CHAPTER VII-10

Microbial Control of Wood-Boring Insects Attacking Forest and Shade Trees

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1 INTRODUCTION

Wood-boring insect pests that feed on the bark, phloem, or xylem (wood) of living trees pose unique management challenges because their immature stages live in cryptic, often inaccessible, habitats within host trees. The eggs of wood borers are laid in or on tree trunks, branches, terminal shoots, or roots. After the eggs hatch, neonates tunnel in and feed on internal target tissues, making infestation both difficult and expensive to detect and control. Adult wood borers typically emerge from the tree to feed, disperse, mate, and oviposit, thus occupying very different habitats and behaviors than immature stages. Both immature and adult wood borers can be relatively long-lived.

The majority of wood-boring insect species that attack live trees are in the order Coleoptera (beetles), with fewer in Lepidoptera (moths) and Hymenoptera (horntails and sawflies), and still fewer in Diptera (flies) (Solomon, 1995). In this chapter, we will review those species for which microbial control has been attempted to control insects that feed in tree bark, phloem, or xylem (wood) of living trees. We will predominantly cover wood borers attacking above-ground woody parts of trees growing in forests or as shade trees or windbreaks, etc. Wood-borers that principally attack orchard trees or woody plants in nurseries are covered elsewhere in this volume (Chapters VII-11, VII-12, VII-13 and VII-16). Insect pests feeding on bark or wood of roots are covered in chapters on citrus trees (VII-13) and woody plants in nurseries (VII-16).

The wood-boring insects covered in this chapter include native species that outbreak periodically in numerous types of habitats: 1) in forested areas due to natural abiotic and biotic conditions such as drought or forest maturation; 2) in monocultures such as fiber farms, tree plantations, nurseries and windbreaks; and 3) in trees under stress after transplantation along streets, in parks, in other urban or suburban environments. We will also review the management of invasive wood-boring beetles, which often become pests after their inadvertent movement between countries in solid-wood packing materials used to transport goods and/or in commodities such as bonsai trees, nursery stock, wooden products, logs, and lumber. The increased movement of non-native wood-boring insects among countries is one consequence of our global economy and threatens the sustainability of forests throughout the world (Chornesky *et al.*, 2005). Between 1985 and 2005, at least 25 exotic species of wood borers were

found to have become established in the continental United States, including two species of Buprestidae (metallic wood-boring beetles), five Cerambycidae (long-horned beetles), and 18 Scolytinae (formerly Scolytidae) (bark beetles) (Haack, 2006). Due to the lack of both natural enemies and coevolved tree-resistance mechanisms to control these borers, these exotic species may become destructive and uncontrollable invasive tree pests. One example is the European woodwasp, *Sirex noctilio* (Hymenoptera: Siricidae), which has become a serious pest in each country to which it has been introduced. For example, in the Australian 'Green Triangle' area, *S. noctilio* killed ca. 4.8 million pine trees in 1987-1989 alone (Bedding and Iede, 2005). Another example is the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), discovered in North America in 2002 (Haack *et al.,* 2002). In Michigan, land managers estimate that larvae of this phloem-feeding beetle have killed more than 20 million ash (*Fraxinus* spp.) trees to date.

Control of wood borers by spraying synthetic chemical insecticides was made more difficult and less effective when chlorinated hydrocarbons, with extended residual persistence, were taken off the market in many countries. Methods to control or eradicate wood-boring insects using conventional materials are still being developed, but the newest methods usually involve application of systemic insecticides, such as imidacloprid through direct trunk injection, soil injection, or soil drench. This chemical moves within trees to reach cryptic larvae as well as affecting adults of some species that feed externally. The desired level of control, however, is seldom achieved by this approach due to unpredictable translocation of insecticides within trees (Poland *et al.*, 2006) and asynchronous larval development, both of which allow many insects to evade treatment. Moreover, chemical insecticides are usually broadly toxic, resulting in risks to non-target organisms and potential groundwater contamination. Many chemical insecticides and formulations require handling by licensed applicators resulting in prohibitive costs. The use of conventional insecticides to control insect borers, therefore, is restricted to a relatively small number of high-value urban trees or periodic borer outbreaks in tree plantations or nurseries and, unfortunately, few management options are available for borers attacking trees in forested and riparian areas. Management of borers in such environmentally-sensitive areas is thus more amenable to microbial control agents (MCAs), which are generally accepted by the public, biodegradable, compatible with other management strategies such as biological control, and which can provide adequate control in these systems with relatively high damage thresholds.

Wood-boring insect pests for which microbial control methods have been developed predominantly occur in the Orders Coleoptera and Lepidoptera (Table 1). This chapter will cover augmentative strategies for control of these pests. Major classical biological control programs have been undertaken to introduce the parasitic nematode *Deladenus* (= *Beddingia*) *siricidicola* for permanent control of *S. noctilio*. This nematode has been introduced and become established in 8 geographic areas (Hajek *et al.*, 2005) in some cases providing excellent long-term control. Classical biological control using *D. siricidicola* is described briefly in Chapter VI-1 and in more detail by Bedding and Iede (2005).

Many types of pathogens and nematodes (see Sections IV and V) are known to naturally infect and parasitize insect pests that bore in wood (Fuxa *et al.*, 1998). However, only some of these species demonstrate a large enough impact to justify

TABLE 1. Major wood-boring pests attacking shade and forest trees for which control using entomopathogens has been evaluated in the field.

Order Family	Pest species, Common name	Country where microbial control investigated	MCA[1]
Order Coleoptera			
Cerambycidae	*Anoplophora glabripennis*, Asian longhorned beetle	China, U.S.	F, N
	Monochamus alternatus, Japanese pine sawyer	Japan, China	F, N
	Plectrodera scalator, Cottonwood borer	U.S.	F
Buprestidae	*Agrilus planipennis*, Emerald ash borer	U.S.	F, B
	Melanophila decastigma, Ten-blotched poplar flatheaded borer	China	N
Curculionidae	*Cryptorhynchus lapathi*, Poplar-and-willow borer	Italy	N
	Hylobius abietis, Large pine weevil	Europe	N
	Ips typographus, European spruce bark beetle	Germany	F
	Scolytus scolytus, European elm bark beetle	United Kingdom	N
Order Lepidoptera			
Cossidae	*Arbela baibarana*, Acacia pseudo carpenter moth	China	F
	Cossus cossus, Willow goat moth	China	N
	Holcocerus insularis, Carpenterworm	China	N
	Prionoxystus robiniae, Carpenterworm	U.S.	B, N
	Zeuzera multistrigata, Leopard moth	China	F, N
Sesiidae	*Paranthrene robiniae*, Western poplar clearwing	U.S.	N
	Paranthrene simulans, Oak clearwing borer	U.S.	B
	Paranthrene tabaniformis, Dusky clearwing	Italy, Poland	F, N
	Podosesia aureocincta, Banded ash clearwing	U.S.	N
	Podosesia syringae, Lilac borer	U.S.	N
	Synanthedon culiciformis, Large red-belted clearwing	U.S.	N
	Synanthedon resplendens, Sycamore borer	U.S.	N
	Synanthedon scitula, Dogwood borer	U.S.	N
Pyralidae	*Euzophera batangensis*, Persimmon bark borer	China	F, N
	Hypsipyla grandella, Mahogany shoot borer	China, Cuba, Honduras, India, Mexico	B, F, N

[1]F = Fungus; N = Nematode; B = Bacterium.

development as MCAs. In other cases, MCAs are developed when preliminary laboratory, greenhouse and field studies demonstrate high levels of mortality. The majority of microbes investigated and utilized as MCAs for control of wood borers are insect-pathogenic fungi, nematodes, and the bacterium, *Bacillus thuringiensis (Bt)*.

