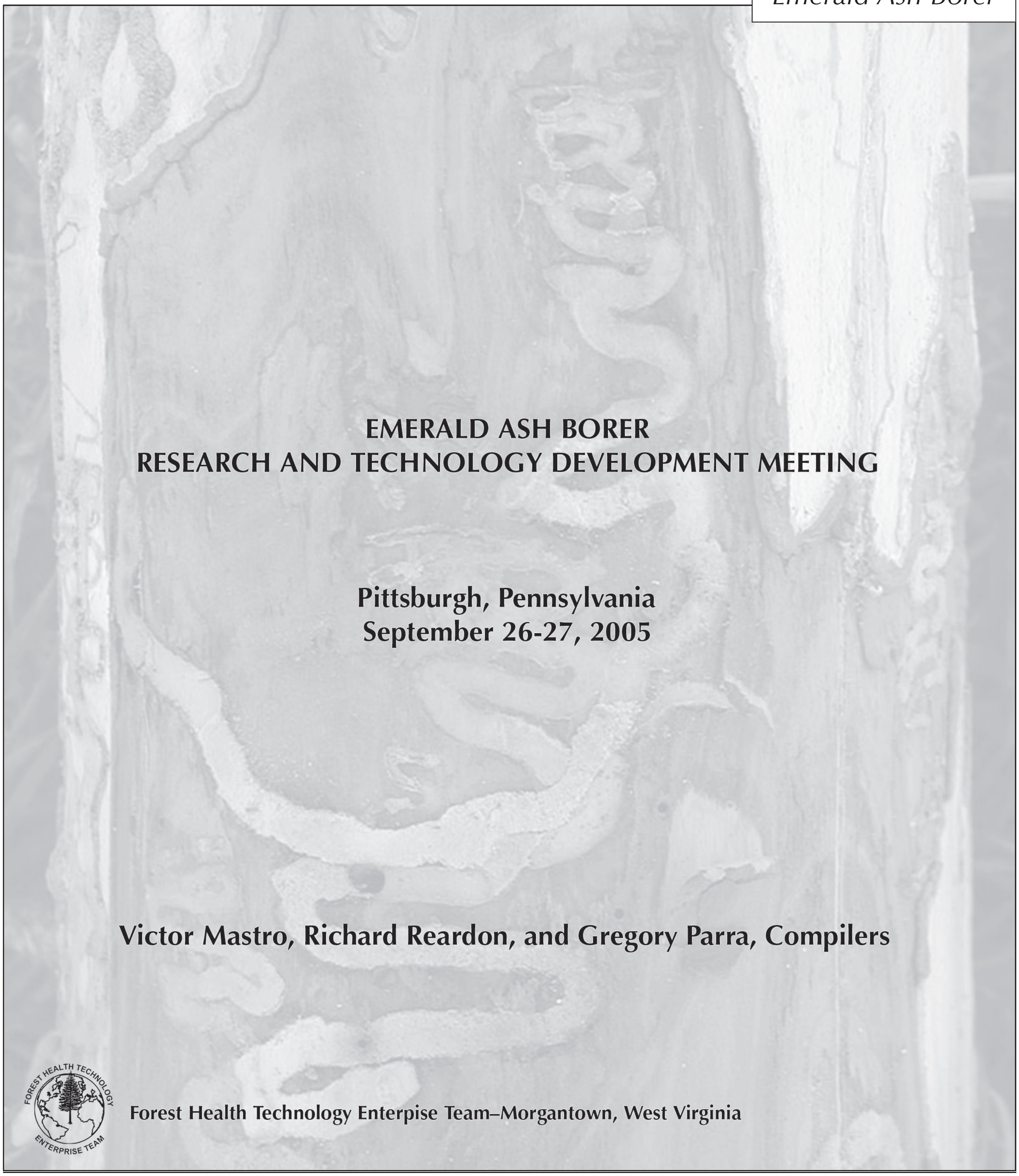
Forest Health Technology Enterprise Team

TECHNOLOGY
TRANSFER

Emerald Ash Borer



EMERALD ASH BORER
RESEARCH AND TECHNOLOGY DEVELOPMENT MEETING

Pittsburgh, Pennsylvania
September 26-27, 2005

Victor Mastro, Richard Reardon, and Gregory Parra, Compilers

Forest Health Technology Enterprise Team–Morgantown, West Virginia

USDA U.S. Department of Agriculture

Forest Service

APHIS Animal and Plant Health Inspection Service

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On the cover: year-old emerald ash borer galleries. Photo by David Cappaert, available at www.forestryimages.org as UGA1460075.

