

Developing fungal bands for control of Asian longhorned beetle, *Anoplophora glabripennis*, in the U S *

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Abstract: An invasive long-horned beetle, *Anoplophora glabripennis*, was first reported in the northeastern and midwestern United States and eastern Canada between 1996 and 2004 and has been given the common name Asian longhorned beetle (ALB). This beetle has also been found in several countries in Europe. ALB is difficult to control because larvae are found within the wood of living trees and the long-lived adults often occur high in tree canopies. This species is native to China and Korea and, because it has been a major tree killer in China, government agencies in the U. S. and Canada are working to eradicate ALB from North America. Our laboratory has been developing a microbial control approach targeting ALB adults, based on the Japanese product Biolisa Kamikiri which is used to control cerambycids in orchards. Entomopathogenic fungi are grown within non-woven fiber bands (= fungal bands) and placed around tree trunks and branches where ALB adults become inoculated when walking across bands. We have conducted bioassays with *Beauveria brongniartii*, *Beauveria bassiana* and *Metarhizium anisopliae* against ALB larvae and adults to identify effective isolates and now focus our efforts on *M. anisopliae* F52 (ARSEF 7711). Caged field trials conducted in China to compare fungal sprays with fungal bands (2000, 2001) demonstrated decreased ALB longevity and fitness for both application methods but longer activity of fungi in cages treated with fungal bands compared with sprays. Uncaged field trials (2001, 2002) yielded faster ALB adult mortality in fungal-treated plots and decreased fitness. Studies in New York City testing the longevity of activity of fungal bands in the field have documented that bands retain $>1 \times 10^7$ conidia $\cdot \text{cm}^{-2}$ (the threshold for activity of Biolisa Kamikiri) for over 3 months. In contrast, studies with unformulated conidia sprayed onto tree trunks in New York documented conidial survival of only a few days. Sublethal effects of exposure of adult female ALB to fungal bands have been investigated further in the laboratory. After either newly eclosed or reproductively active females are exposed to fungal bands, few viable larvae are produced before death of the females. When females are exposed to fungal bands and then caged with males, males become infected.

Key words: microbial control; cerambycidae; wood borers; sublethal effects; non-woven fiber bands

CLC number: S476.12

Document code: A

Article ID: 1672-352X(2007)02-0149-08

1 Introduction

An invasive longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae) was first found in Brooklyn, New York in 1996 (Lingafelter and Hoebeke, 2002). This beetle has been given the common name Asian longhorned beetle, *Anoplophora glabripennis* (ALB), in the U. S. It is native to China

where it has been a major tree-killer, predominantly attacking poplars (*Populus* spp.) and willows (*Salix* spp.). In 1998, ALB was found in Chicago, Illinois and beetle populations were detected near the New Jersey border with New York State in 2002 and 2004 and in Toronto, Ontario in 2003 (Hajek, 2007). The pest has also been found in Austria, Germany and France in recent years. At all of the infested areas in North Ameri-

* 收稿日期: 2006-12-13

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ca, the principal tree species attacked by this polyphagous beetle are species of *Acer* (maple), a common tree genus occurring in U. S forests and also commonly grown in parks, along streets and around homes

ALB larvae are wood borers, feeding in the phloem-cambial region during the first few instars and later instars bore into the xylem. After adults emerge from trees, they spend a period of approximately 9 - 15 days feeding on twigs before they are reproductively mature (Keena, 2002; Smith et al, 2002). In the United States, most females have been observed laying eggs from July to early November (Haack et al, 1997). Adult females chew an egg niche and then turn around and lay an egg in the phloem-cambial area beneath the outer tree bark. It is thought that females may lay eggs in their natal tree and usually do not travel far from their natal trees to lay more eggs if appropriate host trees are nearby (Huang, 1991; Huang and Zhou, 1992). Adults typically begin attacking a tree by laying eggs in the upper trunk and branches, using wood as small as 3 - 4 cm in diameter. As tree crowns begin to die, adults lay eggs in the trunk and even in exposed tree roots (Haack et al, 1997). In the United States, ALB goes through one generation per year although in some areas of China these beetles can undergo 2 generations per year (Lingafelter and Hoebeke, 2002).