Pestiferous borers in their native range seldom kill large healthy trees after initial attacks. However, after repeated attacks, either during the same season, as for scolytines, or during subsequent seasons, as for cerambycids and buprestids, even healthy trees may succumb. Some species are better known for attacking and killing

trees that are already stressed, such as over-mature trees that might already be declining or urban trees exposed to air pollution and with roots stressed by soil compaction and inadequate water and nutrients. The behavior and location of the destructive stages of wood borers, in part, determine which MCAs are most appropriate. Often, the immature stage of a wood borer causes the most damage to live trees due to their feeding on the bark, phloem-cambial region, and/or xylem. In most species of scolytines and buprestids, this stage remains just beneath the tree bark within the phloem-cambial region. In many cerambycids, the immature stages begin feeding just under the bark, and later instars move into the xylem where they are less accessible by MCAs. The larvae of some wood-boring pests, such as cerambycids and buprestids, are solitary, with one immature per gallery, thus limiting horizontal transmission of MCAs. Transmission between individuals is also limited by the oviposition behavior of many bark beetle species that oviposit in a central maternal gallery, and after eclosion, neonate larvae tunnel away from the main gallery as they feed on the phloem and cambium. A greater chance of contact among individuals occurs for some species of Cossidae (carpenterworms), where several larvae share the same gallery. Another factor affecting MCA development for wood borers is the prolonged larval stage, especially for larger wood borers or those predominantly living in the xylem. These larval stages can be long-lived, with some species of wood borers taking several years to develop from egg to adult. In addition, some wood-borer species are quite host specific, while others can have broad host ranges.

Investigations toward development of MCAs begin with laboratory bioassays to determine microbe pathogenicity, followed by comparisons of virulence among different microbial isolates or strains. Thus, many species of wood borers have been challenged with different pathogens and parasitic nematodes in the laboratory. Due to the complexity of larval and adult wood borer habitat and biology, however, laboratory bioassays only demonstrate pathogenicity and virulence under idealized circumstances and cannot reflect effectiveness of MCAs in the field. Therefore, in this chapter we will focus on describing methods and systems for which use of pathogens and nematodes has progressed past laboratory bioassays to field trials evaluating efficacy and developing application methods. Microbial control methods for Coleoptera and Lepidoptera will be surveyed below, followed by in-depth descriptions of methods for microbial control agents to control the Asian longhorned beetle (*Anoplophora glabripennis*) and the emerald ash borer (*A. planipennis*) as case studies.

2 BARK- and WOOD-BORING INSECT PESTS TARGETED WITH MICROBIAL CONTROL

To date, the major groups of pathogens developed for control of wood borers are the fungi and nematodes. Both of these groups of microbes can infect without being eaten by hosts, which is advantageous because applying microbes that require ingestion to infect (*e.g.*, viruses and bacteria) is not appropriate for many wood-borer larvae. For example, late-instar Asian longhorned beetle larvae feed in galleries deep within tree trunks, while the long-lived adults emerge and feed on petioles throughout tree canopies for several months. These characteristics make it difficult for many MCAs to survive long enough or be delivered at an effective rate for ingestion by either larvae or adults.

However, *Bt* has been most successfully evaluated against larvae of some wood-boring Lepidoptera.

Different MCAs can be more appropriate for life stages in different habitats. Species of entomopathogenic nematodes that search for hosts and can remain alive while partially desiccated for some time are more often used to target borer stages living within wood. For applications against wood-boring larvae, cruiser nematodes that disperse to find cryptic hosts within galleries may prove efficacious, although researchers suggest that ambusher nematodes may also prove effective in larval galleries of host species that move in and out of the gallery to feed and expel frass (*e.g*., some cerambycids). In contrast, conidia of fungal pathogens have also often been applied against wood-borer adults or larval stages. Throughout studies conducted in different host/pathogen systems, emphasis has been placed on development of application technologies that are system specific as well as both effective and economically feasible for land managers.

Fungi developed for control of beetles are all conidial fungi (anamorphs = asexual states) now classified in the Family Clavicipitaceae (Order Hypocreales) but previously listed in the Class Hyphomycetes of the Division Deuteromycota (a taxonomic group presently being abandoned as the phylogenetic affinities of so many conidial fungi become known). The predominant species that have been developed into MCAs are *Beauveria bassiana*, *Beauveria brongniartii* and *Metarhizium anisopliae*.

Nematodes that have been investigated for control of wood-boring Coleoptera and Lepidoptera belong to the Order Rhabditida. Most work has been done with species within the genus *Steinernema* (Family Steinernematidae) although in a few instances species of *Heterorhabditis* (Family Heterorhabditidae) have also been investigated, predominantly in the laboratory.

A Coleoptera (Cerambycidae, Buprestidae, Curculionidae)

1. General biologies

Beetles are the most economically important order of insects attacking trees. Within the Coleoptera, species in the families Buprestidae, Cerambycidae, Curculionidae and its Subfamily Scolytinae, are by far the most destructive wood borers. MCAs have targeted species in all of these beetle groups. Larvae of bark beetles and buprestids remain in the phloem-cambial region just below tree bark and kill trees by mass-attacking and girdling them. Larvae of cerambycids often feed just under the bark for some time after which some species bore extensively into the wood; these feeding zones within trees vary by species. Biologies of these families are described in more detail in Solomon (1995).

2. Control with entomopathogenic fungi

a. Japanese pine sawyer. Usually the activity of cerambycids alone kills or weakens trees, but in the case of the Japanese pine sawyer, *Monochamus alternatus*, adult beetles are the problem because they serve as vectors for the pinewood nematode, *Bursaphelenchus xylophilus* the causal agent of pine wilt disease, which kills pines. Interestingly, this cerambycid was not a problem until *B. xylophilus* was inadvertently

introduced from North America to Japan in the early 1900s. *M. alternatus* is now considered a destructive pest of pines throughout Japan and other Asian counties, and the importation of coniferous chips, unseasoned lumber and logs is regulated worldwide to limit the spread of both the insect and vectored nematode. Despite regulation, the Japanese pine sawyer is frequently intercepted at ports of entry throughout the world, *e.g*., live *M. alternatus* adults emerged from infested wood crating in a plumbing warehouse in western New York in June 1998. It is critical to develop management methods for this and related species.

