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Field studies of control of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) using fiber bands containing the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria brongniartii*

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Abstract

The Asian longhorned beetle, *Anoplophora glabripennis*, was first found attacking urban street trees in the United States in 1996 and in Canada in 2003. This tree-killing invasive insect has long been a major pest in China and is difficult to control because immature stages live within wood and long-lived adults are often located high in tree canopies. A microbial control product (Biolisa Kamikiri) consisting of non-woven fiber bands impregnated with cultures of an entomopathogenic fungus, *Beauveria brongniartii*, is marketed in Japan for control of a congeneric orchard pest. Replicated field trials were conducted in Anhui, China to compare Biolisa Kamikiri with similarly prepared bands containing *Metarhizium anisopliae* for control of *A. glabripennis*. One fungal band was placed at 2–2.5 m height, around the stem or major scaffold branch on each of 40 willow trees (*Salix* spp.) per plot, with five plots for each fungal treatment and five control plots. Adult beetles collected from fungal-treated plots 7–22 days after bands were attached to trees died faster than adults from control plots. Beetles exposed to *B. brongniartii* bands consistently died faster than controls throughout this period, while results from plots with *M. anisopliae* bands were not as consistent in differing from controls. Numbers of adult beetles from plots of each fungal species dying in <10 days were greater than controls (16% of beetles) but did not differ between fungal treatments (34–35%). Oviposition in fungal-treated plots was approximately half that in control plots. Locations of adult beetles and oviposition scars within tree canopies were quantified to determine optimal locations for band placement. Most adult beetles were found >3.5-m high in trees, with adults in *B. brongniartii*-treated plots higher within trees than adults in other plots.

Keywords: *Cerambycidae,* Anoplophora glabripennis, *Asian longhorned beetle,* Beauveria brongniartii, Metarhizium anisopliae, *infection, urban forestry*

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Introduction

Non-woven fiber bands impregnated with fungal cultures and attached around tree trunks have been investigated extensively for control of cerambycids in orchards in Japan (e.g., Shimazu et al. 1995; Tsutsumi & Yamanaka 1996; Higuchi et al. 1997; Matsuura et al. 1997). This approach proved to be efficacious and a product named Biolisa Kamikiri, containing *Beauveria brongniartii* (Sacc.) Petch, was developed and has been on the market since 1996 (Higuchi et al. 1997). This product is specifically sold for control of several major pests, including the yellow-spotted longicorn beetle, *Psacothea hilaris* (Pascoe), and the citrus longhorned beetle, *Anoplophora chinensis* (Förster) (=*A. malasiaca* (Thomson)), but is also effective against three other species of cerambycids, the mulberry borer, *Apriona japonica* Thomson, the udo longicorn beetle, *Acalolepta luxuriosa* Bates, and the sugi bark borer, *Semanotus japonicus* Lacordaire. Adults die from fungal infection after exposure to fungal bands but, before dying, can also contaminate mates (Tsutsumi & Yamanaka 1995).

Anoplophora glabripennis Motschulsky (Asian longhorned beetle; ALB) was first discovered in North America in New York in 1996 (Haack et al. 1996) and this discovery was followed by detection in Chicago, Illinois in 1998 (Poland et al. 1998), New Jersey in 2002 (B. Emens, pers. comm.), Toronto, Canada in 2003 (J. Bell, pers. comm.) and a second site in New Jersey in 2004 (B. Emens, pers. comm.). Cerambycids, including *A. glabripennis*, are difficult pests to control because larvae live within wood for relatively long periods and long-lived adults emerge asynchronously and are often high in tree canopies. Based on the severity of problems due to *A. glabripennis* in China where this species is native, eradication programs for elimination of *A. glabripennis* from North America have been intensive. Once infested trees are detected, they are soon felled and chipped and, as a preventive measure, many remaining trees in target areas have been treated with imidacloprid (Merit 75WP for soil injections and Imicide for trunk injections).

The biology of *A. glabripennis* is fairly typical of lamiine cerambycids (Hanks 1999). Lamiine cerambycids often have a preoviposition period during which they feed while becoming reproductively mature after eclosion (=the prematuration period). Smith et al. (2002) evaluated the age-specific fecundity of *A. glabripennis* on three tree species and reported an average prematuration period of 10.6, 16.7, and 15.8 days on Norway maple (*Acer platanoides* L.), red maple (*Acer rubrum* L.) and black willow (*Salix nigra* Marshall), respectively. Both during and after this prematurational period, these large-bodied beetles that do not readily fly often walk on tree trunks and throughout the tree canopy. The goal of using fungal-impregnated bands is to have beetles contact bands during prematurational wandering, while they are maturing and before females begin laying eggs. Such early exposures would result in death of adults, reduction in oviposition, and potential spread of inoculum to conspecifics.