In 2001, Nowak et al. used data on trees growing in nine U. S. cities plus tree cover occurring nationally to estimate the potential effects of ALB on urban trees if this species became established. The maximum potential urban impact in the U. S. was estimated at 34.9% of canopy cover, 30.3% tree mortality and loss of \$669 billion (Nowak et al, 2001). Thus, all areas invaded by ALB in the United States have been the focus of intensive eradication programs costing the U. S. government approximately \$225 million between 1997 and 2006 (US, GAO 2006). ALB larvae are difficult to detect within trees and ALB adults are difficult to detect in tree canopies. No effective long-range pheromones to attract adults have been identified. Several means are used for controlling the low populations of ALB in the U. S. If an infested tree is detected, it is soon felled, chipped and then burned. Another control method is directed at control of adults using the synthetic chemical insecticide imidacloprid which is injected into tree trunks or into

the soil at the bases of trees. Between 2000 and 2004, more than 500 000 trees were treated by trunk or soil injection with imidacloprid (C. Markham, pers. comm.). However, some locations do not allow imidacloprid use or, in some cases, managers do not have access to trees that they think should be treated. It is also questionable whether adults feeding on imidacloprid-injected trees will receive a high enough dose when feeding on twigs because this insecticide is not transported evenly through tree canopies (Poland et al, 2006a) and can act as an antifeedant (Poland et al, 2006b).

Naturally occurring predators, parasitoids and pathogens have not been effective in controlling ALB in China. Fungal entomopathogens are commonly associated with cerambycids and the most common species are anamorphs of the Family Clavicipitaceae, Order Hypocreales (formerly known as Hyphomycetes) (e.g., Shimazu et al, 2002; Dubois, 2003; Haack, 2004). A product based on a fungal pathogen is sold for control of cerambycids in Japan. Non-woven fiber bands made from wood pulp and containing cultures of *Beauveria brongniartii* (Biolisa Kamikiri) are produced by Nitto Denko (Osaka, Japan) for control of *Anoplophora chinensis* (= *m. lasiaca*) and several other cerambycids that are pests in orchards (Higuchi et al, 1997). To make bands, fungal cultures are grown in increasing volumes of media. When the desired volume is achieved, agar is added to the suspension of fungal cells and band material cut to 50 × 500 mm is dipped into the suspension. Bands are then placed on racks at high humidity and the fungus is allowed to grow for approximately 1 week (Higuchi et al, 1997; Dubois et al, 2004a). Bands are ready when the fungus has grown throughout the loose fabric and outer surfaces are covered with conidia. One band per tree is placed around the tree trunk or branch and adult beetles inoculate themselves when walking on tree trunks. These large-bodied adult ALB are reluctant flyers (e.g., H. Bo, personal communication) and often walk on tree trunks and branches (Lance et al, 2003; Hajek et al, 2006; Morewood et al, 2004). This novel application method is advantageous because the fungal bands are localized and long-lived beetles inoculate themselves, hopefully during their pre-maturation period. Bands of *B. brongniartii* are active for at least one month (Higuchi et al, 1997;

Tsutsun i, 1998) and wood pulp bands are biodegradable (Higuchi et al, 1997).

Our studies have focused on application of fungal bands for control of ALB in North America. The goal of U. S. control programs is eradication of ALB. Methods for control of ALB in addition to systemic imidacloprid are clearly needed. This invasive pest occurs in urban areas in North America and the public often requests that biological control be used instead of chemical insecticides. Fungal bands that we have been developing address demand for developing a method for biological control.