Emerald Ash Borer Research and Technology Development Meeting

September 26–27, 2005
Radisson Hotel
Pittsburgh, Pennsylvania

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FOREWORD

The emerald ash borer, *Agrilus planipennis* Fairmaire, a buprestid wood borer, was discovered infesting and killing trees in the area of Detroit, Michigan, in June of 2002. The Michigan quarantine area now includes 20 counties in southeast Michigan and 19 isolated outlying areas. Emerald ash borer was subsequently discovered in Essex Co., Ontario in August of 2002. A number of isolated small populations have also been found in Indiana and Ohio. Most of these are thought to be the result of movement of infested nursery stock, logs, or firewood. Potential impacts of this insect, if allowed to spread, are substantial. In the U.S. alone, there are over 700 million ash trees and a U.S. Forest Service report estimated the loss at between 20 and 60 billion dollars. In response to the discovery of these wood borer populations, federal, state and local authorities held a number of meetings and prepared risk assessments. Both the Canadian and the United States version of the risk assessments conclude that substantial impacts would be the result of this introduction unless actions are undertaken to mitigate them. A Respective Science Panel was convened in each affected country, and their reports have similar recommendations: to develop a plan to contain emerald ash borer (EAB) populations in both countries. The U.S. Science Panel recommended that a strong commitment be made to developing the scientific information and technology necessary to carry out any management programs. A list of areas where research was critically needed was also developed.

As funding from various sources became available for EAB technology development and research, a number of federal, state, provincial and university groups became involved in the work. The first two days of the meeting in Pittsburgh (the Emerald Ash Borer Research and Technology Development Meeting) was the third effort to pull together the many scientists involved in the work in a forum in which they could detail their interest and share their preliminary findings. The second two days of the meeting in Pittsburgh (the Emerald Ash Borer Accelerated Research Review) was the first effort to develop a comprehensive plan for an accelerated research program to produce technologies that will allow for the successful management of emerald ash borer. The abstracts contained in this report represent the first two days of the meeting.

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THE 2005 MICHIGAN EMERALD ASH BORER RESPONSE: AN UPDATE

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The Michigan Emerald Ash Borer Response Project—including the efforts of USDA, Michigan Departments of Agriculture and Natural Resources, and Michigan State University—continues to concentrate on and support a five-pronged approach. This effort includes: Survey, Enforcement, Control, Outreach, and Research components. Survey efforts include the deployment of 12,000 trap trees statewide in both a systematic and risk-based fashion. The gateway strategy which is designed to contain EAB within the lower peninsula of Michigan is utilized as the basis for response decisions. The control objectives include containment within the gateway areas and eradication of isolated populations beyond the gateways.

The Michigan quarantine area now includes 20 counties in Southeast Michigan and 19 isolated outlying areas. Enhanced enforcement efforts include increased state quarantine violation penalties, increased highway blitzes and deployment of an inspection station at the Mackinaw bridge. A multitude of outreach efforts have successfully conveyed the quarantine message and garnered public support. In an effort to reduce the risk of infested ash materials moving out the quarantine area, grinding yards located within the quarantine area have processed over 300,000 tons of ash materials for proper disposal. In efforts to support citizen response to this devastating pest, the cooperators have developed a no-cost municipal tree removal program for property owners in the quarantined area, initiated a restoration effort in cooperation with these communities, and supported an ash reduction program behind, within, and in front of the gateway areas.

INDIANA EMERALD ASH BORER STATUS REPORT

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ABSTRACT

Indiana has two areas infested with emerald ash borer (EAB) in northern Indiana. The largest site is in Lagrange County near the city of Shipshewana, where approximately 12 square miles are infested. The pathway of infestation in this site was believed to have been logs shipped into a mill in the area. Approximately 120, 000 trees have been removed from this largely agricultural site. An estimated 80% of the trees removed have been under 2 inches in diameter. The smaller of the two sites is in Steuben County in the northeast corner of the state.

The area of introduction has two introduction points, each within a mile of one another and each of which are campgrounds. Each site is approximately 1 square mile in size. Introductions are thought to have been through firewood brought in from infested areas and stored at permanent campsites. Of anecdotal interest is that the infestation in Shipshewana is only about two to three years more recent than the proposed Detroit ground zero site. However, apparent movement out of the Shipshewana area is much more limited than observed in Detroit. The limited movement is believed to be strongly associated with the introduction having been located and centered within an Amish community. The difference in spread rates in Detroit and Indiana's Shipshewana site anecdotally points out the role of human mediated movements of wood versus natural spread rates, or versus natural spread rates with limited human mediated movement of wood.