M. alternatus is susceptible to the insect-pathogenic fungus *B. bassiana* (Fig. 1), which has been developed in Japan as an MCA for control of larvae and young adults to prevent transmission of *B. xylophilus* from infected to healthy trees. Spraying *B. bassiana* directly on larval-infested pine logs yielded a maximum of 75% larval mortality (Shimazu and Kushida, 1980), but results were variable during practical use. Infection was improved through release of pine bark beetles, *Cryphalus fulvus*, contaminated with *B. bassiana* conidia; these small bark beetles carried fungal conidia under the bark, resulting in *M. alternatus* larval infections (Shimazu *et al.*, 1999). Another strategy for delivering *B. bassiana* conidia to *M. alternatus* larvae in nematode-infested pines was implantation under host-tree bark of wheat-bran pellets on which *B. bassiana* had been cultured. This strategy yielded 13-81% beetle mortality in logs and 43-45% mortality in standing trees, but was difficult to use in infested standing trees and smaller branches (Shimazu *et al.*, 1992). *B. bassiana* conidia must come into contact with *M. alternatus* larvae living under the bark to infect its host, and the most effective strategy for infecting larvae has been placement of non-woven fabric strips, impregnated with cultures of *B. bassiana* (hereafter called 'fungal bands'), on top of infested logs and branches in piles on the ground (Shimazu *et al.*, 1995) (Fig. 2). These fungal bands were more effective when applied to wood with younger *M. alternatus* larvae because first through third instars feed within the cambial region while later instars bore into the sapwood (Shimazu and Sato, 2003). It is actually not known how conidia reach the larval microhabitat by this method, but it has been hypothesized that water flow from rain or small animals inhabiting this microhabitat move the conidia.

Young *M. alternatus* adults are also a target for MCA development because *B. xylophilus* is not vectored during a period of maturation feeding after adult emergence. Initially, methods for applying *B. bassiana* for control of *M. alternatus* adults involved spraying conidia onto infested pine trees just before adult emergence (Shimazu *et al.*, 1982) and during adult maturation feeding (Shimazu *et al.*, 1983). More recently, fungal bands have been tested against *M. alternatus* adults, and this approach yielded more promising results. A field trial resulting in high levels of infection, involved covering infested trees with a plastic sheet, forcing emerging adults to walk across fungal bands to escape (Okitsu *et al.*, 2000). Adults died from infection within 14 days of emergence, prior to transmission of *B. xylophilus* (M. Shimazu, personal communication). At present, a product based on *B. bassiana* bands for control of *M. alternatus* is being registered for commercialization in Japan. *B. xylophilus* was first discovered in China in 1982 and field trials with fungal band plus host attractants for control of *M. alternatus* to prevent transport of nematodes to healthy pines are being conducted in Anhui Province (Z. Li, personal communication).

b. Asian longhorned beetle. The Asian longhorned beetle, *A. glabripennis*, was accidentally introduced from China to several urban areas in northeastern North America and was first detected in 1996 in New York City area (Hajek, 2007). *A. glabripennis* was subsequently found in Chicago, Toronto and the state of New Jersey and also in Europe. This species is presently the target of eradication programs in the U.S., Canada, and several European countries. *A. glabripennis* kills numerous species of trees and has caused catastrophic levels of damage in China. Although this species is known for killing living trees, this effect results from successive generations repeatedly attacking the same tree. At first upper branches are infested and killed, and in subsequent years, eggs are laid and larval galleries are present on the trunk (Haack *et al.*, 1997). Eventually, with repeated attacks, the entire tree will die.

The principal microbial approach for control of this species has targeted adults using fungal bands placed around tree trunks and branches. After emergence from trees, adults undergo maturation feeding for > 1 week, and during this time, these reluctant fliers walk on tree trunks and contact the fungal bands. In addition, adults contaminated with fungal conidia after contacting a band can transmit conidia to another adult during mating (Hajek, unpublished data). Band production was developed by amending methods developed by Nitto Denko (Osaka, Japan) for commercial production of fungal bands made with *B. brongniartii*, which are used to control cerambycid pests (*e.g.*, *Psacothea hilaris*, *Anoplophora malasiaca*, and *Apriona japonica*) in Japanese orchards (Higuchi *et al.*, 1997); the fungal band product used in Japanese orchards is named Biolisa Kamikiri. For control of *A. malasiaca*, bands are placed at the bases of citrus trees where females oviposit. However, for control of other species such as *P. hilaris* that oviposit higher in trees, bands are placed around limbs in tree canopies (Tsutsumi, 1998).

Field studies on the use of fungi for control of *A. glabripennis* in the U.S. have been conducted in China because field populations of this pest in North America are low, and it is being eradicated. Trials began with a comparison of adults caged with tree trunks that had been sprayed with either *B. bassiana* or *B. brongniartii* or caged with fungal bands of these fungal species (Dubois *et al.*, 2004a) (Fig. 3). Longevity and oviposition of adults receiving the two treatments were similar. However, conidial viability was high on bands 10 days after the experiment began but was drastically reduced from sprays. Thus, subsequent field trials were based on use of fungal bands. Bands containing cultures of *B. brongniartii* and *M. anisopliae* demonstrated faster mortality of adults from treatment plots compared with adults from control plots, as well as greatly reduced oviposition in treatment plots (Dubois *et al.*, 2004b; Hajek *et al.*, 2006). At present, *M. anisopliae* F-52 is being used for fungal bands in the U.S. because this virulent strain was already registered with the U.S. Environmental Protection Agency. Because adults emerge asynchronously for several months, it is difficult to apply materials for control that impact all adults. However, fungal bands are excellent in this regard because activity has been shown to persist for well over one month in the field (Higuchi *et al.*, 1997; Hajek, unpublished data).

c. Emerald ash borer. The emerald ash borer, *A. planipennis*, was identified as the cause of extensive ash mortality (*Fraxinus* spp.) in areas of Michigan and Ontario in 2002 (Haack *et al.*, 2002). Although control programs initially focused on eradication, *A. planipennis* spread into 5 states due to transport of infested nursery stock, firewood,

timber, and natural spread (USDA FS NCRS, 2006), resulting in abandonment of eradication programs in parts of four Midwestern states, which are now considered generally infested. *A. planipennis* typically has a one-year life cycle and overwinters as mature larvae under the bark. After eclosion in spring to early summer, the long-lived adults mate and maturation feed on ash foliage for almost three weeks before females begin to oviposit in bark crevices and between bark layers. Newly hatched larvae bore directly into the bark until reaching the phloem, where they feed in the cambial region, forming characteristic serpentine galleries. High larval densities, as is typically observed in ash trees in North America, result in overlapping galleries, contact between individuals, and cannibalism, providing opportunities for improved efficacy of MCAs through horizontal transmission. Research on conventional insecticides for management and containment or control of *A. planipennis* in high value landscape trees is ongoing (Poland and McCullough, 2006). Suitable methods, however, such as biological and microbial control are also needed for management of this pest in environmentally-sensitive forested and riparian areas.

Comparative laboratory bioassays demonstrated *B. bassiana* strain GHA, registered as BotaniGard® for control of a variety of pests, was highly virulent against *A. planipennis* immatures and adults compared to other fungal isolates (Liu and Bauer, 2006). Median lethal concentrations of petroleum- or vegetable-based formulations ranged from 17 to 800 conidia/cm^2 and lethal times ranging from 4 to 10 days. In the greenhouse, BotaniGard application methods for control of *A. planipennis* were compared at a single rate (1×10^{14} conidia/ha) applied in a spray tower to 1) potted ash trees or 2) uninfested ash logs which were then caged with *A. planipennis* adults, or 3) infested ash logs prior to beetle emergence. When adults were caged with treated ash trees or logs, only 10% or 18% of adults became infected, whereas 61% of adults became infected when emerging from treated logs. Pre-emergent BotaniGard sprays were then field tested by spraying and caging infested ash trunks at two rates (1×10^{14} and 1×10^{15} conidia/ha), resulting in 43% and 76% fungal infection of emerging adults, respectively (Bauer *et al.*, 2004). In a separate experiment, fungal bands grown with *B. bassiana* GHA were evaluated on infested, caged ash trunks, resulting in 32% infection of the emergent adults (Liu and Bauer, unpublished data). Fall applications of BotaniGard (3×10^{14} conidia/ha) applied to infested ash trunks resulted in ca. 20% larval infection, presumably through infiltration of sprays into cracks and splits that form in ash tree trunks and branches after *A. planipennis* attack. These findings led to a larger field trial in a 20-year old infested ash plantation in which infested ash trees (canopy and trunk) were sprayed with BotaniGard (3×10^{14} conidia/ha) to drip four times during the *A. planipennis* emergence period. These sprays resulted in 36% fewer larvae infesting the trunks in the fall and 68% fewer adults emerging in the spring compared to control trees (Liu and Bauer, 2006), and ca. 26% less decline in condition of the ash canopy in the treated trees when compared to untreated trees (Liu and Bauer, unpublished data). Additional field trials are ongoing to increase the efficacy of BotaniGard for *A. planipennis* management with fewer applications.