Investigations of the use of fungal-impregnated bands to control *A. glabripennis* began in the laboratory in 1999 and continued with caged and non-caged trials in the field in China (Dubois 2003; Dubois et al. 2004a,b). Throughout these studies, emphasis was on the *B. brongniartii*-based commercial product. However, because *B. brongniartii* could not be confirmed as native to the US (R.A. Humber, pers. comm.), we assumed that registration of this fungal species for pest control in the US could be more difficult. During bioassays and caged studies, additional fungal species and strains had been compared with *B. brongniartii* and results demonstrated that isolates of *Metarhizium anisopliae* could be efficacious against *A. glabripennis* adults

(Dubois 2003). The present study was conducted, in part, to compare non-woven fiber bands containing cultures of *M. anisopliae* versus *B. brongniartii* in the field.

In Japan, band placement is dependent on the cerambycid species being targeted. *A. chinensis* lays eggs under tree bark near the ground, so to control this beetle, orchard managers attach fungal bands around lower tree trunks (Kashio & Ujiie 1988; Hashimoto et al. 1989). The behavior of *P. hilaris* differs from *A. chinensis* because this species lays eggs higher in the tree and bands to control *P. hilaris* are hung from branch crotches within the tree canopy (Tsutsumi & Yamada 1991). Detailed studies of oviposition site preference in *A. glabripennis* have not been conducted but beetles are known to oviposit under the bark of tree trunks as well as in branches of various sizes (A.E.H. & M.T.S., unpubl. data). In the present study, fungal bands were always attached mid-way within the tree canopy but locations and activities of adult beetles were always recorded to begin to understand locations for optimal placement of bands.

In this study, our overall goal was to compare the efficacy of two fungal isolates applied in fiber bands attached to trees. We also sought to quantify adult behaviors in treated and untreated plots. We compared the efficacy of commercially prepared *B. brongniartii*-impregnated bands with laboratory-produced non-woven fiber bands impregnated with cultures of *M. anisopliae* for infecting and killing ALB adults. Fungal bands were attached to trees and *A. glabripennis* adults were subsequently collected and reared. Evidence of beetle emergence and oviposition and aspects of adult behavior were also monitored in treatment versus control plots.

Materials and methods

Study site

Studies were conducted along ca. 2 km of Shengli Road (20 m wide) in Bengbu, northern Anhui Province, in central eastern China. The predominant tree species, *Salix matsudana* Koidz., was interplanted with *Salix babylonica* L., four to five trees deep on both sides of the road, between the road and adjacent fields. Trees were 14.9 ± 0.7 cm (mean $\pm$ SE; $n = 600$ trees) diameter at breast height and a representative sample of 30 trees averaged 8.5 ± 0.4 m in height (range: 4.3–14.5 m). Trees were spaced 4.8 ± 1.4 m apart (average of 45 randomly chosen pairs of trees) and canopies from adjacent trees usually overlapped. These trees had been planted 16 years before this study, which was conducted in 2002. The *A. glabripennis* population had been developing in the study trees for some years before this study, with an average of 822.9 ± 224.1 oviposition scars at < 3.5 m in height per 10-tree plot.

Fungal bands

Bands impregnated with *Beauveria brongniartii* (Saccardo) Petch and *Metarhizium anisopliae* (Metchnikoff) Sorokin were compared in these studies. The *B. brongniartii* strain was originally isolated from *P. hilaris* in Japan (NBL 851) and was grown in 50×500 mm nonwoven wood pulp bands by Nitto Denko (Osaka, Japan), as described by Higuchi et al. (1997). The *M. anisopliae* strain (VD 1; ARSEF 7234) was originally isolated from an *A. glabripennis* adult that had developed in wood from an infested tree from Chicago and emerged in the USDA, Forest Service quarantine facility in Ansonia, CT. Bioassays with this fungal isolate had shown that it is virulent against *A. glabripennis* (Dubois 2003; A.E.H. & J. Lund, unpubl. data).

M. anisopliae bands were produced in Anhui Agricultural University using polyester as the nonwoven fiber material (5.3 ± 2.3 mm thick, based on 13 representative measurements), with band production procedures described in detail in Dubois (2003) and summarized in Dubois et al. (2004b). Basically, fungal cultures are grown in liquid media, non-woven fiber material (a textile similar to uncompressed felt, with numerous uses including batting for quilts) cut into bands is then soaked in the suspension of fungal cells, the bands are laid on racks at high humidity and the fungus grows throughout the bands and produces conidia on the surfaces. *M. anisopliae* bands were the same dimensions as *B. brongniartii* bands. Using methods for quantifying conidial densities in bands developed by Higuchi et al. (1997), *B. brongniartii* and *M. anisopliae* bands initially contained $3.03 \times 10^8 \pm 0.16 \times 10^8$ and $1.24 \times 10^8 \pm 0.27 \times 10^8$ conidia/mm^2, respectively. One band was attached to each treated tree at either 2 or 2.5 m height, based on the location of major branching from the trunk; $89.7 \pm 3.2\%$ of bands were attached at 2.5 m and the remainder were attached at 2.0 m. Bands completely encircled either the trunk or a main branch, with $77.7 \pm 5.0\%$ of bands around major branches instead of trunks because scaffold branches diverged from the trunk at an average of 203.9 ± 3.3 cm height. Trees had from two to 10 major scaffold branches with an average of 3.6 ± 0.1. The lengths of bands encircling the trunk or main branch averaged 39.6 ± 2.2 cm (range: $13-105$ cm). Bands were attached to trees between 7 and 12 July, 2002 in five plots for each fungal treatment and no bands were attached in the five control plots.

