Evaluation of a Microbial Insecticide to Control Emerald Ash Borer

Summary of Research Conducted in 2002-2003

Background

Study of emerald ash borer natural enemies in Michigan:

Emerald ash borer (EAB) is generally not considered a pest in the Asian countries where it is native. In North America, EAB is thriving due to the lack of natural enemies (parasites, predators, insect pathogens) that prey on EAB in Asia. It is also likely that our native ash trees lack mechanisms to resist EAB attack.

To learn what natural enemies are killing EAB under the bark of ash trees, we felled infested ash trees every other week in a woodlot in Livonia, Michigan from August 2002 through July 2003. From paired logs cut from the same ash tree, we (1) allowed EAB and insect natural enemies to develop to the adult stage or (2) removed all EAB life stages from the logs; dead EAB were cultured for insect-pathogenic fungi and live EAB were allowed to continue development on diet.

We isolated and identified a variety of insect-pathogenic fungi from EAB cadavers during this study, although the prevalence was surprisingly low considering the high EAB populations found under the bark of infested ash trees (Table 1).

Table 1. Fungal pathogens isolated from infected emerald ash borer larvae, prepupae, and pupae in southeastern Michigan (n = 5817)

Species of Insect-Pathogenic Fungi	Infected EAB (n)	Infection Prevalence (%)
Beauveria bassiana	24	0.41
Metarhizium anisopliae	2	0.03
Paecilomyces farinosus	30	0.51
Paecilomyces fumosoroseus	7	0.12
Verticillium lecanii	36	0.62
Total	**99**	**1.70**

Insect Disease and Microbial Insecticides

Disease in insects is relatively common, especially when population densities are high. As with other animals, insect diseases generally result from infections by various microorganisms such as bacteria, viruses, and fungi. Insect pathogens serve as important natural enemies of insects in nature. Several of the more virulent insect pathogens have been developed into “microbial insecticides” and are registered by the U.S. Environmental Protection Agency (EPA).

Microbial insecticides offer several advantages over conventional insecticides including (1) good safety record for humans and other mammals; (2) narrow host range; (3) compatibility with other control methods; (4) biodegradability; (5) self-propagating; and (6) transmissible providing for natural spread of disease-causing microorganisms throughout the insect population. Some disadvantages of microbial insecticides when compared to conventional chemical insecticides may include higher cost, lower efficacy, shorter shelf life and field persistence, and slower knock down of insects.

The results of our EAB natural enemy survey in Michigan revealed the importance of insect-pathogenic fungi as EAB natural enemies. Insect-pathogenic fungi are relatively common in the environment, and when live spores come into contact and attach to the body of a susceptible insect, a fatal infection is likely to occur. For wood-infesting insects, the moist environment under tree bark facilitates both infection and spore production. Therefore, we began testing BotaniGard as a possible EAB-management tool for use by homeowners and managers of municipalities, parks, and other environmentally sensitive areas such as forests, woodlots, and wetlands. In such environments, where relatively high pest populations can be tolerated, microbial insecticides are used because of their good safety record in humans and other nontarget animals.

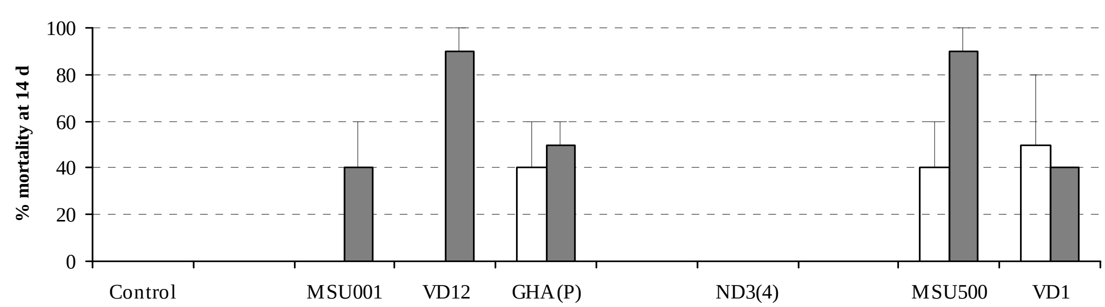
Comparing the infectivity of insect-pathogenic fungi in EAB:

Infectivity to immature EAB: We compared the infectivity of six insect-pathogenic fungi in immature EAB. All fungi were isolated from wood-boring beetles except *B. bassiana* GHA, which was isolated from a grasshopper and is commercially available as the registered microbial insecticide BotaniGard®. These fungi are native to North America, except *B. brongniartii* ND3(4) which is native to Japan. (Table 2)