d. Cottonwood borer. Taking a different approach to cerambycid control using fungi, *B. bassiana* was applied at different rates as a soil drench for control of the cottonwood borer, *Plectrodera scalator*, in a nursery where adults laid eggs into root collars and larvae tunneled in roots (Forschler and Nordin, 1989). After application, adults were

collected and reared throughout the flight period (June-August) and > 60% of adults were infected with *B. bassiana*. The authors hypothesized that adults became infected both when emerging from the rootstocks but also from contact with *B. bassiana* contaminated soil in the treated plots. Among larvae for the highest rate applied (1 x 10^{11} colony forming units/m^2), neonate larval establishment was lower in the treatment than controls. However, there were no differences among treatments for second-year larvae.

e. European spruce bark beetle. A *B. bassiana* product from Fytovita (Praha, Czech Republic) named Boverol has been investigated for control of the European spruce bark beetle (*Ips typographus*) (Kreutz *et al.*, 2004). Field-cage studies in a spruce stand demonstrated horizontal transmission among adults after *B. bassiana*-treated beetles were released into a healthy population. As a result of the treatment, the length of maternal galleries and numbers of *I. typographus* larvae and pupae were reduced. In a second study, beetles were lured into cages using commercial pheromone traps and some of those adults entering cages became contaminated with *B. bassiana* conidia. Numbers of holes constructed by females to form egg galleries and lengths of maternal galleries were reduced, and no larvae or pupae were found under the bark. The authors state that the next step is to determine whether *B. bassiana* plus pheromones is more effective for population control than mass trapping using pheromones alone.

f. Poplar-and-willow borer. Larvae of the weevil *Cryptorhynchus lapathi* bore into wood of poplars and willows and can kill, weaken or deform trees. Larvae tunnel in xylem and the openings of their galleries are plugged with aggregations of wood particles. In one study in Italy, wood plugs were treated with suspensions of *B. bassiana* conidia in growing poplars, resulting in about 50% larval mortality (Cavalcaselle, 1975).

3. Use of entomopathogenic nematodes for control of wood-boring beetles

a. Japanese pine sawyer. Entomopathogenic nematodes have been investigated for control of cerambycid larvae within wood. *Steinernema carpocapsae* was sprayed onto horizontally oriented logs to control *M. alternatus*, the vector of *B. xylophilus*, in Japan; spray volume, timing and rate were investigated (Yamanaka, 1993). Larval mortality was greater (69.2-72.2%) after exposure to *S. carpocapsae* than when fenitrothion was applied. However, the most efficacious nematode concentration was much higher than concentrations used commercially for control of other coleopteran pests. Although nematodes were applied only to the tops of logs, *M. alternatus* larvae under bark and within tunnels became infected, both those on tops of logs and those beneath logs; the nematodes clearly dispersed to find hosts. The author stated that use of nematodes to control larvae of *M. alternatus* needed to be part of an integrated control approach because nematode applications alone would not prevent spread of *B. xylophilus*.

b. Asian longhorned beetle. Species of *Steinernema* were also tested against *A. glabripennis* larvae in China. Using *S. feltiae*, 2000 infective juveniles (IJs)/ml were injected into galleries of later instars until galleries were full, resulting in an average 62% larval mortality (Qin *et al.*, 1988). Galleries of *A. glabripennis* larvae do not

intersect and are constructed upwards, so injection of nematodes into larval tunnels is not practical. It was likely that *A. glabripennis* larvae became infected with entomopathogenic nematodes when they traveled to the bark surface to discard frass from their galleries (Qin *et al.*, 1988). In another study, IJs were injected directly into the gallery openings from which larvae expel frass or sponges soaked in a suspension of IJs were placed in the openings; different concentrations and application methods for two strains of *S. carpocapsae* were compared in the field. Injection of 7,500 IJs/ml into each gallery opening resulted in an 86.4% reduction in active frass removal, suggesting larval mortality (Liu *et al.*, 1998). Laboratory studies have shown that *S. carpocapsae* infectivity is inhibited by exposure to aqueous extracts from *A. glabripennis* although pathogenicity was not affected (Fallon *et al.*, 2004). *S. feltiae* juveniles were positively attracted to *A. glabripennis* frass extracts, in agreement with laboratory bioassays during which *S. feltiae* IJs were more infectious than *S. carpocapsae.*

c. European elm bark beetle. For control of the bark beetle *Scolytus scolytus*, a suspension of *S. carpocapsae* sprayed onto heavily infested logs in the field was considered ineffective (Finney, 1977; Finney and Walker, 1979). Although logs treated in the summer yielded some infected larvae and adults beneath the bark, no overall difference in numbers of emergence holes were found in comparisons with controls.

d. Ten-blotched poplar flatheaded borer. The only wood-boring buprestid tested with entomopathogenic nematodes has been *Melanophila decastigma* in poplars in China (Liu *et al.*, 1998). Two strains of *S. carpocapsae* were injected into gallery openings, which active larvae had formed to eject frass. Equivalent results were obtained for different strains, with a maximum reduction of 89.5% active larval gallery openings after application of 10,000 nematodes in 1 ml. However, the authors suggested that for economic reasons, 5,000 nematodes in 1 ml should be applied, although in their study this only yielded a 66.7% reduction in active galleries.

e. Large pine weevil. Among the weevils, a large program using entomopathogenic nematodes has been developed in Europe for control of the large pine weevil, *Hylobius abietis* (Torr *et al.*, 2005). Larvae of this species develop within stumps and roots of dying and dead conifers and can reach high population densities after clear cutting a stand. Most damage is inflicted after young trees are transplanted at a site when adults feed on bark and phloem of transplants, at times girdling them. Transplants can be weakened or killed by the long-lived *H. abietis* adults, resulting in up to 100% loss of plantation restocks in some sites. To reduce populations of this weevil, larvae are targeted by nematodes that can search within stumps for hosts. However, it is critically important that the phenology of *H. abietis* is monitored because nematodes should be applied close to the time when larvae are pupating in stumps, because this is a particularly vulnerable stage. At present, researchers recommend applying a single application of *S. carpocapsae* at 3.5×10^6 nematodes in 500 ml water around the base of each stump. Because it can be difficult to reach all stumps after extensive tree felling has occurred, Torr *et al.* (2005) describes use of a forwarder-mounted spray rig to deliver nematodes to target stumps using hand-held lances. This equipment can treat 5 ha per day and was used for treatment of 200 ha in the United Kingdom in 2003. Using this methodology, Torr *et al.* (2005) estimate adult emergence is reduced by 60-75%.

f. Poplar-and-willow borer. Nematodes have also been applied against another weevil, *C. lapathi*, whose larvae bore in stems of young willows and poplars. When cotton swabs soaked with suspensions of 20,000 IJs of *S. carpocapsae* were applied to entrances of larval galleries, 100% mortality was obtained (Cavalcaselle and Deseö, 1984). These trials were followed with application of three species of nematodes at different concentrations, applied to bark before larvae entered the wood. However, the highest mortality resulting from these applications (75% from *S. feltiae*) was not considered sufficient for practical control.