Study plots and data collection

Fifteen plots of 40 trees each were established along either side of the tree-lined road. The three treatments (*B. brongniartii* bands, *M. anisopliae* bands and controls) were randomly assigned to five plots for each treatment. Adjacent plots were separated by $23-100$ m (mean $\pm$ SE; 49.0 ± 6.7 m). Five plots were directly across the road from an additional five plots and were separated by 20 m of asphalt. Thirty trees within the center of each plot were only used for collection of adult beetles and associated data and five trees on either side of each plot were only used for collection of oviposition and emergence data. Therefore, adult beetle data were not collected from peripheral trees that were climbed to count oviposition scars and exit holes. Throughout the study, markings were not made on trees to avoid impacting beetle behavior (e.g., host or mate finding).

Sampling was extremely time-consuming so sampling was organized so that one of three groups of plots was sampled on one day. Consecutive sampling resulted in $2-3$-day intervals between samplings for the different groups, but all groups were sampled with 5-day intervals between sample dates for that group.

From 23 to 29 July, 300.3 mm of rain fell (50.1 ± 25.6 mm/day during this period) resulting in flooding that prevented data collection from 29 July to 1 August. The study was terminated on $19-20$ August because the numbers of adult beetles had decreased to an average of 2.3 ± 0.4 beetles collected per plot per sample date. After bands had been attached to trees, 12 of the 15 plots were sampled for a total of 42 days and three of the plots were sampled for 37 days.

Adult beetle location, behavior and rearing. Adult beetles were collected from within plots 3 days before bands were attached, 2 days after bands were attached, and thereafter

at 5-day intervals for the duration of the study. Sampling was conducted between 08:00–12:00 and 14:00–18:00 h. At the time of collection, the following adult beetle data were recorded: (1) height above ground level within tree, with adults >3.5 m height all being recorded as >3.5 m, (2) gender, and (3) activity at the time of collection (stationary, walking, male on female, or ovipositing). Two samplers spent approximately 2 h attempting to collect at least 10 adult beetles from each plot on each sampling date for collecting adults. A maximum of 13 beetles was collected once in one plot, but usually fewer than 10 beetles were found; collections of ≥ 10 beetles per plot on one sample date occurred in only five instances. On each adult sampling date, each tree in a plot was observed from the ground and from a 2-m ladder, to detect adult beetles. Once seen, adults were collected by hand from the tree or by knocking them from trees with a 3.5-m bamboo pole. Samplers were able to reach all beetles using combinations of ladders, climbing and bamboo poles. The shade-loving *A. glabripennis* adults do not readily fly and, throughout the course of the study, beetles that were seen rarely flew away. Collected adults were kept individually in 650 mL polypropylene containers (10.9 cm diam. $\times 8.1$ cm height) with clear lids punched with three holes for ventilation, at $25°C$ and approximately 16:8 (L:D). Adults were provided with fresh *Salix* twigs for food every 2 days and were monitored daily for death for up to 75 days after collection. After death, beetle cadavers were placed in cups with non-ventilated lids and a saturated cotton plug to provide moisture for outgrowth of fungi, which was then recorded.

Oviposition scars and exit holes. As external indications of the presence of *A. glabripennis*, small oviposition scars are evident in the bark and, after eclosion, adults chew out of the tree leaving an exit hole. The numbers of oviposition scars and exit holes were quantified from the peripheral trees by examining tree trunks and branches to a height of 350 cm. These data were only recorded up to 350 cm above ground level because it was not possible to see all bark surfaces above that height and counts above 350 cm therefore could not be accurate. Counts were made 1–2 days before adults were collected and at 5-day intervals thereafter. When beetles and data were initially collected prior to hanging bands, all oviposition scars and exit holes that appeared to have already been made during the 2002 season and before were recorded separately. From that time forward, oviposition scars and exit holes were recorded at each 5-day interval and the numbers of oviposition scars and exit holes for each sampling date were calculated by subtracting numbers from the previous sampling periods.