Table 2. Insect-pathogenic fungi tested in EAB		
Fungal species isolate	**Insect Host order: family**	**Origin**
Beauveria bassiana		
MSU001	Coleoptera: Cerambycidae	USA
VD12	Coleoptera: Cerambycidae	USA
GHA	Orthoptera: Acrididae	USA
Beauveria brongniartii		
ND3(4)	Coleoptera: Cerambycidae	Japan
Metarhizium anisopliae		
MSU500	Coleoptera: Cerambycidae	USA
VD1	Coleoptera: Cerambycidae	USA

Using a laboratory bioassay, we determined the virulence of these six fungi in EAB prepupae (the insect-life stage between larva and pupa). We found all fungal isolates screened were infective and fatal to EAB prepupae, except *B. brongniartii* ND3(4) (Fig. 1).

Fig. 1. Mortality of EAB prepupae (10 EAB/concentration) exposed to $1x10^6$ (white bar) or $1x10^7$ spores/ml (grey bar) of six insect-pathogenic fungi 14 days after treatment



Infectivity to EAB adults: We further screened the five isolates infective to EAB prepupae (Fig. 1) against EAB adults. For the adult bioassays, we used the same spore concentrations and method of inoculation, although the bioassay ended after seven days due to high virulence.

Adult EAB were more susceptible to fungal infection than were prepupae, and $1x10^7$ spores/ml resulted in 100% mortality of the treated EAB adults (data not shown). No differences in virulence were detected between these isolates at $1x10^6$ spores/ml (Table 3).

Table 3. Mortality and median lethal times (LT_{50}) of EAB adults (40 adults/concentration) to $1x10^6$ spores/ml of five insect-pathogenic fungi 7 days after treatment

Fungal species isolate	**Mortality (%)**	**LT_{50}**	**95% CL**
Beauveria bassiana			
MSU001	88.6 ± 7.5	4.9	4.7 – 5.2
VD12	85.7 ± 11.4	5.0	4.7 – 5.2
GHA (powder)	94.3 ± 5.7	4.1	3.3 – 4.7
Metarhizium anisopliae			
MSU500	97.1 ± 2.9	4.9	4.7 – 5.1
VD1	100.0 ± 0.0	4.4	4.1 – 4.6

Studies of BotaniGard®, a microbial insecticide formulated with *Beauveria bassiana* GHA

Beauveria bassiana GHA is virulent against EAB (Fig. 1, Table 2) and is the active ingredient of BotaniGard, a biopesticide registered by the EPA in 1999 for control of certain insect pests in agriculture, horticulture, and forestry. When BotaniGard is sprayed on plants, the spores adhere to the leaves, branches, and other plant parts. When a susceptible insect comes into contact with these spores, they stick to its, and a fatal infection may result. We are testing BotaniGard as a possible management tool for control or suppression of EAB in parks, woodlots, and riparian areas. At the present time, no EAB control methods are available for ash trees in such environmentally sensitive areas.

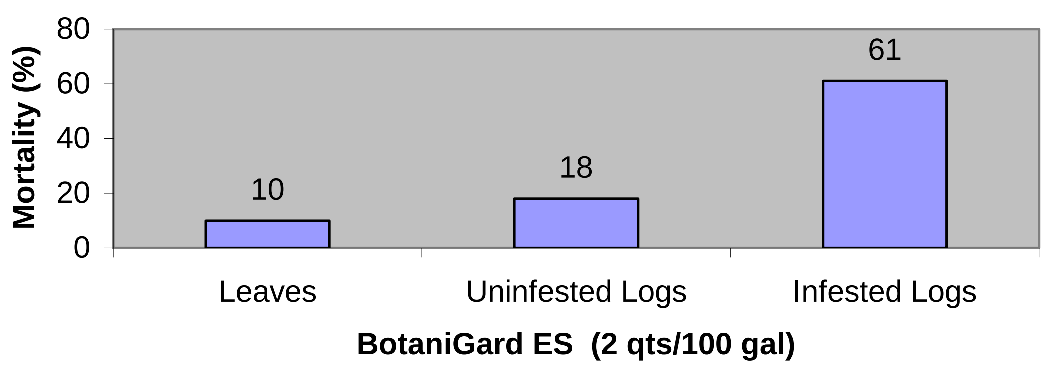
Laboratory comparison of BotaniGard formulations: First, we compared the efficacy of two BotaniGard formulations against EAB: BotaniGard ES is formulated with petroleum-based oils; BotaniGard O is formulated with vegetable oils and used by growers of organic food. This bioassay involved 24-hr exposure of EAB adults to ash foliage sprayed with serial dilutions of BotaniGard using a spray tower at the rate of 20 gal water/acre. After death, EAB were cultured for *B. bassiana* infection and the median lethal concentration (LC_{50}) of each formulation was determined. These values demonstrate high virulence of this fungal strain against EAB, and no significant difference between the two formulations (Table 4). The LT_{50}s for BotaniGard ranged from 4 to 10 days and was inversely correlated with spore concentration.