2 Bioassays testing fungal entomopathogens for control of ALB

The first step in development of fungal bands was to conduct bioassays comparing susceptibility of larvae and adults and to compare virulence of different fungal isolates. Using field-collected insects in China, Dubois (2003) compared 4 isolates of *B. bassiana* (one of which is registered for control in the U. S.; GH-1) and the strain of *B. brongniartii* used in fungal bands by Nitto Denko (NBL 851). NBL 851 was originally isolated from the cerambycid *Psacothea hilaris* in Japan. Bioassays with adults and larvae were conducted by dipping insects into agitated conidial suspensions of 10^6 or 10^8 conidia \cdot mL⁻¹. Results were more consistent for adults than larvae; since the application method we planned targets adults and it is uncertain how to apply fungal pathogens to larvae that live within living trees, all further bioassays utilized adults only. Bioassays were continued and, in total, adult 'dipping' bioassays conducted by Dubois (2003) compared 9 isolates of *B. bassiana* (originating from North America and China), 3 isolates of *B. brongniartii* (from China and Japan) and one isolate of *Metarhizium anisopliae* (from China). All isolates of *B. brongniartii* killed adults quickly [LT₅₀s of 6.1 and 7.3 days for WU 20 (ARSEF 6827) and NBL 851, respectively]. In particular, LT₅₀s for strains of *B. bassiana* (VD 11; isolate lost and replaced with VD 12 isolated from the same host species and location) and *M. anisopliae* (VD 1; ARSEF 7234) isolated from ALB adults in the U. S. did not differ significantly from NBL 851.

Due to the superior performance of *B. brongniartii*

strains, we decided to continue studies with isolates of this fungal species from North America. Surprisingly, although we found 9 citations in the published literature and isolates in culture collections reporting *B. brongniartii* in North America we were unable to prove that any of these isolates were truly *B. brongniartii*. Thus, to use *B. brongniartii* NBL 851 in the United States, we would be using an exotic strain of a species that we could not confirm is native to the United States. Registering a microbial strain with the U. S. Environmental Protection Agency (EPA) so that it can be used as a biopesticide can take a long time and requires significant investment, even for native microbial strains. The prospect of being able to register *B. brongniartii* NBL 851 with the U. S. EPA seemed highly questionable. Therefore, we changed our focus and concentrated only on strains of *B. bassiana* and *M. anisopliae* instead of *B. brongniartii*.

Throughout this time, bioassays were difficult because adult ALB were often not readily available. We conducted studies to determine how to rear ALB on artificial diet and began a colony in the USDA, ARS quarantine on Cornell University campus (Dubois et al, 2002). Replicated bioassays comparing strains of *M. anisopliae* and *B. bassiana* isolated from ALB adults in the United States yielded average times to death of 8.2 and 14.5 days, respectively (unpublished data). Additional bioassays that were not replicated but utilized numerous strains of *B. bassiana* (4 strains) and *M. anisopliae* (8 strains) showed a consistent trend of faster mortality with the strains of *M. anisopliae* we tested.

Next, we compared a Chinese strain of *M. anisopliae* isolated from ALB that had been proven virulent in previous studies (VD 1) with two strains of *M. anisopliae* already registered with the US EPA (ESC 1 from EcoScience and F 52 from Earth Biosciences). Days to death for adults were 5.6 ± 0.3 (mean + SE) for the Chinese strain VD 1, 5.8 ± 0.3 for F 52 and 6.6 ± 0.5 for ESC 1 (unpublished data). No company was producing ESC 1 and registration for this strain with EPA had lapsed. In contrast, a company (Earth Biosciences) was producing F 52 and registration for this strain was up to date. Therefore, since 2004 *M. anisopliae* F 52 (ARSEF 7711) has been the focus of our field studies in China.

3 Field trials with fungal bands

Methods for evaluating fungal bands for control of ALB in the field can be difficult (general methods are described in Hajek and Bauer, 2007). Studies have all been conducted in China because ALB populations are far too low in the U. S. and, whenever an infested tree is located, it is cut down, chipped and burned soon thereafter. In China, it can be very difficult to locate existing ALB populations that are dense enough for our studies because heavily infested stands are often cut down. Some of the areas with consistently high ALB population are located in desert and semi-desert regions and we have avoided working in these areas; we are testing fungal entomopathogens that require moisture to be active and this pathogen would be used in areas of the United States that are not arid so it only makes sense to conduct studies in non-arid regions of China.