Data analysis. Survival of the adult beetles collected and reared during this study provided a measure of the number of beetles contaminated with sufficient inoculum to initiate disease and mortality under the laboratory-caged conditions. Survival of each group of beetles collected on each sampling date was used to provide a snapshot of field survival, until a new group of beetles was collected which was then substituted, to represent survival over the next interval. Overall mortality of adult beetles collected during the study was calculated as the product of a series of interval survival rates (see Elkinton et al. 1992; Royama 2001).

   Counts of days to death for adult beetles that were collected from 2 to 37 days after bands were attached to trees were analyzed using a generalized linear mixed model (Proc GLIMMIX) with repeated measures and a negative binomial distribution. *Post hoc* comparisons were tested with least squares means (SAS Institute 2004). Days

to death for beetles collected during the pre-sample before bands were attached, were transformed using log+1 and compared using a general linear model. To compare days to death for male versus female beetles, individual dates were merged to achieve adequate sample sizes. Data were transformed to log +1 and analyzed using analysis of variance to test for effects of sex and treatment using the Bonferroni correction for *post hoc* tests. To compare percent beetles collected that died in <10 days across treatments, data were analyzed by logistic regression, accounting for overdispersion, using the GENMOD procedure (SAS Institute 2004).

To compare oviposition across treatments, data were totaled across dates and trees due to the low numbers of new oviposition scars and exit holes in the 10 trees from each plot. To sum oviposition scars by treatment, the first sample date after bands were attached to trees was not included because, even if females had been inoculated with conidia the first days that bands were attached to trees, any effects on oviposition would not have been seen only 2 days later (A.E.H. & J. Lund, unpubl. data). Therefore, the numbers of oviposition scars from the second sample after bands were attached to trees until the final sample were used for analyses. The count of total exit holes made in 2002 for each treatment was used to estimate the numbers of these long-lived beetles in trees. To estimate the numbers of females, numbers of exit holes were divided by 2, because previous studies demonstrated virtually equal numbers of males and females (Dubois et al. 2004b). The numbers of oviposition scars per female from controls were considered the expected number for this period and Chi-squared tests were used to compare observed oviposition in treatments with expected.

To begin to address questions about optimal locations for fungal bands, data for locations where adult beetles were collected were analyzed by three groups (tree boles and usually the first major junction of scaffold branches (<2.5 m), lower sections of scaffold branches (2.5–3.5 m) and smaller branches higher in the tree canopy (>3.5 m)) by treatment using Chi-squared tests. Preference of adult beetles for individual trees was analyzed using a variance:mean ratio to evaluate distribution in control trees (Pielou 1977).

Results

Adult density and mortality

Initial densities of adult beetles collected in plots before bands were attached to trees did not differ among plots (mean $\pm$SE $=6.9\pm0.7$ beetles per plot) ($F_{2,12}=0.26$; $P=0.7716$). After bands were attached to trees, an average of 38 ± 3 adults per plot (range: 28–66) were collected throughout this study (range of total adults collected per treatment: 178–210). By 19–20 August (37–42 days after bands had been attached to trees), the population of adult beetles had declined, with ≤2 adult beetles found in each of nine of the 15 plots (Figure 1), and sampling was discontinued. Across the study, the male:female ratio was nearly equal (52.8% females). However, earlier in the study, there were slightly more males (19–21 July and before $=51.4\%$ males) and after 21 July, the ratio shifted to more females (60.7%).

Mortality and days to death

Study-long mortality of the population differed among treatments, with the greatest mortality in plots where *B. brongniartii* bands were attached to trees and lowest
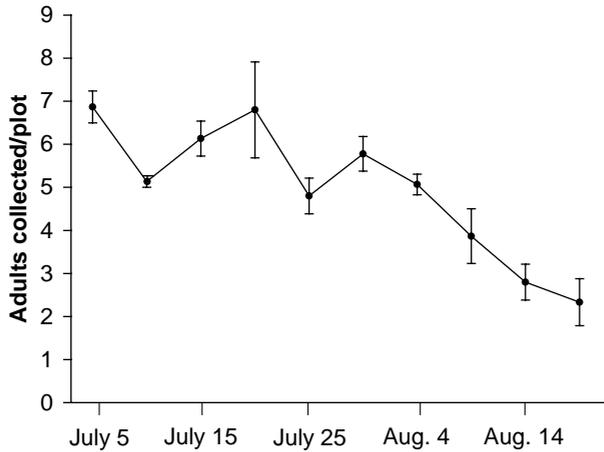
Figure 1. Seasonal dynamics of *Anoplophora glabripennis* adults found at 5-day intervals across three treatments (mean $\pm$ SE) in Bengbu, Anhui, China, 2002.

mortality among controls (Table I). Based on total population mortality, the numbers of males dying did not differ from females ($\chi^2 = 0.1802$; df $= 1$; $P = 0.6712$). Cadavers of adult beetles were rarely found in the field.