Table 4. Median lethal concentration (LC_{50}) of BotaniGard ES and BotaniGard O

Formulation	LC_{50} (conidia/cm^2)	95% FL	Slope ± SEM
BotaniGard ES	4.9	1.9 – 9.4	0.94 ± 0.16
BotaniGard O	4.7	0.3 - 21.9	0.63 ± 0.15

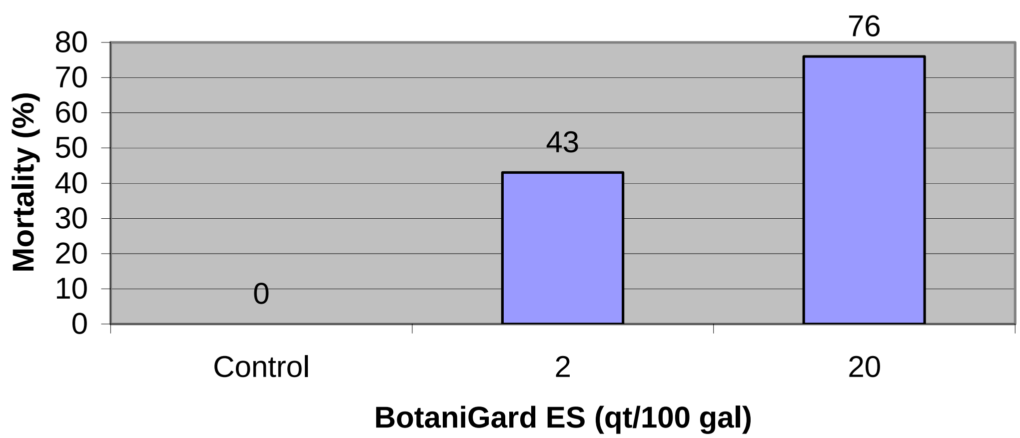
Greenhouse study of BotaniGard treatments for EAB adults in 2003: In our laboratory studies, both BotaniGard formulations were equally virulent (Table 4). Therefore, we used BotaniGard ES, the petroleum-based formulation, for subsequent studies. Our first objective was to compare the infectivity of spores applied to leaves vs. bark. Using the same spray tower described above, we applied 2 qts BotaniGard/100 gal (1.25 tablespoons/ gallon) to: (1) leaves of small potted ash trees; (2) uninfested ash logs; (3) pre-emergent infested ash logs; and (4) unsprayed controls. EAB adults were placed in cages with the treated ash in a greenhouse, held until beetle death, and cultured for fungal infection. We determined that BotaniGard infected and killed 10% of adults caged with sprayed trees, 18% of adults with sprayed logs, 61% of adults emerging from sprayed logs, but none of the control insects were infected (Fig. 2). Many of these beetles became infected while chewing through the treated bark.

Fig. 2. Application of BotaniGard ES to the leaves of potted ash trees, uninfested, or infested ash logs caged with emerald ash borer adults in the greenhouse



Field evaluation of BotaniGard trunk sprays for EAB adult control in 2003: The results of our greenhouse studies showed that BotaniGard was most effective when applied to tree trunks prior to EAB emergence (Fig. 2). We field-tested this method in an ash plantation at Fox Hill Golf Course in Ann Arbor, MI. Using a hand-held sprayer, we applied 2- or 20-qts BotaniGard/100 gal to five EAB-infested tree trunks before EAB emergence (June). We then stapled a screen cage around each tree trunk with epicormic shoots containing leaves to serve as food for the beetles. The beetles emerged from the tree trunks into the cage, and after death were cultured for fungal infection. We found that 43% and 76% of EAB adults became infected at 2- and 20-qts/100 gal, respectively; no infected EAB were found on control trees (Fig. 3). The trunks of these trees are currently being dissected to evaluate the prevalence of *B. bassiana* infection among EAB larvae in the sprayed trunks. Additionally, adult EAB that failed to emerge due to *B. bassiana* infestation will also being determined.