MANAGING THE EMERALD ASH BORER IN CANADA

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Despite aggressive control actions, the Emerald Ash Borer (EAB) continues to spread in Canada. In early 2004, an estimated 85,000 ash trees were removed in western Chatham-Kent County to establish an "Ash-Free Zone." Unfortunately, surveys conducted by the Canadian Food Inspection Agency (CFIA) in late 2004 and early 2005 revealed that EAB was present at some 80 outliers immediately east of the zone. This necessitated the removal of an estimated 50,000 ash trees around these sites in the late winter of 2005. Investigations conducted by the CFIA indicated that these sites were likely the result of the movement of infested forest products and firewood to the area prior to the establishment of the ash-free zone rather than through natural dispersal from Essex County.

Surveys conducted by CFIA and its partners in 2005 detected EAB in western Lambton County, Ontario, along the St. Clair River (and including Walpole Island). Populations on Walpole Island are well established and may have been there for up to five years – tree mortality is in evidence. Other populations along the Ontario side of the St. Clair River appear to be the result of natural dispersal from St. Clair County, Michigan.

In October of 2005, an outlier population was detected in Elgin County, to the immediate east of Chatham-Kent County. Subsequent surveys and investigation have revealed that this is most likely the result of an introduction at a rest stop, possibly as long as five years ago. Delimitation surveys are continuing in this area and there is no evidence to date that EAB is established elsewhere in the county.

Intense risk-based surveys were conducted throughout S/W Ontario and at lower levels throughout the remainder of Ontario in 2005. To date, EAB has not been detected anywhere else in Canada other than Essex, Lambton and Elgin counties, and the Municipality of Chatham-Kent (Ontario) and there is no reason to believe it is established elsewhere in Canada at this time.

Current CFIA policy is to "slow the spread" through intensive surveillance at high risk sites, aggressive control actions around known outliers, enforcement and compliance actions and effective communications. In spite of these on-going efforts, it is anticipated that EAB will continue to spread and become increasingly destructive in Canada. At risk are an estimated 1 billion trees in Ontario and at least 2 billion in Canada.

Since the discovery of EAB in 2002, the CFIA has placed a high priority on working with its regulatory and research partners in Canada and the US to ensure that research needs are clearly identified and the "best" science is incorporated into surveillance and control strategies.

The CFIA is currently deciding on its next steps. A management plan is currently being drafted for review by CFIA senior management and which will reflect recommendations from the Canadian EAB Science and Survey Committee. The inability to effectively detect EAB at low population levels is seen as the single most important impediment to effective control of EAB.

INVASION GENETICS OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS* FAIRMAIRE) IN NORTH AMERICA

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ABSTRACT

Emerald ash borer (EAB) was first detected in Michigan and Canada in 2002. Efforts to eradicate this destructive pest by federal and state regulatory agencies continue. Knowledge of EAB genetics will be useful in understanding the invasion dynamics of the beetle and to help identify geographic localities of potential biocontrol agents. Genetic techniques, such as mtDNA gene sequencing, amplified fragment length polymorphisms (AFLP), nuclear gene sequencing, and microsatellite analysis will help determine the geographic origin of EAB in its native range throughout eastern Asia.

To date, we have obtained approx. 2,100 EAB individuals, mainly from 32 localities in Michigan, but also from three localities in Ohio, one locality each in Indiana and Canada, seven localities in China, and four localities in South Korea (kindly loaned to us by Dr. Dave Williams, USDA-APHIS). We also have obtained one adult from Shiroishi, Japan (kindly loaned to us by Dr. Paul Schaefer, USDA-ARS). Mitochondrial cytochrome oxidase I (COI) sequences (485 bp) from all North American EAB (Michigan: 76, Ohio: 2, Indiana: 4, and Canada: 6), all EAB from China (Dagong: 5, Hangu: 1, Heilongjiang Province: 4, Hebei: 2, Jilin: 2, and Liaonging: 2), and six EAB individuals from three localities in South Korea were identical. However, mitochondrial COI sequences from five individuals in two populations in South Korea differed from this common haplotype by two to four nucleotides, and the Japanese sample differed from the common EAB mtDNA haplotype by 3.7%. Therefore, the mtDNA COI sequence of the Japanese sample is very different from any other individual sampled, and there is COI haplotype variation in two of the three localities in South Korea. We have also obtained AFLP profiles from EAB individuals from Michigan (46), Ohio (2), Ontario, Canada (6), South Korea (4), Dagong (4), Heilongjiang Province (3), Liaonging (2), Hebei (2), Jilin (2), and one individual from Japan (four selective AFLP primer pairs; 139 scoreable loci).