Days to death for beetles sampled before bands were attached to trees did not differ among plots ($F_{12,90} = 1.47$; $P = 0.1490$), with these adults living an average of 31.2 ± 1.5 days (range: 4–78 days) after collection (data not shown). Longevity of beetles differed significantly among treatments 7, 12, 17 and 22 days after bands were attached to trees (least squares means tests; all $P < 0.05$) (Figure 2). Throughout this time, adult beetles from *B. brongniartii*-treated plots died faster than controls. Days to death for adult ALB from *M. anisopliae*-treated plots did not differ from the *B. brongniartii* treatment but were lower than controls on days 7 and 22. On sample day 12, days to death for beetles from *M. anisopliae*-treated plots did not differ from *B. brongniartii*-treated plots ($t = -0.78$; $P = 0.4370$) and were nearly significantly shorter than controls ($t = 1.89$; $P = 0.0589$). On sample day 17, days to death for beetles from *M. anisopliae*-treated plots did not differ from controls ($t = 0.75$; $P = 0.4519$) and were significantly greater than *B. brongniartii*-treated plots ($t = -2.14$; $P = 0.0329$). For all treatments, days to death for males did not differ significantly from females either across all dates or considering only days 7–22 (ANOVA; $P > 0.05$) (data not shown).

Table I. Total percent population mortality during the study and mean percent mortality <10 days after collection of *Anoplophora glabripennis* adults in plots treated with fungal bands and in control plots in Bengbu, Anhui, China, 2002.[a, b]

Treatment	n	Total% population mortality[c]	Mean% mortality (95% limits) in <10 days
B. brongniartii NBL 851[d]	178	83.4 a	34.8 (21.6–46.5) a
Metarhizium anisopliae VD 1	210	60.8 a	34.1 (27.1–52.0) a
Control	189	32.1 b	16.2 (10.3–32.1) b

[a]Values followed by different letters are significantly different ($P < 0.05$). [b]Values are estimates based on field-collected insects reared under laboratory conditions. [c]Season-long mortality calculated as in Elkinton et al. (1992) and Royama (2001). Chi-squared tests used to compare percentages. [d]Commercial product Biolisa Kamikiri (Nitto Denko, Osaka, Japan).
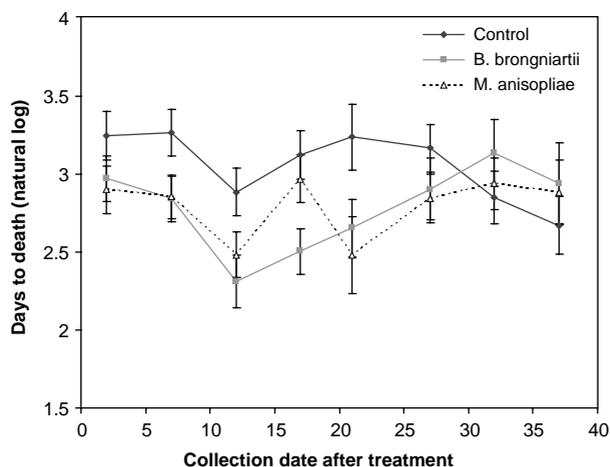
Figure 2. Days to death (natural logarithm) for adult *Anoplophora glabripennis* adults collected from 2 to 37 days after fungal impregnated bands were attached to trees, comparing *M. anisopliae* (VD 1) and *B. brongniartii* (NBL 851) with controls.

Investigating mortality in beetles collected from fungal-treated plots more closely, more beetles from the fungal treatments died in <10 days after collection compared with beetles from control plots ($F_{2,12} = 5.46$; $P = 0.025$), while numbers of beetles dying in <10 days did not differ between the two fungal treatments ($F_{1,8} = 0.35$; $P = 0.5682$) (Table I). Numbers of males dying in <10 days after collection did not differ from numbers of females in any treatment (χ^2-tests; $P > 0.05$) (data not shown).

For many ALB dying in fungal treatment plots, fungi did not grow out of resulting cadavers (Table II). However, by far the greatest numbers of cadavers with fungal outgrowth occurred in plots where bands of that specific fungal species had been applied. For beetles collected before bands were applied in the field, there was a consistent low percentage of cadavers producing fungal outgrowth after death (all plots <4%). After bands were attached to trees, low percentages of cadavers with outgrowth of other fungal species than the species released in those plots were found. However, instances of *M. anisopliae* outgrowth from beetles collected *in*

Table II. Species of fungi growing from cadavers of *Anoplophora glabripennis* reared after collection from treatment and control plots, Bengbu, Anhui, China, 2002.