4. Use of bacteria for control of wood-boring beetles

Among MCAs targeting all insect pests, the most widely used is the insect-pathogenic bacterium *Bacillus thuringiensis* (*Bt*). Although most products target larval insect pests in the orders of Lepidoptera and Diptera, many *Bt* strains are active against species of Coleoptera. *Bt* isolates showing potential for control of wood-boring beetles in laboratory bioassays include isolates of *Bt* subsp. *tenebrionis* for species of Bostrichidae, Cerambycidae, Curculionidae, and Scolytidae (Cane *et al.*, 1995; Beegle, 1996; Weathersbee *et al.*, 2002; Chen *et al.*, 2005); *Bt* subsp. *thuringiensis* and *Bt* subsp. *entomocidus* for species of Scolytidae (Jassim *et al.*, 1990); and *Bt* subsp. *israelensis* for species of Cerambycidae and Scolytidae (Alfazairy, 1986; Méndez-López *et al.*, 2003). The use of *Bt* for controlling larvae of wood borers, however, may only be achieved through the expression of *Bt* Cry toxin genes in genetically modified trees.

a. Asian longhorned beetle. Use of *B. thuringiensis* was proposed for management of *A. glabripennis* and mulberry longicorn beetle, *Apriona germari*, in China with a Cry3Aa toxin gene cloned from *Bt* strain 866 (Chen *et al.*, 2005).

b. Emerald ash borer. After the discovery of *A. planipennis* in North America, four registered *Bt*-based MCAs were evaluated for activity against adults, which feed on ash leaves throughout their adult life (Bauer *et al.*, 2004). Although some activity was observed at high rates of formulated *Bt*s in laboratory and small field trials, further laboratory bioassays demonstrated that the major Cry toxins from *Bt* subsp. *kurstaki*, *Bt* subsp. *tenebrionis*, and *Bt* subsp. a*izawai* were not toxic to adult *A. planipennis*. Therefore, different *Bt* isolates, with known coleopteran activity, were acquired from public and private culture collections and are now being screened for activity against this buprestid (Bauer *et al.*, 2006).

B Lepidoptera (Cossidae, Sesiidae, Pyralidae)

1. General biologies

Larvae of numerous lepidopteran families bore in trees (Solomon, 1995). Larvae of species in 3 families have been targets for MCAs. The Cossidae includes carpenterworms, with caterpillars of some species living gregariously in tunnels within wood. Also within the Cossidae are the leopard moths, named for the spotted patterns

on wings and bodies of adults. Larvae of species in the family Sesiidae, the clearwing moths, bore in wood, living one per tunnel. Larvae of wood-boring pyralids feeding in shoots and in cambium often cause the most damage in young trees. General biologies of cossids and sesiids are described briefly in Chapter VII-16 and in Solomon (1995).

2. Control with entomopathogenic nematodes

a. Cossidae in China. Entomopathogenic nematodes have been investigated for control of cossids and sesiids in both China and the U.S., based on the ability of nematodes to survive in the moist larval galleries within wood and travel to reach larvae within the wood. In northern China, the carpenterworm *Holcocerus insularis* is a major pest of ash (*Fraxinus pennsylvanica*), the Chinese scholar tree (*Sophora japonica*) and willows (*Salix* spp.) which are grown as shade trees, as well as Chinese hawthorn, *Crataegus pinnatifida* var. *major*, which is grown for fruit and medicine. This wood borer often occurs with several immatures per gallery within tree trunks. In southern China, the leopard moth *Zeuzera multistrigata* is a pest of Australian pine, *Casuarina equisetifolia*, a tree species planted extensively for windbreaks. *Z. multistrigata* attacks younger trees and has only one larva per tree but larvae will migrate from tree to tree several times, causing serious damage before completing development (Kaya *et al.*, 2006). In Gansu Province in northwestern China, *Cossus cossus* is a major pest of willows. Four species of entomopathogenic nematodes have been tested against *H. insularis*, demonstrating that *S. carpocapsae* was the most effective species (Yang *et al.*, 1993). Field trials with *Z. multistrigata* demonstrated that *S. carpocapsae* is also effective against this species. Studies included comparisons of application rates, demonstrating highest susceptibility of *Z. multistrigata* (50 IJs in 2 ml for 93% mortality), intermediate susceptibility of *H. insularis* (100,000 IJs in 100 ml for > 90% mortality), while *C. cossus* was quite resistant (800,000 IJs in 100 ml killed only 80%). The authors suggested that while innate susceptibility could explain these results, factors such as depth and structure of galleries, densities and ages of larvae and moisture levels within galleries would also affect the ability of nematodes to disperse to borer larvae and infect. Methods for the application of nematodes were compared using *Z. multistrigata*, demonstrating equivalent results from injection of nematodes into borer tunnels and blocking the borer holes with a sponge laced with a suspension of *S. carpocapsae*. The sponge plug method was easier to use and was applied to 25.3 ha in 1990, resulting in 90% larval mortality (Yang *et al.*, 1990). Comparison of control by *S. carpocapsae* versus dichlorvos, an organophosphate fumigant, against *H. insularis* demonstrated higher levels of mortality due to *S. carpocapsae* than the insecticide after 40 days (96% versus 76%, respectively). Interestingly, one Chinese study challenging *H. insularis* with *S. carpocapsae* in the field demonstrated two peaks in infection, showing that this MCA completes two life cycles, with numbers of dead larvae peaking 2-4 days after application and then again on the 14th day after application (Qin *et al.*, 1988). Following demonstrated success in controlling *H. insularis* using *S. carpocapsae*, this nematode was used extensively in five areas in China (Yang *et al.*, 1993). Control has been so successful that this nematode has been used to protect street trees in Tianjin since 1987, reducing infestations from 12.6 to 4% of trees (Kaya *et al.*, 2006).

b. Carpenterworm in the U.S. In the U.S., two methods for applying *Steinernema carpocapsae* and *S. feltiae* were compared for control of the carpenterworm, *Prionoxystus robiniae* (Cossidae) (Forschler and Nordin, 1988). Gallery injection yielded mortality of 70-100% of 5^{th} and 6^{th} instar larvae, while results from bark surface application were more variable. Because gallery injection is more labor intensive, only bark surface applications were tested further although this method requires that nematodes survive on the bark surface to reach and enter galleries. Concentrations of *S. carpocapsae* ranging from 0.5-2.9 x 10^4 nematodes/gallery applied to the bark surface resulted in 50-85% larval mortality. Percent mortality from applications made during moist weather conditions at ca. 21-23^{o}C was higher than when applications were made during drier and cooler weather; researchers hypothesized that more nematodes survived to disperse on bark and reach galleries under the moister and warmer weather conditions.