We have observed differences in AFLP profiles both within populations from the same location as well as between all populations. Neighbor-joining analysis of the 139-band AFLP data set indicates that individuals from Michigan cluster more often with individuals from China, while the Japan individual fell into a separate, more distantly related group. However, we cannot rule out South Korea as the geographic origin of North American EAB. Despite the observed COI haplotypes diversity in South Korea, the common haplotypes that is shared by all Chinese and North American EAB individuals exists in each of the Korean populations sampled. Thus, it will be necessary to increase our sampling of Asian populations if we hope to make valid inferences about the geographic location(s) of source populations that gave rise to the North American EAB infestations.

We are continuing to expand our AFLP data set to include data from a fifth and sixth selective primer pair. We are also working to obtain DNA sequences from the nuclear genes wingless (*Wg*), phosphoenolpyruvate carboxykinase (*Pep*CK), cytochrome c (*Cytc*), and elongation factor-1α (EF-1α). Finally, microsatellite markers are being developed for EAB to incorporate these highly polymorphic markers into the data set. We expect that analysis of the expanded data set will improve resolution of the EAB populations and allow us to determine which populations are most closely related to each other and if there was a single introduction or multiple introductions of this pest.

GUT MICROBIAL FLORA OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS* FAIRMAIRE

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ABSTRACT

Gut symbionts play important roles in insect development, survival, and reproduction. Thus they offer promising targets that can be manipulated to adversely affect pest insect performance. For example, our previous work with gypsy moth demonstrated that activity of *Bacillus thuringiensis* var. *kurstaki* (Btk) to can be greatly synergized by administering materials that alter their gut communities (1, 2). In addition, some gut symbionts may be obligate or facultative pathogens, and so an understanding of these microbiota facilitates both the discovery of pathogenic agents and possibilities for altering gut communities to increase predisposition to resident facultative pathogens. We have been evaluating the gut flora of the emerald ash borer (EAB) for the past two years using a combination of molecular and culturing methods. This is part of a larger project involving seven species of invasive and native wood borers, bark beetles, and caterpillars (3, 4, 5). Using culture-independent methods, we have found 11 bacterial species from EAB larvae and 18 from adults, representing eight bacterial classes. Using culture dependent methods, we found eight bacterial species from EAB larvae and 13 from adults, representing five bacterial classes. Some of these bacteria appear to exhibit cellulolytic activity as we observed for Asian longhorned beetle and linden borer (6). Our ongoing and future work includes a) screening additional 16S rDNA libraries constructed from individual guts of larvae and adults, b) investigating functions such improved digestion of nutrients, detoxification of plant compounds, etc., c) quantifying sources of variation; d) localizing various bacterial types to different parts of the gut, and e) conducting manipulations to test effects on EAB survival, development, and behavior.

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EMERALD ASH BORER DISPERSAL – A RELEASE AND RECAPTURE STUDY

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ABSTRACT

This particular study was designed to investigate the emerald ash borer's dispersal behavior. We investigated distance dispersal from a central release point and landing height on trees.

At approximately 15, 30, and 60 meters out from the release location, we chose 4, 8, and 16 ash trees, respectively. A series of metal mesh panels (1 meter by 0.5 meters) covered in tangle foot strung together by 0.5 meter lengths of rope was hung along the length of each tree. The traps were strung vertically parallel to the tree's trunk by climbing a maximum of 10 meters and attaching a pulley to the tree. A rope was then strung through the pulley and the chain of traps was tied to the rope and pulled up along the length of the tree. A rope was tied to the tree holding the last two panels tightly to the tree in order to keep the panels closely associated with the trunk of the tree.

Beetles used in this study were collected in the laboratory. Heavily infested ash wood was brought into the laboratory and placed in barrels. The adult emerald ash borer emerging from these barrels were collected and marked with day-glow powder. They were then placed in jars with ash leaves and water until their time of release. On the release date, the leaves and water were taken out of the jars and the jars were left at the release point to allow the beetles to fly out at will.

Through the summer, a total of roughly 6,000 marked beetles were released in this study. Of the beetles released, 210 were recaptured. Of the 28 trees, 20 recaptured at least one released beetle; the most a single tree recaptured in total was 29 marked beetles. The tree located farthest out from the release point, at 76 meters, recaptured two beetles. No trends in flight patterns were apparent from recapture rates: they dispersed at random. Furthermore, some trees located more closely to the release point recaptured no beetles while others, further from the release point and along the same direct flight path, did. The trees were either black or green ash and were in various states of decline. A Chi-square analysis of total number of beetles caught in black and green ash was significantly different from random, with more beetles caught on black ash trees. A Chi-square analysis of the total number of beetles caught on trees and the tree's percent relative dieback was also performed. The results were significantly different from random. Trees with 100% dieback (dead trees) caught the most beetles. We also analyzed the position of the panels along the length of the trunk and number of beetles detected on each panel using a Chi-square test. The results were significantly different from random. The panels located lowest along the tree trunk caught the fewest beetles and those located highest caught the most beetles. This was a consistent and prominent pattern.