Treatments	Total cadavers	No. (%) with *B. brongniartii*	No. (%) with *M. anisopliae*
		Fungal outgrowth	
Before bands were attached to trees			
B. brongniartii NBL851 (Biolisa Kamikiri)	40	1 (2.5%)	1 (2.5%)
Metarhizium anisopliae VD 1	35	1 (2.9%)	0 (0%)
Control	31	1 (3.1%)	1 (3.1%)
After bands were attached to trees			
B. brongniartii NBL851 (Biolisa Kamikiri)	178	48 (27.3%)	5 (2.8%)
Metarhizium anisopliae VD 1	210	8 (3.8%)	63 (30.0%)
Control	189	14 (7.3%)	7 (3.6%)

B. brongniartii-treated plots and vice versa, plus control cadavers with *M. anisopliae* outgrowth were all <4%. The exception was 7.3% of cadavers of beetles collected in control plots after bands had been attached to trees yielded outgrowth of *B. brongniartii*.

Oviposition by treatment

Initial counts of oviposition scars and exit holes did not differ significantly for the different treatments (Table III). Numbers of new oviposition scars declined through time and by days 37 and 42, no new oviposition scars were found in 13 and 11 of the 15 plots, respectively. The numbers of oviposition scars per female in both fungal treatments were approximately half those found in controls (χ^2-tests; $P < 0.05$) (Table III).

Adult location and behavior

In the control plots, adult beetles were found throughout the study on only 58% of the trees sampled for adults ($n = 150$ trees sampled 10 times during the study). The variance:mean ratio of 2.838 suggests that the distribution of adult beetles on individual trees was not random but aggregated among trees. Among those trees on which ALB adults were found ($n = 87$), beetles were still seldom seen; adults were observed only 1–3 times on 78.2% of the trees on which adult beetles were seen. Thus, there were relatively few trees where adults were repeatedly observed on the same tree, with numbers peaking at 10 and 12 beetles recorded across the study from two different trees. These two trees did not differ from others in size and architecture. For 41.4% of trees on which beetles were found, only one beetle was found on one sampling date on that tree and it was alone on the tree at that time. In the instances where more than one beetle was seen on a tree at the same time ($n = 34$ instances), it was most common to find a male and female on the same tree at the same time (70.6%).

Throughout the study, behavior of a total of 697 beetles was recorded. The majority of adults seen (total females $n = 376$; total males $n = 321$) were motionless on the tree (43.6% of females and 58.9% of males) while 18.1 and 11.2% of males and females,

Table III. Oviposition and eclosion for 10 trees/plot (means ±SE) for fungal treatment and control plots in Bengbu, Anhui, China, 2002.

	Initial population counts[a, b]		Throughout season
	Oviposition scars	Exit holes	Oviposition/female[c]
Beauveria brongniartii NBL 851[d]	3.4 ± 0.8 a	17.0 ± 3.4 a	10.2 a
Metarhizium anisopliae VD 1	2.2 ± 1.5 a	50.2 ± 24.5 a	10.4 a
Controls	1.4 ± 0.2 a	24.0 ± 4.4 a	20.0 b

[a]Initial populations quantified as pre-sample counts plus counts taken 2 days after bands were attached to trees, at which time fungal treatments would not yet have affected oviposition. [b]Log-transformed values for oviposition scars or exit holes per plot tested using a general linear model. [c]Calculated as total oviposition including and after the second sample date after bands were attached to trees, divided by the number of females in the plot, calculated as half of total exit holes throughout the sampling period. Data for plots were merged due to low numbers of exit holes in some plots. [d]Commercial product Biolisa Kamikiri (Nitto Denko, Osaka, Japan).

respectively, were walking on bark surfaces. Among females observed, 26.6% appeared to be laying eggs while about a fifth of males (21.8%) and females (18.6%) were either mating or males were near females, guarding them. After bands were attached, the number of adult females that appeared to be ovipositing ($n = 104$) and the number of females observed together with males ($n = 70$) did not differ between fungal treatment plots and controls (χ^2-tests; $P > 0.05$).

Vertical location of adults within trees did not differ by sex for any treatment (χ^2 tests; $P > 0.05$), so sexes were merged for further analyses. Throughout the study, 60.9% of the adult beetles observed were found at >3.5 m height within the tree canopy and only 1.8% of the total adults collected were found at <2 m. After bands were attached, adults collected in *B. brongniartii*-treated plots differed in distribution by height within trees compared with adults from both *M. anisopliae*-treated and control plots (χ^2-tests; $P < 0.05$) (Figure 3). In *B. brongniartii*-treated plots, the percentage of adults at >3.5 m height, was greater than in either *M. anisopliae*-treated plots or controls. In *B. brongniartii*-treated plots, more adult females appeared to be laying eggs at >3.5 m (61.8%, $n = 34$) compared with *M. anisopliae*-treated plots (21.9%, $n = 32$) or controls (35%, $n = 40$) ($\chi^2 = 11.8093$, df $= 4$, $P = 0.0188$). Greater proportions of adults were also seen with males and females together at >3.5 m in *B. brongniartii*-treated plots (76.5%, $n = 17$ pairs total) compared with *M. anisopliae*-treated plots (55.6%, $n = 27$ pairs) or controls (54.2%; $n = 24$ pairs), although differences were not statistically significant ($\chi^2 = 2.4878$, df $= 4$, $P = 0.2883$).

