Liande Li, Jim McNeil, Katie Poole, and Alison Burke and data were summarized by Sana Gardescu. Technical assistance was generously provided during laboratory bioassays by Sana Gardescu, Joshua Hannam, Rebecca Lomachinsky, James Reilly, and Alice Vandel. We thank J. Rennells of the Northeast Regional Climate Center at Cornell University for providing weather data. Francoise Vermeylen, David Saville, Patrick Tobin and Frank Rinkevich assisted with analyses. We thank two anonymous reviewers for their helpful comments. Field studies were funded by the New York State Department of Agriculture and Markets, and both field and laboratory bioassays would not have been possible without the *A. glabripennis* colony supported by the Alphawood Foundation.

References

Daoust, R.A., Pereira, R.M., 1986. Survival of *Beauveria bassiana* (Deuteromycetes: Moniliales) conidia on cadavers of cowpea pests stored outdoors and in laboratory in Brazil. Environmental Entomology 15, 642–647.

Dubois, T.L.M., 2003. Biological control of the Asian longhorned beetle, *Anoplophora glabripennis*, with entomopathogenic fungi. Ph.D. dissertation. Cornell University, Ithaca, New York, 200 p. Available from: University Microfilms, Ann Arbor, MI 3104525.

Dubois, T., Hajek, A.E., Jiafu, H., Li, Z., 2004a. Evaluating the efficiency 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., Hajek, A.E., Smith, S., 2002. Methods for rearing the Asian longhorned beetle (Coleoptera: Cerambycidae) on artificial diet. Annals of the Entomological Society of America 95, 223–230.

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

Dubois, T., Lund, J., Bauer, L.S., Hajek, A.E., 2008. Virulence of entomopathogenic hypocrealean fungi infecting *Anoplophora glabripennis*. BioControl 53, 517–529.

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

Haack, R.A., Bauer, L.S., Gao, R.-T., McCarthy, J.J., Miller, D.L., Petrice, T.R., Poland, T.M., 2006. *Anoplophora glabripennis* within-tree distribution, seasonal development, and host suitability in China and Chicago. The Great Lakes Entomologist 39, 169–183.

Hajek, A., Dubois, T., Lund, J., Smith, M., Bauer, L., Zengzhi, L., 2007. Developing fungal bands for control of Asian longhorned beetle, *Anoplophora glabripennis*, in the US. Journal of Anhui Agricultural University 34, 149–156.

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

Hajek, A.E., Lund, J., Smith, M.T., 2008. Reduction in fitness of female Asian longhorned beetle (*Anoplophora glabripennis*) infected with *Metarhizium anisopliae*. Journal of Invertebrate Pathology 98, 198–205.

Higuchi, T., Takeshi, S., Shuji, 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.

Hu, J., Angeli, S., Schuetz, S., Luo, Y., Hajek, A.E., 2009. Ecology and management of exotic and endemic *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Agricultural and Forest Entomology, in press.

Iba, M., 1993. Studies on the ecology and control of the yellowspotted longicorn beetle, *Psacothea hilaris* Pascoe (Coleoptera; Cerambycidae), in the mulberry fields. Bulletin of the National Institute of Sericulture and Entomological Science 81, 1–119.

Jaronski, S.T., 1997. New paradigms in formulating mycoinsecticides. In: Goss, R.G., Hopkinson, M.J., Collins, H.M. (Eds.), Pesticide Formulations and Applications Systems, vol. 17. ASTM STP 1328, American Society for Testing Materials, West Conshohocken, PA, pp. 99–112.

Keena, M.A., 2005. Pourable artificial diet for rearing *Anoplophora glabripennis* (Coleoptera: Cerambycidae) and methods to optimize larval survival and synchronize development. Annals of the Entomological Society of America 98, 536–547.

Keena, M.A., 2006. Effects of temperature on *Anoplophora glabripennis* (Coleoptera: Cerambycidae) adult survival, reproduction, and egg hatch. Environmental Entomology 34, 912–921.

Matsuura, K., Fukui, K., Adachi, T., 1997. The effect of the nonwoven fabric sheet with an entomogenous fungus *Beauveria brongniartii* against the yellow-spotted longicorn beetle, *Psacothea hilaris* and the mulberry borer, *Apronia japonica* on the fig field. Bulletin of the Hyogo Prefectural Agricultural Institute. Agriculture Section 45, 45–48.

Meikle, W.G., Jaronski, S.T., Mercadier, G., Quimby, P.C., 2003. A distributed delay routine-based simulation model of *B. bassiana* conidial stability in response to environmental stressors. BioControl 48, 561–578.

Ninomiya, Y., Higuchi, T., 1998. Development of biorational pest control formulation against longicorn beetles using a fungus *Beauveria brongniartii* (Sacc.) Petch. In: Proceedings of the Society for Invertebrate Pathology, Sapporo, Japan, pp. 199–202.

Nowak, D.J., Pasek, J.E., Sequeira, R.A., Crane, D.E., Mastro, V.C., 2001. Potential effect of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) on urban trees in the United States. Journal of Economic Entomology 94, 116–122.