SPREAD AND DISPERSAL OF EMERALD ASH BORER: A DENDROCHRONOLOGICAL APPROACH

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ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), was discovered in North America in 2002 and has since been found to be responsible for the death and decline of over 15 million ash trees in southeastern lower Michigan. We are using tree ring analyses to reconstruct the historical dispersal patterns and rates of spread of EAB throughout the core EAB infestation in southeastern lower Michigan and several outlier EAB populations. Increment cores or cross-sections from EAB-killed ash trees were collected on at least a 3.0 × 3.0 mile grid over an area greater than 5,800 square miles encompassing the core EAB infestation. EAB-killed trees were preferentially sampled over declining or non-stressed ash trees. Increment cores and cross-sections have been prepared using standard dendrochronological techniques. Skeleton-plotting of increment cores and cross-sections is currently in progress to identify the year that EAB killed the trees. Additional dendrochronological analyses are in progress to determine when EAB initially infested individual trees. Crossdating and other dendrochronological analyses are in progress that will reveal when and where EAB initially became established in southeastern lower Michigan and how it spread historically. A case study was also presented to illustrate how we are using dendrochronological techniques to date when infestations began and to evaluate the spread, dispersal, and population dynamics of EAB at several outlier sites.

SPREAD AND DISPERSAL OF EMERALD ASH BORER: A COUPLED MAP LATTICE MODEL APPROACH

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ABSTRACT

The ability to predict the temporal and spatial dynamics of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in outlier populations is needed to continue development of effective management strategies for improved control of EAB. We are using a coupled map lattice model approach to model the spread and dispersal of EAB. Coupled map lattice models create an artificial environment in which several iterations of EAB dynamics may be performed, representing the spatial spread of EAB over time. The general EAB spread model involves initial dispersal from a point source (e.g. infested firewood), loss of ash phloem resource due to larval feeding, population growth, and subsequent dispersal of new EAB adults. Advantages of using this approach to model EAB dynamics include being able to vary the density and distribution of ash and initial EAB infestation levels. To develop a realistic model of EAB spread and dispersal, model parameters are currently being fit to match EAB dynamics observed at several outlier sites. Potential applications of this approach include: 1) evaluating management techniques and strategies at distinctly different sites (e.g. forest, urban, riparian, etc.); 2) determining ash removal zones at EAB eradication sites given the ash distribution, infestation levels, and number of years infested; 3) predicting EAB dynamics following varying degrees of ash removal; and 4) evaluating the effectiveness of biological controls. Implications of this research were discussed in relation to future management guidelines.

MODELING POTENTIAL EMERALD ASH BORER SPREAD THROUGH GIS/CELL-BASED/GRAVITY MODELS WITH DATA BOLSTERED BY WEB-BASED INPUTS

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ABSTRACT

As everyone knows at this conference, enormous economic and ecologic consequences across North America are at stake due to the introduction of the emerald ash borer. We present two aspects of work related to assessing the potential and actual spread of this organism. First, we model the susceptibility and potential spread of the organism across the eastern United States and especially through Michigan and Ohio using Forest Inventory and Analysis (FIA) data to account for ash distribution and abundance at a 20 x 20 km resolution (Prasad and Iverson, ongoing). This effort produced a general map of relative importance of rural ash to the emerald ash borer. When combined with maps of percent forest cover, we were able to estimate the availability of the ash resource to the invasive species. These maps show a high level of ash availability in the zones surrounding the borer's current range. We are also developing a cell-based model for the potential spread of the organism. This model is primarily based on a modification of an earlier model we developed to assess migration of tree species under climate change (Schwartz 1993, Iverson et al. 1999, Schwartz et al. 2001). A series of 'what if' scenarios are being developed to assess potential spread ranges. In addition, we are using a gravity model approach (Bossenbroek et al. 2001) to predict long-distance dispersal events based on human movement patterns.

The second aspect of our work involves acquiring current data on the location of the organism. Besides acquiring field data ourselves and from other agencies, we have developed a Web-based tool for public agencies and private individuals to enter the locations of their ash trees that have (or have not yet) been infested by the organism, which will support es-

timates of the rate of spread. To serve maps on the Web, we used a public domain software developed by the University of Minnesota called MapServer. People can enter their ash tree information at this site (*eabserver.osu.edu*) by street, administrative boundary, quadrangle, digital orthophoto, or GPS coordinates. In addition to the flash tutorial on the website to educate users on the proper use of the web-based map server, detailed instructions for use have been published (Sydnor et al. 2005). Using these data and GIS tools, we hope that the EAB community will better understand the current and potential rate of spread, which will guide management decisions to help contain this destructive pest.