c. Sesiidae. Entomopathogenic nematodes have also been successfully used for control of sesiids in diverse hardwood tree species (see Table 1). Among the hosts that have been investigated, 88.9% of western poplar clearwing moths (*Paranthrene robiniae*) were controlled by *S. carpocapsae* on heavily infested birch and poplar (Kaya and Lindegren, 1983). *S. carpocapsae* was also used against the dusky clearwing, *Paranthrene tabaniformis*, in poplars, applying nematodes with cotton swabs soaked with suspensions of 2 x 10^4 IJs to entrances of larval galleries to yield 97.5% larval mortality (Cavalcaselle and Deseö, 1984). *S. carpocapsae* was sprayed on the bark of lightly infested dogwood trees (*Cornus* spp.) to control dogwood borer (*Synanthedon scitula*), resulting in an 84.6% reduction in borer abundance (Davidson *et al.*, 1992). Percent control of the large red-belted clearwing, *Synanthedon culiciformis*, in alder reached 77-84% when 6.5 or 11.5 x 10^6 *S. carpocapsae* IJs were applied directly to bark. However, control increased to 86-93% when 1.8 or 3.6 x 10^4 IJs were applied directly to each gallery opening (Kaya and Brown, 1986). These researchers found that *S. carpocapsae* was more effective at controlling sycamore borer, *Synanthedon resplendens*, than *S. feltiae* and hypothesized it was more difficult for the larger *S. feltiae* to enter the smaller gallery openings of this host species. Results suggested that entomopathogenic nematodes were more effective against sesiid hosts with larvae living in moist heartwood habitats (*e.g.*, *S. culiciformis*) compared with species with larvae living in drier bark galleries (*e.g.*, *S. resplendens*) (Kaya and Brown, 1986; Kaya, 1988).

Several nematode species have been assayed against the banded ash clearwing moth, *Podosesia aureocincta*, and the lilac borer, *Podosesia syringae*, attacking ash (*Fraxinus* spp.) trees (Gill *et al.*, 1994; Smith-Fiola *et al.*, 1996). Nematode applications reduced numbers of living larvae when tree bark was thoroughly sprayed with water prior to application whereas control was meager when bark was not sprayed with water prior to nematode applications (Smith-Fiola *et al.*, 1996). The authors hypothesized the water spray improved nematode survival, allowing more to disperse into larval galleries.

d. Persimmon bark borer. Eight strains of *Steinernema* were tested against this pyralid, *Euzophera batangensis*, in a coastal casuarina windbreak in Fujian, China. *S. carpocapsae* provided the best control at 72% larval mortality (Huang, 1995).

2. Control with entomopathogenic fungi

a. Cossids and sesiids. Relatively few trials have been conducted using entomopathogenic fungi against wood-boring Lepidoptera. In one study in China, cossid larvae were targeted using a novel method to apply conidia so that these would reach larvae within the wood (Huang *et al.*, 1990). *B. bassiana* conidia were mixed with waste molasses and sweet potato starch to form a paste which was smeared into excretion holds of larvae of the cossids *Z. multistrigata* and *Arbela baibarana* in casuarina trees. Resulting larval mortality was 93.6-96.8%. This paste retained conidial infectivity for 90 days at room temperature. In 1988-89, the paste was applied to 583.7 ha of forests at a cost of 0.128 yuan (U.S. $0.02) per ha, resulting in 88.5-98.4% larval mortality.

Beauveria bassiana was used against larvae of the clearwing *P. tabaniformis* in 3-year-old infested poplars in Poland. Conidial suspensions injected into larval galleries in July, yielded 94-96% control (Schnaiderowa and Swiezynska, 1977).

b. Pyralids. *Beauveria bassiana* has been tested against mahogany shoot borers in diverse countries by spraying conidia on trees. When applied to a plantation of 10-month old red cedars (*Cederela odorata*) in Mexico once a month and once every 3 months, 71% mortality of *Hypsipyla. grandella* larvae was reported (Sanchez-Monsalvo and Velazquez-Estrada, 1998). In Uttar Pradesh, India, 80% mortality of *Hypsipyla robusta* larvae in shoots of young red cedars (*Toonia ciliata*) was recorded (Misra, 1993). In Cuba, *B. bassiana* and *M. anisopliae* have been tested in nurseries and plantations at 4 kg of suspended spore powder/ha. In the plantation, 40.7% and 39.6% infection was achieved for *B. bassiana* and *M. anisopliae*, respectively. Also in Cuba, a mixture of *B. bassiana*, chemical insecticides during the first year after planting and silvicultural treatments are recommended to control *H. grandella* in mahogany plantations (Casanova *et al.*, 2001).

When *B. bassiana* conidia were applied in water and diesel oil against persimmon bark borer, *Euzophera batangensis*, in coastal windbreaks of casuarinas in Fujian, China, 86.1-100% control was reported (Huang, 1995).

3. Control with entomopathogenic bacteria

a. Cossids and sesiids. As many isolates of *Bt* provide effective microbial control of lepidopteran larvae, *Bt* has been investigated for control of cossid and sesiid larvae. *Bt* was injected into phloem-cambial mines and galleries of the cossid *P. robiniae* and the sesiid *Paranthrene simulans* but was ineffective even at high rates (Solomon, 1985).

b. Mahogany shoot borer. *Bacillus thuringiensis* was evaluated for control of *H. grandella* in one- and two-year old mahogany plantations in Honduras. In these trials, *Bt* that was sprayed on entire trees weekly provided better control than untreated trees (Goulet *et al.*, 2005). *Bt* was also tested against *H. grandella* in Mexico at different frequencies. When applied every month, 91% of larvae died, while mortality dropped to 67% when Bt was applied every 3 months (Sanchez-Monsalvo and Velazquez-Estrada, 1998).

3 APPLICATION AND EVALUATION OF ENTOMOPATHOGENS FOR CONTROL OF COLEOPTERA

A Case Study: Fungal control of *Anoplophora glabripennis*: use of fungal bands

1. Preparation of inoculum

This application method targets adult *A. glabripennis* that contaminate themselves when walking on tree trunks and branches. Fungi are applied as non-woven fiber bands impregnated with fungal cultures that are wrapped around trees. For these studies, the methodology for growing bands of *B. brongniartii* for control of *A. glabripennis* was amended from the method developed by Nitto Denko (Osaka, Japan) for control of orchard pests (Higuchi *et al.*, 1997). We could not confirm that *B. brongniartii* is native to North America (Hajek, unpublished data). Therefore, registration of this fungal species by the U.S. Environmental Protection Agency (EPA) would probably be lengthy, if this was even eventually possible. Bioassays demonstrated that *M. anisopliae* F-52, a strain already registered with the EPA for control of other pest species and marketed by Earth Biosciences (New Haven, Connecticut), was virulent against *A. glabripennis* adults. Therefore, this strain was chosen for further development.

To produce fungal bands, fungal cells are grown in liquid media on shakers (Dubois *et al.*, 2004a). Once cell number is maximal, additional media and molten agar are added. Quickly, pieces of non-woven fiber material are soaked in media and then laid on racks at 100% RH until surfaces of the bands are covered with conidia (approximately 7-10 days depending on the media composition and temperature). It is important that after sporulation, bands are allowed to dry slowly under high humidity, usually for several days. Bands are 5 cm wide and usually 50 cm long. The material for bands that is used in Japan is created from wood pulp and this is advantageous because bands are then biodegradable and do not have to be removed from trees. We have been unable to find comparable biodegradable material in the U.S. and therefore have substituted non-woven polyester-based quilt batting (*e.g.*, Soft and Bright, The Warm Co., Seattle, WA).