Poland, T.M., Haack, R.A., Petrice, T.R., 2001. Evaluation of systemic insecticides to control *Anoplophora glabripennis* (Coleoptera: Cerambycidae). In: Fosbroke S.C.L., Gottschalk, K.W. (Eds.), US Department of Agriculture (USDA) Forest Service General Technical Report NE-285, pp. 105–106.

Poland, T.M., Haack, R.A., Petrice, T.R., Miller, D.L., Bauer, L.S., 2006. Laboratory evaluation of the toxicity of systemic insecticides for control of *Anoplophora glabripennis* and *Plectrodera scalator* (Coleoptera: Cerambycidae). Journal of Economic Entomology 99, 85–93.

SAS Institute, 2004. SAS/STAT 9.1 User's Guide. SAS, Cary, North Carolina.

Sawyer, A.J., Ramos, M.E., Poprawski, T.J., Soper, R.S., Carruthers, R.I., 1997. Seasonal patterns of cadaver persistence and sporulation by the fungal pathogen *Entomophaga grylli* (Fresenius) Batko (Entomophthorales: Entomophthoraceae) infecting *Camnula pellucida* (Scudder) (Orthoptera: Acrididae). Memoirs of the Entomological Society of Canada 171, 355–374.

Shanley, R.P., 2007. Using non-woven bands impregnated with the entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) for biological control of the Asian Longhorned Beetle, *Anoplophora glabripennis* (Motschulsky) (MS thesis). Cornell University, Ithaca, New York, 55 pp.

Shibata, E., Higuchi, T., 1988. Application of an entomogenous fungus. *Beauveria brongniartii* (Sacc.) Petch, for control of the adult sugi borer, *Semanotus japonicus* Lacordaire (Coleoptera: Cerambycidae). Applied Entomology and Zoology 23, 199–201.

Shibata, E., Yoneda, Y., Higuchi, T., Ishinose, H., Yamada, N., 1991. Control method of the adult sugi bark borer, *Semanotus japonicus* Lacordaire (Coleoptera: Cerambycidae), using the nonwoven fabric sheet with an entomogenous fungus. *Beauveria brongniartii* (Sacc.) Petch, in Japanese cedar. Applied Entomology and Zoology 26, 587–590.

Shimazu, M., 2004. A novel technique to inoculate conidia of entomopathogenic fungi and its application for investigation of susceptibility of the Japanese pine sawyer, *Monochamus alternatus*, to *Beauveria bassiana*. Applied Entomology and Zoology 39, 485–490.

Shimazu, M., 2009. Use of microbes for control of *Monochamus alternatus*, vector of the invasive pinewood nematode. In: Hajek, A.E., Glare, T.R., O'Callaghan, M. (Eds.), Use of Microbes for Control and Eradication of Invasive Arthropods. Springer, Dordrecht, Netherlands, pp. 141–157.

Shimazu, M., Takatsuka, J., 2006. Factors affecting poor mycelial growth on cadavers of *Monochamus alternatus* infected with an entomopathogenic fungus, *Beauveria bassiana*. Bulletin of the Forestry and Forest Products Research Institute 5, 235–242.

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

Sokal, R.R., Rohlf, F.J., 1981. Biometry, second ed. W.H. Freeman and Co., San Francisco.

Sprenkel, R.K., Brooks, W.M., 1977. Winter survival of the entomogenous fungus *Nomuraea rileyi* in North Carolina. Journal of Invertebrate Pathology 29, 262–266.

Starr, H., 1997. Probit-Logit Analysis, version 2.0. Praxeme RandD, Montpellier, France.

Thomas, M.B., Gbongboui, C., Lomer, C.J., 1996. Between-season survival of the grasshopper pathogen *Metarhizium anisopliae* in the Sahel. Biocontrol Science and Technology 6, 569–573.

Tsutsumi, T., 1998. Microbial control of longicorn beetles using non-woven fabric sheet containing, *Beauveria brongniartii* in the orchard. In: Proceedings of the VIIth International Colloquium on Invertebrate Pathology and Microbial Control, August 23–28, Sapporo, Japan, pp. 203–209.

Tsutsumi, R., Yamanaka, M., 1995. Sexual transmission of entomogenous fungus, *Beauveria brongniartii* GSES, between yellow spotted longicorn beetle *Psacothea hilaris* adults by mating behavior. Japanese Journal of Applied Entomology and Zoology 39, 267–269.

USDA APHIS (United States Department of Agriculture, Animal Plant Health Inspection Service). 2007. USDA treats New York and New Jersey trees against Asian Longhorned Beetle. United States Department of Agriculture, Animal and Plant Health Inspection Service Newsroom (May 3). <http://www.aphis.usda.gov/newsroom/content/2007/05/albNY.shtml> (accessed 16.05.08).

Xu, J.Z., 2002. Study on application of entomogenous fungi against *Monochamus alternatus* and *Anoplophora glabripennis*. (MS thesis). Anhui Agricultural University, Hefei, Anhui, China.

Xu, J.Z., Fan, M.Z., Li, Z.Z., 2003. Comparative studies of different ways of releasing *Beauveria brongniartii* against Asian longhorned beetles. Chinese Journal of Biological Control 19 (1), 27–30.

Zhang, B., Liu, Y., 1996. Experiment report on the infection of *Beauveria bassiana* and *B. brongniartii* to *Anoplophora glabripennis*. Ningxia Forest Protect Research 2, 1–4.