If you have access to ash tree data and their condition, we'd love to work with you to help populate this wide-access database!

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IS EMERALD ASH BORER AN OBLIGATE MIGRANT?

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ABSTRACT

Computer-monitored flight mills with tethered emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), adults to measure flight speed, duration, and periodicity showed tethered beetles flew up to 5.2 km in two days at flight speed of at least 1.5 m/sec (3.5 mph). Females fly twice as far as males ($P < 0.002$), and mated females fly twice as far as unmated females ($P < 0.0001$). The discovery that mated females fly longer, further, and faster than either males or unmated females suggests that females are programmed to make a post-teneral dispersal flight. This is supported by the absence of a correlation ($R^2 = 0.007$) between distance flown and female size (Taylor et al. 2004).

Further study was done to better understand dispersal of mated females. After eclosion, female *A. planipennis* were allowed to maturation feed for 10 days and mate. One to three days later, 24 mated females were flown 8 h/day and allowed to rest, feed, and drink for 16 h for up to five days. The distance flown by these beetles ranged from 277 m to 9.84 km in two to five days. Their average flight speed over three days was 1.5 km/day, but 50% flew >4 km, and 10% flew >7 km. Of the latter group, the average flight speed was ~2.5 km/day. The distance flown declined from day to day, except for one mated female that flew >2 km per day for a total of 9.84 km in four days; she had been placed on the mill the day after mating. These results are consistent with our earlier conclusion that female *A. planipennis* engage in post-teneral migratory flight.

These studies were conducted in a more or less stimulus-free environment; thus, we have no knowledge of what external factors might influence flight thresholds and parameters. A better understanding of factors influencing flight behavior may facilitate development of effective management strategies for this highly mobile pest.

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EMERALD ASH BORER HOST RANGE AND PREFERENCE STUDIES

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ABSTRACT

Previous literature on the emerald ash borer (EAB) suggests that, in its native range in Asia, EAB will attack species other than ash (*Fraxinus*), including *Ulmus* sp., *Juglans* sp., and *Pterocarya* sp. In North America, as ash trees die in the core zone of infestation, concern has been raised about the potential for species other than ash to act as a suitable host for this pest. If an alternate host species were to be discovered, the impact on forest resources would increase dramatically in North America.

Our objectives were to 1) determine if EAB can oviposit and develop on potential alternate host species and 2) evaluate preference among four North American species of ash. We evaluated early instar development on logs of ash and potential alternate hosts that were placed on infested ash trees in field settings and in no-choice laboratory bioassays using cut branches and live nursery trees. We studied four ash species common in Michigan: green ash (*F. pennsylvanica)*, white ash (*F. americana)*, black ash (*F. nigra)*, and blue ash (*F. quadrangulata).* Potential alternate host species included American elm (*U. americana)*, black walnut (*J. nigra)*, hackberry *(Celtis occidentalis)*, Japanese tree lilac *(Syringa reticulata),* hickory *(Carya sp.)* and privet *(Ligustrum sp*.). We also assessed EAB host preference at field sites with multiple species of ash growing in close proximity.

In the no-choice laboratory bioassays conducted in 2003 and 2004, female EAB laid eggs on all species. There was larval feeding under the bark on all species except hickory. Larval development on the ash species appeared normal, but development on the non-ash species was highly impaired and resulted in larval death, with the exception of privet. Larvae on privet survived to the second instar and gallery development appeared. Additional studies to address larval development on privet are currently underway.

In 2004, 40 nursery trees including 10 green ash, 10 white ash, 10 Japanese tree lilac, and 10 black walnut, were transplanted in Ann Arbor, Michigan. Male and female beetles were caged on the lower 1-m section of the stem of each tree throughout the summer. The un-caged portions of the trees were exposed to wild beetle populations for the duration of the natural flight season. All trees were harvested and dissected during the winter. There were approximately 35 galleries/m^2 on the caged green ash stems, 0.75 galleries/m^2 on the caged white ash stems, and no galleries on tree lilac or walnut. On the upper, uncaged sections of the trees, we recorded approximately 150 and 75 galleries/m^2 on green and white ash trees,

respectively. No galleries were found on the un-caged portions of tree lilac or walnut. More than 65% of the galleries on green ash trees were on trees with rough bark.