Counts of oviposition scars could not be made accurately at >3.5 m, but below this level, oviposition scars were always more abundant between 2.5 and 3.5 m in height (69.1% of total oviposition scars quantified) compared with <2.5 m (30.9%).

Discussion

Adult *A. glabripennis* collected 7–22 days after fungal bands were attached to trees died faster than adults collected in control plots. During this time interval, beetles
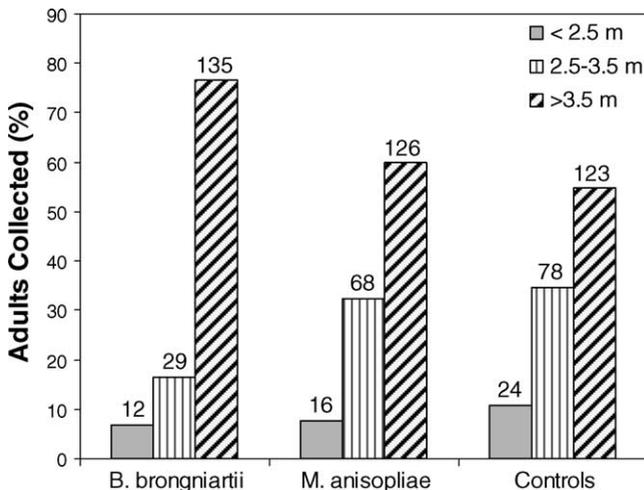

Figure 3. Distribution of *Anoplophora glabripennis* adults by height within canopies of trees banded with fungal bands versus controls in Bengbu, Anhui, China, 2002. Values above bars are numbers of individual beetles.

collected from plots with *B. brongniartii* (Biolisa Kamikiri) bands always died more quickly than beetles from controls, while beetles from plots treated with *M. anisopliae* bands died more quickly than controls on two of the four sample dates in this period. The initial conidial densities of the two types of bands differed, with *B. brongniartii* bands starting with >2.4 times greater conidial densities than *M. anisopliae* bands and we cannot say whether the differences in conidial densities on bands account for observed differences in mortality patterns. Both fungal treatments killed similar percentages of adults in <10 days of collection and these percentages were greater than controls. During studies in a quarantine facility in the US, the preovipositional period of *A. glabripennis* averaged 15.8 days on black willow, *S. nigra* (Smith et al. 2002). Hypothetically, if adult beetles emerge from trees and soon thereafter contact bands during their prematurational wandering, females could die from fungal infections before laying eggs. However, oviposition could still have occurred in fungal-treated plots under several scenarios. Some oviposition would still occur before females die from infections if females become infected, either from walking across bands or mating with inoculated males (Tsutsumi & Yamanaka 1995), later during their preovipositional period rather than soon after emergence. Also, oviposition could occur if reproductively mature females that had mated fly into the area. However, in these cases, females would still have been inoculated with fungal conidia and would die prematurely and not lay all their eggs. Laboratory bioassays (A.E.H. & J. Lund, unpubl. data) and caged studies in China (Dubois et al. 2004a) have documented that after a female becomes infected, the number of eggs laid before that female dies is reduced. In the present study, we found a population-level trend supporting these findings because after band placement, oviposition still occurred in fungal treatment plots but was substantially reduced compared with oviposition in control plots.

In a previous field trial comparing two *B. brongniartii* strains in China in 2001, adult beetles collected from fungal treatment plots also died faster than controls (Dubois et al. 2004b). However, fewer adults were present in the plots used in 2001 and these differences were not statistically significant. *M. anisopliae* bands were not included in 2001 field trials. *M. anisopliae* bands were included in the present study because no strains of *B. brongniartii* are registered for pest control in the US but strains of *M. anisopliae* have been registered, making it much easier for development of this species for use in the field. Both the 2001 study and the present study were also consistent in finding decreased oviposition in fungal-band treated plots (Dubois et al. 2004b).

One interesting result from this study was that adults were found higher in trees in plots treated with *B. brongniartii* compared with *M. anisopliae* or controls. *A. glabripennis* adults have large eyes and are thought to find mates in part using vision. *M. anisopliae* spores are green and *B. brongniartii* spores are white so these bands were different colours. Whether the different appearance of the two types of bands influenced beetle behavior is not known, although a study of *A. glabripennis* behavior has shown that adults were more attracted to green than white (J. Lund, pers. comm.). In addition, some species of insects are known to avoid fungal pathogens (e.g., Villani et al. 1994; Thompson & Brandenburg 2005) but there have been no previous reports documenting such behavior for *A. glabripennis*. Regardless of this differential distribution of beetles by height within trees, adult beetles collected from *B. brongniartii*-treated plots died significantly faster than beetles from *M. anisopliae*-treated plots on only one of four sample dates between 7 and 22 days after bands were

hung and died faster than beetles from control plots throughout this period. Whether adults are being repelled or deterred by *B. brongniartii* should be studied further to determine whether avoiding this effect would improve control using these bands or, conversely, whether this effect could be utilized to protect trees.