Fig. 3. Application of BotaniGard to infested caged ash tree trunks prior to EAB emergence in Ann Arbor, MI



Field study of BotaniGard trunk sprays for EAB larval control in 2003: Pre-emergent trunks sprays of BotaniGard have the potential to reduce populations of adults attacking ash trees in an area (Fig. 3). EAB damage to ash trees, however, occurs the year prior to adult emergence when larvae are feeding under the bark. In the fall, we observed cracks over larval galleries on infested tree trunks. Therefore, we applied 14-qts BotaniGard/acre using a hand-held sprayer to the trunks of 13 infested trees in Meridian Township, MI in October. We are currently dissecting these trees to evaluate the efficacy of BotaniGard to infect larvae under tree bark. Although these dissections are ongoing, we have found 10-30% of the larvae are infected with *B. bassiana* in the sprayed trees; no infected EAB were found in the control trees. The prevalence of infected EAB was higher in trees with higher numbers of EAB larvae, probably due to more abundant tree cracks and overlapping galleries. Based on the results of this study, however, we suggest BotaniGard be applied to ash trunk in August or early September, before EAB larvae tunnel deeper into the sapwood where they overwinter.

Summary: In 2003, we learned that BotaniGard, at rates of 2 and 20 quarts/100 gallons (equivalent to 1.25 and 12.5 tablespoons/ gallon), kills EAB adults and larvae when applied to infested tree trunks. As with other insecticides, higher rates achieve better control. Some reduction in local EAB populations with this method may be possible, although EAB larval damage may continue to exceed the tolerance threshold of the treated trees. Thus, protection of trees with BotaniGard is not assured because EAB adults are highly mobile, and immigration from adjacent areas is likely. The level of tree protection afforded by BotaniGard will likely increase if control of EAB larvae is improved by (1) repeat applications during the summer and (2) EAB are controlled over a large area.

Conclusions: Development of control strategies for EAB is proving difficult, even with conventional insecticides, because EAB larvae feed within the tree trunk. Thus, trees with external evidence of EAB attack may not survive regardless of the treatment due to extensive internal injury caused by EAB. Methods to evaluate the extent of EAB damage within an ash tree have not been developed. Careful observation of ash trees after leaf flush (during May in southeastern Michigan) may provide signs of EAB attack such as (1) weak, dying, or dead crown; (2) epicormic shoots (weak branches sprouting from the main trunk); and (3) D-shaped emergence holes on the trunk where EAB adults emerged. Death of the main trunk is imminent for trees with these symptoms, and insecticide treatments are unlikely to be successful.

Homeowners should consider selecting only healthy and EAB-free ash trees for EAB control due to the time, expense, hazard, and uncertainty of success. Annual insecticide applications may be only partially effective in controlling EAB. Therefore, removal of dying and dead ash will reduce the number of EAB emerging and attacking adjacent trees. In addition, healthy trees may be less attractive and more tolerant of EAB attack. Therefore, we suggest maintaining or improving the health of your trees by fertilizing, watering, mulching, and avoiding trunk damage caused by lawn mowers, soil compaction, nearby construction, grazing animals, etc.

Research Plans for 2004. BotaniGard will be further field-tested on infested and uninfested ash in 2004. Applications will begin in early to mid June, before peak emergence of EAB adults. The entire tree trunk and upper branches will be sprayed because we now know that EAB attacks the upper crown of trees first. Trees will be sprayed until wet, to assure good protection of BotaniGard from UV by infiltration of the fungal spores into bark layers, cracks, and crevices. We will spray every 2 weeks through July when EAB are laying eggs and bark-cracks are forming over larval galleries. To evaluate the impact of this spray regimen on EAB and ash, we will compare the numbers of adult EAB and larval galleries in sprayed vs. unsprayed research plots.

Pros:

1. The active ingredient of BotaniGard has no known negative effects on plants, birds, fish, mammals, and many beneficial insects, such as bees.
2. Homeowners can apply this material themselves. No license, permit, or application equipment are required.
3. EAB adults contaminated with fungal spores may transmit the disease to other EAB during physical contact such as mating and while laying eggs.
4. Dead EAB will release spores that are infective to healthy EAB.
5. BotaniGard is active against EAB at the time of application. It does not require time to be translocated by the tree.
6. No ground water contamination concerns from the use of BotaniGard are known.

Cons:

1. BotaniGard may be more difficult to find at stores because of its limited market distribution when compared to conventional sprays.
2. Fungal spores are sensitive to UV degradation in sunlight resulting in low persistence and the need for repeat applications.
3. Insects may take longer to die when exposed to BotaniGard than for conventional insecticides, thus oviposition may continue prior to insect death.

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