In 2003, logs of green ash, walnut, and elm were attached to the main stem of infested green ash trees, 5 to 7 meters above the ground. In 2004, white ash and blue ash logs were added to the study and logs were attached to infested white ash trees. For both studies, less than four galleries were found on walnut and none were found on elm. Nearly 200 galleries per m^2 were found on green ash in 2003 and on white ash in 2004. This study was repeated in 2005 at two sites with predominately green ash trees and two sites with predominately white ash trees. One green and one white ash log were placed in each tree along with a third log that was either black or blue ash or black walnut. Logs from this study are currently being dissected to evaluate larval development and density.

Host preference was evaluated in 2003 and 2004 at three sites in the core zone that had both green and white ash street trees growing in close proximity. At all sites for both years, there were more exit holes per m^2 in the green ash trees than in the white ash trees and the green ash trees showed significantly more canopy dieback than the white ash trees. Similar studies were conducted in two woodlots that contained white and blue ash trees growing close together. At both sites, the number of EAB exits and woodpecker attacks were significantly higher in the white ash trees than in blue ash trees.

Overall, preliminary results suggest that EAB females will land and oviposit on species other than ash. Early instar feeding is limited and development is substantially impaired in non-ash species in both lab and field studies. Results also suggest that in situations where they are growing in close proximity, EAB prefer green ash over white ash and white ash over blue ash. Studies that are still in progress include a two-choice leaf-feeding bioassay using four North American species of ash.

INTERSPECIFIC VARIATION IN ASH RESISTANCE TO EMERALD ASH BORER

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ABSTRACT

Emerald ash borer (EAB) is an aggressive killer of even healthy ash in North America. However, reports suggest that EAB does not devastate ash in Asia, but rather that isolated outbreaks occur in response to stresses such as drought. Thus, emerald ash borer seems to behave in Asia much as its close native buprestid relatives do in North America, colonizing only stressed trees. This implies that Asian ashes may be generally resistant, with weakened trees preferentially colonized. Native trees may be more resistant to native pests because of natural defenses that have developed over their long coevolutionary history.

To test the hypothesis in the case of ash resistance to EAB, a replicated common garden planting containing North American, European, and Asian ashes was established at the Michigan State University Tollgate Education Center in Novi, Michigan with the following objectives: (1) compare resistance of major North American, European, and Asian ash species to emerald ash borer, (2) identify mechanisms of resistance/susceptibility of ash species to EAB, and (3) determine the effects of drought and other stress on susceptibility of ash species to EAB, as well as North American ash borers. After two years, Manchurian ash (*Fraxinus mandshurica*), which shares an evolutionary history with EAB, had significantly fewer EAB exit holes and less EAB induced-dieback than did white (*F. americana)* and green ash (*F. pennsylvanica*) cultivars, as well as Northern Treasure ash (*F*. x 'Northern Treasure'), which is a hybrid between native black ash (*F. nigra*) and Manchurian ash. These preliminary results are consistent with the hypothesis that Manchurian ash is a source of resistance genes to EAB by virtue of their coevolutionary history. However, it remains to be seen if this pattern will hold over time. Work is underway to determine whether this pattern has a phytochemical basis.

LIVING WITH EMERALD ASH BORER: MODELING ASH PHLOEM REMOVAL FROM FORESTS

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ABSTRACT

The exotic insect, emerald ash borer (*Agrilus planipennis*), is established in Michigan and in some surrounding states. At high population densities, this insect is capable of attacking and killing green, black, and white ash trees. Long-term prospects for management include biological control to reduce population densities of the insect and resistance or tolerance of ash trees to reduced population densities of the pest. In the short term, reduction of pest populations in local areas is achievable by removal of its breeding substrate.

Removal of ash from high priority areas such as those stands in close proximity to outlier populations will reduce the population density of this insect. Emerald ash borer larvae develop in the phloem of ash trees in stems and branches above approximately 2.5 cm diameter. We can estimate the amount of phloem available to the insect in a forest stand containing ash, and work to reduce the amount of ash phloem in the stand. We are currently developing models of the amount of ash tree removal necessary to reduce the breeding substrate by a target percentage.

Data have been collected over the summer of 2005 from standing white, green and black ash to estimate the surface area and to measure phloem thickness. Additional data on the condition of the tree and the position of the tree in the forest canopy and in relation to the forest edge have also been collected. Over the fall and winter of 2005, trap trees in Michigan and Northern Wisconsin will be cut, and diameter and phloem thickness of the trees and their branches will be determined. We anticipate up to 300 trees being added to our database from this source.