2. Experimental design

Evaluating the efficacy of fungal band applications is difficult because *A. glabripennis* is a long-lived beetle that is cryptic as a larva, living within wood. Adults are not easily seen in tree canopies. Adults preferentially feed on the bark of small twigs, often high in tree canopies, and they are not attracted to lights. Long distance pheromones or host attractants for quantifying or collecting adults have not been identified (Hajek, 2007). Adults lay eggs under the bark in branches as small as 3 cm in diameter but also in tree trunks. During field studies, many adults were found at > 3.5 m high in trees that were ca. 8 m tall, with fewer adults found at 2 m or lower on trunks. Thus, infested wood is often high in trees, also making sampling more difficult.

Ovipositing females chew shallow niches or pits in the bark, which they use to lay eggs slightly under the bark. Early instars feed in the phloem-cambium directly beneath the bark while later instars tunnel into the xylem. Until larvae reach later instars that created holes in the bark for discarding frass from galleries, there is little evidence that

larvae are present in trees (oviposition niches are very small). Thus, sampling larvae requires cutting down trees and carefully dissecting the wood to collect living or dead larvae. It is not always possible to fell trees but even if this is possible, it can be difficult to split large diameter wood without damaging larvae under the bark and within the sapwood. Thus, to evaluate the effects of fungal bands, we have used the methods described below (Hajek *et al.*, 2006; Dubois *et al.*, 2004a, b).

Because *A. glabripennis* is being eradicated in the U.S. and populations are very low, field trials have all been conducted in China. Most importantly, stands of trees with abundant *A. glabripennis* populations must be located for conducting studies. Experienced professionals locate infested sites during winter; experts are needed to ensure that the borers in trees are indeed the species of interest. To do this, a few sample trees are cut down, dissected, cerambycid larvae are collected from within trees, and identified to species. It is critical to locate sites with adequate densities of *A. glabripennis*, which can be estimated from numbers of larvae within trees that were felled.

At appropriate sites where we have conducted studies, groups of 40 trees adjacent to each other were chosen as plots within larger plantings of trees. For our most basic studies, 3-5 replicate plots where fungal bands were hung on tree trunks were established along with 3-5 control plots (*e.g.*, Hajek *et al.*, 2006). We have tried to separate replicate plots from each other by at least 50-100 m to try to eliminate, or at least minimize, movement of adults and fungal conidia among plots; although *A. glabripennis* adults can fly, they are reluctant fliers and it is not thought that they regularly disperse very far in the presence of abundant host trees (Huang, 1991; Huang and Zhou, 1992).

3. Fungal application

Fungal bands are hung around tree trunks or branches, usually attaching them with nails or staples. In our studies using 15-19 cm diameter poplars and willows in China, we attached bands at a height of 2.0-2.5 m. It is critical that fungal bands not be hung in the sun or conidia will quickly die. Therefore, band height should be adapted to make sure bands are shaded. All studies in China have been conducted with one band/tree, as is recommended for use of Biolisa Kamikiri in Japanese orchards (Higuchi *et al.*, 1997). These guidelines from Japan were created for small citrus and fig trees and perhaps more bands/tree should be applied for larger shade trees in the U.S. However, we hypothesize that addition of attractants to bands, as is our eventual goal, would reduce the numbers of fungal bands that are needed per tree.

4. Timing

Adults of *A. glabripennis* emerge from trees asynchronously over several months although the peak numbers of adults at field sites in Anhui Province in 2002, occurred during late June throughout July to early August (Hajek *et al.*, 2006). For our studies, bands were hung early in July when many adults had only recently emerged. Our collections of adults continued over 37-42 days, ending 19-20 August.

5. Evaluation

Following, we will outline the different procedures required to evaluate fungal band treatments:

1. Collect adults (5 per plot) before bands are hung as a pre-application sample of natural levels of fungal infection. We have collected these large adults by climbing trees or by using long bamboo poles to knock beetles out of trees. Adults are reared individually in plastic cups between 20 and 25°C, providing fresh twigs from host trees every 5 days as food. After collection, beetles are monitored daily for 40 days. It is important to open these pre-treatment rearing cups as seldom as possible to prevent potential cross-contamination.

2. After bands are hung, adult beetles are collected every 5 days and reared to detect fungal infections. During 2000 and 2002, we established a maximum of 5 beetles for each collection per plot, but often were unable to find this maximum number. This limit was established to ensure that we were collecting a small enough proportion of the population on each sampling date so that productive sampling could continue through that season (populations of these beetles are often not sufficiently abundant that large numbers of beetles could be removed from the population at regular intervals throughout the season) without depleting the population before the end of the season. For the first 24 hours after collection, a wet cotton ball is placed in each adult rearing cup to allow high humidity so that any *M. anisopliae* conidia potentially on the beetle cuticle will be able to germinate. Rearing procedures are as described above. Any beetles dying are maintained under moist conditions and fungal outgrowth from cadavers is used to identify the species of entomopathogenic fungus killing the beetle.

3. The background population densities of adult beetles can be monitored by regularly walking transects through the replicate plots, using binoculars to record locations and numbers of adult beetles. During studies conducted in 2006, approximately 43% of the trees in each plot were scanned by experienced researchers every 5 days to count adults, which were on tree trunks and in tree canopies.

4. To evaluate the effects of fungal band treatments on oviposition, we used ladders or climbed trees to count oviposition niches and emergence holes. To make sure that adult beetles in plots are disturbed as little as possible, we created a subset of trees where adults were collected and a subset of trees where oviposition and emergence data were taken. In this way, we were not counting oviposition and emergence on the same trees where we were hoping to collect adults to determine whether they were infected. It is important that this sampling is done before bands are hung to count oviposition niches and emergence holes from previous years as baseline data. After bands are hung, trees are ascended to quantify oviposition and emergence every 5 days.
To estimate the numbers of adult females that are active in a plot, we divided the number of emergence holes in all trees in that plot by two (because the sex ratio is approximately 1:1). When using ladders and climbing trees, for each replicate plot,

the number of new oviposition niches is divided by the number of females in that plot to derive the number of eggs laid per female.

5. During 2006, we wanted to evaluate entire trees (when climbing trees or using ladders, we could not evaluate smaller diameter wood) so we arranged with tree owners and cut down a subsample of trees from each treatment plot approximately 20 days after bands were hung. Trees were then carefully dissected to count the numbers of oviposition niches and emergence holes as well as numbers and locations of larvae in the wood. Eggs were maintained under moist conditions on moist filter paper until hatch or for 10 days. Larvae are placed in cups with artificial diet (Dubois *et al.*, 2002) and monitored daily for 30 days. Necropsies were performed on all unhatched eggs and larval or pupal cadavers to determine the cause of death. This type of sampling would be enhanced if trees were felled for quantification before bands were hung as well as several times after bands are hung. However, permission to cut trees and cost of trees can limit this type of sampling.