We conducted studies in the field in China in 2000, 2001 and 2002. In 2000, ALB adults were placed in window screening cages surrounding 1 m long sections of boles of willow trees (Dubois et al, 2004a). Two commercially available fungi, *B. brongniartii* NBL 851 and *B. bassiana* GHA were sprayed on tree trunks within cages or bands containing cultures of these fungi were placed around trunks within cages. Both fungi killed adults more quickly than controls and NBL 851 killed adults more quickly than GHA, although application method had no effect on time to death. Oviposition scars per cage and oviposition rate per female were reduced for both fungal strains, demonstrating a sublethal effect of fungal infection. When adults were removed from cages after 10 days, conidial viability was still high on bands but was drastically reduced for conidia sprayed onto tree trunks.

Caged studies were repeated in 2001, comparing fungal bands of *B. brongniartii* NBL 851 with bands of *B. brongniartii* (WU 20; ARSEF 6827) from China and *B. bassiana* (VD 12; ARSEF 6393) and *M. anisopliae* VD 1 isolated from ALB in North America. Field temperatures were higher during 2001 (range of maxima: 30.2 - 38.5°C) than 2000 (range of maxima: 30.1 - 33.2°C) and mean humidities were lower (2001: 74.0% - 89.0% RH, 2000: 60.0% - 80.0%). Longevity differed between sexes with females living longer

than males. Treated males died more quickly than controls only for *B. brongniartii* NBL 851 and treated females died more quickly than controls only for *B. bassiana* VD 12 and *M. anisopliae* VD 1. A small study of survival of fungal bands was also conducted. When beetles were exposed to bands that had already been hanging on trees for 15 days, under these hot and dry conditions no fungal band treatments killed adults more quickly than controls.

In 2001, uncaged studies using fungal bands were also conducted in China but, due to difficulties in finding sites with abundant enough host populations, treatments were conducted at only two sites, which had very different population densities (Dubois et al, 2004b). Each site included three separated treatment areas, each composed of 100 trees. Fungal strains that were compared were *B. brongniartii* NBL 851 and *B. brongniartii* WU 20. Adults collected in the areas with *B. brongniartii* WU 20 bands died more quickly than adults collected in areas with *B. brongniartii* NBL 851 bands or controls. At the site with higher ALB populations, a sublethal effect was seen once more, with decreased oviposition in fungal band treatments compared with controls. The decrease in oviposition occurred 5 days earlier for adults collected from *B. brongniartii* NBL 851-treated trees compared with *B. brongniartii* WU 20-treated trees.

In 2002, uncaged field studies were conducted at a site with higher host populations over a larger area than the 2001 study. Five replicate plots of 40 trees per treatment were used to compare three treatments: bands of *Beauveria brongniartii* NBL 851, *Metarhizium anisopliae* VD 1 and controls (Hajek et al, 2006). Adult beetles were collected every 5 days from plots and reared to determine time to death. Adults collected from fungal-treated plots 7 - 22 days after bands were hung died more quickly than controls although results were more consistent for the *B. brongniartii* NBL-851 treatment. Numbers of adults dying in less than 10 days in fungal treated plots (33% - 34%) were greater than in control plots (16%). Once again, an effect on reproduction was recorded. Oviposition in fungal-treated plots was significantly lower than in control plots.

During field studies, smaller investigations have been conducted to evaluate behavior of beetles relative

to fungal bands Adult longevity decreased when adults walked on *B. brongniartii* NBL 851 bands for 5 seconds and for 25 seconds on *B. brongniartii* WU 20 and *M. anisopliae* VD 1 (Dubois et al, 2004b). In the 2001 field site, ALB were usually located > 3.5 m high in trees but adults in plots with *B. brongniartii* NBL 851-treated trees occurred higher in trees than adults in plots with *M. anisopliae* VD1 and controls (Hajek et al, 2006).

4 Fungal bands and sprays

Fungal bands (unpublished data). In 2001, Dubois et al (2004a) found that conidial densities on bands of different fungal strains varied significantly based on fungal strain Directly after production, bands of *B. brongniartii* WU 20 and *M. anisopliae* VD 1 were covered with fewer conidia than bands of *B. brongniartii* NBL 851 and *B. bassiana* VD 12 Conidial densities on bands of all strains on bands decreased over 15 days in the field Conidial densities decreased fastest on *B. brongniartii* NBL 851 bands and slowest on *M. anisopliae* VD 1 and *B. bassiana* VD 12 bands After 15 days, conidial densities of all bands were much lower than *B. bassiana* VD 12 bands (Dubois et al, 2004a).