During this study, a disparity was seen between the percent mortality of adults from fungal treatment plots and the percent of resulting cadavers from which entomopathogenic fungi grew; fungal outgrowth was not seen from many cadavers of *A. glabripennis* adults exposed to fungal treatments. This syndrome has been reported previously for other lamiine cerambycids (Kawakami 1978; Shimazu 1994) and was also previously found with *A. glabripennis* both during laboratory bioassays and in the field (Dubois 2003). Shimazu (1994) has hypothesized that although fungal pathogens may kill cerambycids, after host death the fungus cannot always grow out through the thick cuticle and so no external fungal growth is evident on many cadavers. Therefore, while the fungus may not erupt from cadavers, the beetles could still have been killed by these fungal pathogens. While this hypothesis is consistent with our results, during future studies, soon after host death, dissection of cadavers or molecular analysis of cadaver contents could help to verify that fungal pathogens are causing host death.

Levels of fungal infection from cadavers of adults collected from controls suggest that (1) both the fungal species being studied occur naturally, (2) fungal spores are dispersing from treatment plots into controls or (3) infected beetles are flying from treatment plots to controls. We know that these fungal species are commonly occurring natural enemies attacking cerambycids (see Dubois 2003). Higuchi et al. (1997) suggested that spores originating from Biolisa Kamikiri bands did not abundantly disperse by wind from field sites, based on absence of infections in control sites 0.5 km from treated areas where high levels of infection were recorded. While *A. glabripennis* adults can fly distances up to 2644 m (Smith et al. 2001, 2004), when host trees are densely planted, as in this study, little dispersal by adult *A. glabripennis* occurs (Huang 1991; Huang & Zhou 1992). Therefore, the most likely hypothesis for explaining the fungal infection in beetles from control plots is that these are naturally occurring background levels of infection by native entomopathogenic fungi. However, to prove this we would need to differentiate fungal strains applied during this study from native strains, which would require molecular analyses (St. Leger & Joshi 1997).

The question arises as to whether bands are needed on each tree for control of ALB. In Japan, it is recommended that Biolisa Kamikiri bands should be placed on every tree within orchards being treated for control of *A. chinensis* and *P. hilaris*. During this study, bands were applied to each tree in groups of 40 trees, and adjacent trees of the same planting were untreated. The extent to which beetles from untreated areas dispersed into banded plots to mate and lay eggs as well as the extent to which beetles left the treatment plots are not known. While we cannot yet answer how densely bands must be applied for different levels of control, at present, application to each tree would be most prudent, especially if highly valued trees are densely planted. In addition, in this study the trunk was banded in some cases but for many trees, only one of several scaffold branches was banded. In depth studies of adult dispersal within a tree have not been conducted but it seems logical that placement of bands on each scaffold branch would improve the chances of contacting wandering adults.

A. glabripennis is known to lay eggs in small diameter branches as well as in tree trunks (A.E.H. & M.S., unpubl. data). During this study, oviposition by

A. glabripennis was greater at 2.5–3.5 m compared with <2.5 m and, unfortunately, we were unable to count oviposition scars at >3.5 m. This nontraditional methodology for application of entomopathogenic fungi in non-woven fiber bands was developed for control of *A. chinensis* and *P. hilaris*. *A. chinensis* lays eggs near the soil surface and this specific and restricted distribution of oviposition makes it is easy to target adults with bands (e.g., Kashio & Ujiie 1988; Hashimoto et al. 1989). However, Biolisa Kamikiri is also effective for control of *P. hilaris*, which lays its eggs higher in the tree (Tsutsumi & Yamada 1991). This study has shown that although *A. glabripennis* also lays eggs higher in the tree, bands attached to trees at 2–2.5 m height can provide some control. Future studies will investigate whether hanging bands higher would improve the efficacy of this method for applying entomopathogenic fungi for control of *A. glabripennis*. However, in addition, future studies should try to include larger trees that would be more representative of mature urban and forest trees in North America.

Seasonal dynamics of *A. glabripennis* populations in Anhui Province appear to differ from previously published reports from the more northern province of Gansu (Smith et al. 2001), where peak numbers of adults were found in mid-July. While the timing of the adult peak in Anhui is not known exactly for 2002 because studies did not start early enough, maximum numbers of beetles were present 4–6 July and 19–21 July. In both provinces, by 19–24 August, numbers of *A. glabripennis* adults were clearly declining. This information is pertinent to eradication efforts in North America, where *A. glabripennis* occurs on a south-north gradient, from Carteret, New Jersey to Toronto, Ontario, but in such low populations that phenology of adults is very difficult to study.

Acknowledgements

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