6. Persistence studies

To evaluate persistence of activity of conidia on bands, bands were placed at approximately 3 m in the shade, around trunks of *A. glabripennis* host trees (predominantly *Acer* spp.) in Queens, New York. We chose this location because it was a location where *A. glabripennis* is present in the field. At 1-4 week intervals for up to 3 months after band placement on trees, three fungal bands are removed from trees and brought to the laboratory. If bands were wet from rain and could not be processed immediately, they were dried briefly at high humidity before transport. Densities of conidia on bands were then quantified by blending several 5 cm^2 pieces of bands, filtering through cheesecloth and counting conidia with a hemocytometer. Germination of conidia from bands is quantified by spreading conidia on water agar for 24 hours. The density of germinated conidia per cm^2 of band is plotted by time to evaluate the persistence of living conidia on fungal bands.

B Case study: fungal control of *Agrilus planipennis*: pre-emergent trunk sprays

1. Preparation of inoculum

BotaniGard ES (BioWorks, Inc., Fairport, NY), a petroleum-based conidial suspension of *B. bassiana* GHA was suspended in water within label rates prior to field application.

2. Experimental design

Initially, BotaniGard trunk sprays were evaluated for efficacy against emerging *A. planipennis* adults in a 20-year old plantation of green ash trees (*Fraxinus pennsylvanica*) moderately infested with 50 to 100 larvae/m^2 of bark. The trees, spaced 2 m apart, ranged in diameter from 9 to 14 cm and in height from 8 to 10 m. A completely randomized block design was used to compare the efficacy of the three treatments (two fungal concentrations and the control) with five trees/treatment. In

early June, prior to emergence of *A. planipennis* adults, BotaniGard was applied to 180-cm trunk sections, which were then caged with aluminum screening (Brite-Kote™, 18 × 16 mesh, Phifer Wire Products, Inc, Tuscaloosa, AL) to contain emerging *A. planipennis* adults. Untreated, caged trees were used as controls (Fig. 4).

In the same ash plantation the following year, a field trial targeting both adult and immature *A. planipennis* was conducted by spraying "to drip" the canopies and trunks of ca. 180 infested green ash trees (now heavily infested with >100 larvae/m^2) with BotaniGard. Trees were sprayed individually every two weeks from late June to early August within the treatment plot; trees in the control plot were not sprayed. The condition of each tree crown was estimated in early June the year of treatment as well as one year after treatment. Crown condition was based on standardized observations of ash crown die-back observed after *A. planipennis* attack, using a scale of 0 to 100%. Trees with low, medium, and high infestation levels exhibit crown die-back of 0-24%, 25-50%, and >50%, respectively. Due to the high variability in *A. planipennis* attack rates between individual trees, 50 sample trees (25 treatment and 25 control trees) were selected by crown die-back category to include 30 with low infestation, 10 with medium, and 10 with high infestation. The 50 samples trees were felled at the end of the study to quantify larval infestation levels and adult emergence. Measures of treatment efficacy included a) crown condition before and after treatment; b) *A. planipennis* larval density and fungal infection prevalence; and 3) numbers of *A. planipennis* adults emerging the following year.

3. Fungal application

For the initial study, the trunk treatment targeting *A. planipennis* adults was sprayed with a professional 11.4-L sprayer equipped with a flat fan nozzle and pressure gauge for delivery of a known quantity of *B. bassiana* GHA conidial suspension to the bark surface of each tree. Fungal suspensions were applied at 35 psi (300 kPa) to the north and south sides of each trunk at two application rates (1×10^{14} and 1×10^{15} conidia/ha); control trees were untreated. An average of 166 ml (5×10^7 conidia/ml) and 169 ml (5×10^8 conidia/ml) were sprayed on each tree to achieve the two treatment application rates. Before treatment, the surface area was calculated for each trunk section and used to calibrate the amount of fungal suspension needed for each tree at the designated application rate.

The following year, tree canopy and trunk applications targeting all *A. planipennis* life stages required a truck-mounted hydraulic sprayer to accommodate the volume needed to spray 8-11 liters of BotaniGard suspension on each tree to achieve the rate of approximately 3×10^{14} conidia/ha.

4. Timing

For the initial study, trunk treatments targeting adults, sprays were conducted several days before *A. planipennis* adults were expected to emerge (26 June, 2003); adult emergence was estimated by periodic dissection of trees in the plantation and removal of pupae to determine their age. Adults were removed from the screen cages 45 days after spraying on 11 August. At this time adult emergence was complete. Adults were evaluated for mortality and fungal infection prevalence.

The following year, tree canopy and trunk applications targeting all life stages were sprayed every two weeks starting 23 June and ending 3 August 2004, for a total of 4 treatments. Crown conditions were evaluated 31 May and 14 June 2005. Sample trees were dissected for immature stages between December 2004 and March 2005, while adult emergence from these trees was monitored during summer 2005 from logs cut and held in the laboratory. In addition, canopy condition was monitored in summer 2005.

5. Evaluation

For the initial study, trunk sprays targeting emerging adults were evaluated following adult emergence in the field and death within cages, ca. 6 weeks later. When screen cages were removed all *A. planipennis* adults were collected, including those on the bark, inside bark crevices, and in exit holes on treated trunk section (Fig. 5). Tools used for handling insects were surface sterilized with 70% alcohol between uses. *A. planipennis* adults were placed in individual wells of sterile 24-well plastic plates under saturated humidity conditions to assess cause of death. Mycosis was confirmed after two weeks if a white conidial bloom appeared on the cadaver.

To evaluate the canopy and trunk applications, 25 treatment and 25 control trees from each plot were felled with a chainsaw. The main trunk was cut into 100-cm log sections from the base up to wood that was 2 cm in diameter. Each 100-cm section was cut again so that 30-cm log-sections could be transported to the laboratory for dissection and removal of insects. Live and dead *A. planipennis* larvae, prepupae, pupae, and adults were collected from each trunk-section and assessed for fungal infection as described above. The remaining 70-cm log sections from 15 treatment and 15 control trees were incubated for adult emergence in individual cardboard-rearing tubes (20-30 cm in diameter, 80 cm in length) for 8 weeks at room temperature. Data on each *A. planipennis* life stage were collected and recorded from each log. Crown conditions of the remaining trees in both the treated and control plot were reassessed one year after the initial assessment.

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Figure 1. *Monochamus alternatus* adult killed by *Beauveria bassiana* (photo taken by Mitsuaki Shimazu).

Figure 2. Applications of *Beauveria bassiana* bands to logs for control of *Monochamus alternatus* larvae within the wood (photo taken by Mitsuaki Shimazu).

Figure 3. Cage containing a *Beauveria brongniartii* band and 5 *Anoplophora glabripennis* adults to evaluate effects of fungal bands on adult longevity and oviposition (photo taken by Thomas Dubois and reprinted from Dubois *et al.* 2004a by permission of the Entomological Society of America).

Figure 4. Screen cages trapped *Agrilus planipennis* emerging from infested green ash trees, *Fraxinus pennsylvanica*, following a pre-emergent spray of *Beauveria bassiana* strain GHA in late June. After emergence was complete, cadavers of *A. planipennis* adults were evaluated for fungal infection in the laboratory (photo taken by Houping Liu).

Figure 5. Adult *Agrilus planipennis* killed by *Beauveria bassiana* strain GHA while emerging from an ash tree trunk after pre-emergent trunk spray with BotaniGard ES. It is hypothesized that adults became infected after eclosion, while chewing out of trees. Adults are approximately 1.3 cm long (photo taken by Houping Liu).