To evaluate the length of time that fungal bands were retain activity against ALB adults, studies were conducted using mature trees in Calvary Cemetery, Queens, New York City Bands were placed around trunks of trees at 2.5 - 3.5 m in height in the shade with one band per tree At varying intervals, three randomly chosen bands were removed from tree trunks and shipped to Cornell University for evaluation At Cornell, replicates of 25 cm² pieces of band were used to count the total number of conidia and the germinability of these conidia to determine the density of viable conidia Based on the Higuchi et al (1997) study, we assumed that the threshold conidial density for bands that would kill adult cerambycids walking over them is 1×10^7 conidia · cm⁻².

In 2001, we began field trials 27 July and 18 August, using *B. brongniartii* NBL 851 but, due to the 9/11/01 disaster, the steady sampling that we had planned was interrupted (unpublished data). Therefore, we placed another set of bands in the field on 22 October, very late in relation to the normal ALB adult field sea-

son The first replicate ended 33 days after bands had been hung in trees but, even at this time, bands contained 1×10^8 conidia · cm⁻². The second replicate ended after 47 days on trees (in October), still having 5 times the threshold density of conidia We concluded sampling the 22 October trial after 58 days The conidial density had dropped below the activity threshold in early December but this is extremely late in the season and ALB adults would not be active in the field at this time At the same time that densities of viable conidia were evaluated, bioassays with bands were conducted using adult beetles in our quarantine colony These bioassay data confirmed band activity throughout the study but, because we did not always have large numbers of adults available, sample sizes were sometimes low and were variable among dates, making analysis of bioassay data difficult

We followed the same protocol for studies in 2002 but used different fungal strains, as we were at that time questioning whether *B. brongniartii* is native to North America Using the same study area, 30 bands of *B. bassiana* VD 12 and *M. anisopliae* VD 1 were placed on tree trunks on 18 - 19 July Bands were sampled a total of 63 and 77 times for *M. anisopliae* VD 1 and *B. bassiana* VD 12, respectively Throughout this time, densities of viable conidia never dropped below the activity threshold of 1×10^7 conidia · cm⁻². For each sample date, between 6 - 9 adults were exposed to pieces of bands Days to death for beetles censored at ≤ 21 days averaged 12.6 ± 0.7 for *M. anisopliae* VD 1 and 11.1 ± 0.8 for *B. bassiana* VD 12 An average of $95.6\% \pm 2.5\%$ of adults exposed to *M. anisopliae* VD 1 died before 21 days while only $56.5\% \pm 8.8\%$ of adults exposed to *B. bassiana* VD 12 died before 21 days

In 2003, as we realized how expensive and time-consuming it would be for the isolates we were using to be registered with the U. S. EPA, we decided to focus on strains that were registered already In addition, since the conidia on bands were remaining dense and viable for the periods of time we previously were sampling, we extended sampling to cover almost 3 months The bands used in 2003 were made with *M. anisopliae* ESC 1 Bands were placed on trees June 30 and were sampled a total of 13 times, with this study ending after 119 days on 27 October Densities of viable conidia on bands were

$8.6 \times 10^7 \pm 7.7 \times 10^6$ per cm^2 when hung on trees and $2.5 \times 10^7 \pm 3.5 \times 10^6$ after 119 days. Thus, after 3 months in the field, conidial densities were still above the action threshold. Bioassays at day 0 yielded 100% adult ALB mortality in ≤ 30 days, with an average of 9.9 ± 0.3 days to death. After 119 days in the field, all adults exposed to bands still died in < 30 days, with an average of 14.7 ± 1.5 days to death.

Our method for estimating the number of viable conidia per band involves blending the entire band and then counting with a hemocytometer and plating to determine percent germination. Thus we were mixing the conidia positioned on the band next to the tree with conidia on the outside of the band. We evaluated whether the side of the band (i.e., toward the tree trunk or away from the trunk) differed in the density of viable conidia using bioassay. We exposed adults to different sides of pieces of bands. We found that adults exposed to the 'tree side' of bands died in 12.8 ± 0.5 days ($n = 44$; 100.0% mortality in < 30 days) while adults exposed to 'exposed side' of bands died in 13.9 ± 1.0 ($n = 28$; 96.4% mortality in < 30 days), which suggests that the conidia were surviving equally well on both sides of the bands.

In 2004, the same protocol was used to evaluate bands made with *M. anisopliae* F 52. The company producing the non-woven fiber band material that we had been using was discontinuing this product ($203.4 \text{ g} \cdot \text{m}^{-2}$; Poly-fil Ultraft Batting, 100% polyester, Fairfield Processing Company, Danbury Ct.). We could not find a replacement that was a similar weight so we settled on a thinner material ($135.6 \text{ g} \cdot \text{m}^{-2}$; Soft and Bright, The Warm Company, Seattle, WA). Bands were grown using each type of material for comparison. Due to problems with growing bands ourselves, viable bands were placed on trees a little later than we considered optimal, 10 August. We evaluated densities of germinable conidia 10 times for thicker material and 5 times for thinner material, leaving bands on trees for 112 days total. Bands began the experiment with $1.55 \times 10^8 \pm 1.62 \times 10^7$ conidia $\cdot \text{cm}^{-2}$ (thick bands) and $1.45 \times 10^8 \pm 1.57 \times 10^7$ conidia $\cdot \text{cm}^{-2}$ (thin bands). After 112 days in the field, thick bands had $2.0 \times 10^7 \pm 2.26 \times 10^6$ conidia $\cdot \text{cm}^{-2}$ while on thin bands, conidial densities were only slightly less ($9.1 \times 10^6 \pm 3.95 \times 10^6$ conidia $\cdot \text{cm}^{-2}$).

cm^{-2}).

Sprays The federal agency controlling decisions regarding methods for control of ALB, the USDA Animal and Plant Health Inspection Service (APHIS), was never happy about having to use ladders to climb into trees to place bands in trees. USDA, APHIS repeatedly requested that we investigate spraying fungal conidia on tree trunks. I repeatedly explained to them that the longevity of band activity far surpasses the length of time the conidia would survive on tree bark. Nevertheless, due to their continued insistence that they were not equipped to climb trees to hang bands but could spray trees easily, in 2004, we conducted a study to evaluate the length of time that sprays of *M. anisopliae* F 52 survived on mature Norway maples (*Acer platanoides*) and sugar maples (*Acer saccharum*). On 6 - 9 August, 2005 1.5 m long sections of tree trunk plus adjacent leaves, twigs and branches were sprayed with an emulsifiable concentrate of *M. anisopliae* F 52. Areas of trees that were sprayed were always the lowest completely shaded area within the tree canopy which was approximately 2.9 - 5.4 m high. Samples were taken before spraying, directly after spraying and on 5 - 6 later dates, up to 18 days after the spray. To sample, a drill with a 1.25 inch (3.18 cm) diameter bit was used to take circular samples of smooth, thin bark and thick trunk bark from each cardinal direction. Samples were also taken of leaves and twigs and soil beneath trees. Standard procedures were used to plate samples on drosophila selective media (Sneh, 1991) without benomyl. Conidia deposited from our initial sprays (maximum = 1.9×10^5 conidia $\cdot \text{cm}^{-2}$) were much less dense than conidial densities on fungal bands. Regardless of the species of tree or the type of sample, densities of viable conidia plummeted after the spray date so that by 2 days later $< 5\%$ of the initial concentration was present (unpublished data). Our conclusions from this study were that *M. anisopliae* F 52 conidia do not survive very long when sprayed into the tree canopy and onto tree bark, even if these substrates are shaded.

5 Indirect effects of exposure to bands

As mentioned above, during field trials, we found a sublethal effect of fungal infection (Dubois et al, 2004a; b; Hajek et al, 2006); before females died from

fungal infections, oviposition decreased significantly. To investigate this further, we asked how many surviving larvae a female could produce before dying from a fungal infection. Studies were conducted both with females that had only recently melanized and were therefore in their pre-maturation feeding period (= 'new' females), and with females that had already been laying eggs for a while (= 'mature' females). New females that were inoculated with fungal spores on average produced < 1 larva that survived for 4 weeks after fungal exposure. Mature females that were already laying eggs when inoculated produced 4.5 ± 1.3 surviving larva after fungal exposure while controls produced an average of 9.2 ± 0.5 surviving larvae.

Previous studies of citrus longhorned beetle, *Anoplophora malasiaca*, in Japan showed that when adults were exposed to entomopathogenic fungi, they could pass spores to mates (Tsutsumi, 1998). We conducted a similar study by exposing females to fungal bands (*M. anisopliae* F 52) and then caging males with females. This study was conducted with both 'new' females and males and mature females and males. Males caged with treated females died long before control males caged with untreated females and this result was consistent for both ages of beetles.

6 Conclusions

We have identified fungal isolates virulent to ALB adults, including an isolate that is already registered with the U. S. EPA and is being mass-produced by industry (*M. anisopliae* F 52). We have tested fungal bands in the field in New York City and found that densities of gemmable conidia on bands remained above the threshold of 1×10^7 conidia $\cdot \text{cm}^{-2}$ for at least 112 days. Lastly, our caged and uncaged field studies in China demonstrated both faster mortality of adult beetles and reduced reproduction in fungal band-treated plots. Importantly, fungal bands can affect beetles that don't walk across them. When an adult beetle walks across a fungal band, she can carry fungal spores to infect a mate and she will reproduce very little before dying. We are presently also investigating the extent to which inoculated adults move conidia onto the tree bark.

Why are fungal bands not applied on trees in the U. S. ?

1. In the U. S. , the trees infested with ALB are often much larger than the average infested trees available for our studies in China. Based on our experience in China, we therefore cannot answer the important question of how many bands must be hung on each tree. USDA, APHIS, the governmental organization in charge of ALB eradication programs, worries that with only one band per tree, chances are too low that all adult beetles will contact bands (and since the goal for control is eradication it is important that at least most beetles become infected). In addition, because adults are often high in the tree canopy, it is difficult to put bands at all of the locations where adult ALB might spend most of their time. In response to this criticism, we are working on applying a host attractant with fungal bands to attract adult beetles to bands. Presently, several scientists are working on a long-distance host attractant and we plan to use their most effective compounds that have been identified for our studies.

2. USDA, APHIS has been injecting trees and the soil at the bases of trees with imidacloprid for control of ALB adults. USDA, APHIS has developed this methodology and is reluctant to have to climb trees to apply bands, especially if they have to later climb trees again to remove bands. Bands used in Japan are made from wood pulp and therefore are biodegradable and thus do not need to be removed. We have not been able to find non-woven needle-punched fiber bands made of biodegradable products in the U. S. , but we will continue looking for such a product.

3. Lastly, at present there are no North American industries growing fungal bands. We need to continue discussion with industry so that fungal bands will be available from industry if USDA, APHIS wants to order them. In addition, USDA, APHIS is of course interested in how much use of fungal bands would cost both in terms of purchasing the bands and time to apply them. In response, the longevity of activity of fungal bands makes them extremely attractive for control of the long-lived adult ALB; many alternative control options would not retain activity for the entire field season, as fungal bands will.

The general attitude of USDA, APHIS to use of fungal bands for control of ALB in the U. S. is positive but the issues and questions posed above need to be ad-

dressed before fungal bands will be ordered by USDA, APHIS and hung in trees for ALB control in the U. S.

Acknowledgments: We thank the many people who have assisted with studies including in particular Peng Fan, Huang Bo, Fan Meizhen and Hu Jiafu of Anhui Agricultural University and Micheal Wheeler, Alison Burke, and Ryan Shanley of Cornell University. We thank Nitto Denko, Jarrod Leland (USDA, Agricultural Research Service) and Earth Biosciences for supplying fungal bands. This research was funded by the Alphawood Foundation, the Millstein Litwin Foundation, and the USDA, Agricultural Research Service, the USDA, Forest Service and the New York State Department of Agriculture and Markets.

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