The ash phloem model will consider tree species, position of the tree in the forest canopy, and geographic location among other parameters. Forest resource professionals will be able to access the model to input stand data and ash phloem reduction targets. Model outputs will include the diameter limit for removal of ash to achieve the specific phloem reduction target. By reducing emerald ash borer populations through phloem reduction, this model will enable genetic diversity of ash to be maintained by not requiring the removal of the smaller trees in a stand.

BIOLOGICAL COST OF ERADICATION: CONSEQUENCES TO THE NATIVE PLANT COMMUNITY

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ABSTRACT

This project focuses on the biological consequences of the implementation of the emerald ash borer eradication program. Specifically, the goal is to examine how changes in the light environment and soil compaction influence changes on the native plant community structure. This project is conducted at Pearson Metropark, located in Lucas County in North West Ohio, where the Ohio Department of Agriculture has instated a quarantine protocol that calls for the removal of every ash tree within a ½-mile radius around a positively infested tree. Due to the discovery of three infested trees identified in Pearson in the spring of 2005, the Park was chosen to receive aggressive eradication with 17,000 ash trees tagged for removal. The eradication protocol, applied April-July of 2005, was supposed to be implemented with as minimal impact to the habitat as possible.

However, inevitably the altered state of the habitat is immediate with drastic environmental changes. Gap formations will occur very fast and on a much larger scale because multiple trees are being removed from area at a time. This contributes to an increase in duration and intensity of light reaching the forest floor. In addition, the effect of heavy vehicles used to remove trees may lead to an increase in soil compaction. Each of these factors is likely to influence the composition of existing plant community. This project is designed to compare species composition and structure of plant communities between cut and uncut areas.

The sampling design consists of fourteen 20x25m plots (eight uncut plots and six cut plots). A baseline of the plant community structure was assessed before cutting began and then again after cutting was completed. Measurements for overstory composition and structure were collected with similar information gathered for the shrub layer and the herbaceous understory. The light environment was assessed using hemispherical photography to determine solar radiation regimes (direct and diffuse radiation) and plant canopy characteristics. Each fisheye image is 10 m in diameter, and six pictures were taken from every plot (four corners and two from the center). Soil compaction measurements were also taken in all 14 plots. A soil penetrometer was use to determine the pressure in pounds per square inch (PSI) needed

to penetrate through the soil profile. Readings were taken at five different depths 3, 6, 9, 12, and 15 inches. Six readings were taken from each plot.

Preliminary results show significant differences in the light environment and soil compaction between the cut and uncut plots. There is an increase in the amount of light that is reaching the forest floor in cut plots compared to uncut plots. In addition, the degree of soil compaction is not only greater in the cut vs. uncut plots, but at every depth there are higher levels of compaction between the cut and uncut plots. These changes to the abiotic environment are likely to influence the dominance and distribution of plant community members. Monitoring the altered habitat and resultant community structure will continue over the next several years. However, some of the changes observed thus far create ideal opportunities for the spread of invasive plant species.

GUIDELINES FOR EX-SITU SEED COLLECTION IN THE STATE OF OHIO

ABSTRACT

This continuing project is an effort to develop guidelines for *ex situ* seed collection. The aim of *ex situ* conservation is to capture and preserve the genetic variation and the biological and economic potential of species that are of interest for human societies. This conservation strategy is relevant at times when there are major threats to the preservation of plant genetic resources—for example, in the spread of the emerald ash borer. *Ex situ* methods have contributed to the conservation of genetic resources of several important crops and trees.

In this study, microsatellite markers are being used to assess the genetic structure of green ash and white ash. In addition, other breeding parameters, such as the mean number pollen donors contributing to the seed crop of each trees will be estimated. These factors will be taken into consideration in order to develop a germplasm sampling design. An optimal design will provide information about locations for intensive collection, the number trees to be sampled in that area, and the number of seeds collected per tree in order to accurately represent the genetic diversity for those species.

IMPACT OF EMERALD ASH BORER AND OTHER DISTURBANCES ON RURAL AND URBAN FORESTS IN MICHIGAN

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ABSTRACT

Two ash monitoring systems are being established throughout rural forests and recreational areas of Michigan to address three objectives: 1) detect presence of emerald ash borer (EAB), 2) monitor current conditions and changes over time in rural forests and recreational sites throughout Michigan with and without EAB, and 3) determine other factors responsible for variations in ash health over the state.

The Rural Ash Monitoring Plot System (RAMPS) was initiated during the summers of 2004 and 2005 with the establishment of over 300 plots along five gradients running through the state of Michigan. At these plots, variables measured include stand age, soil texture, live and dead basal area by species, crown variables (live crown ratio, light exposure, transparency, density, and dieback), tree vigor, presence of EAB, and other types of tree damage. During 2004 in the Lower Peninsula, the percent of dead ash varied from 0% to 100% by plot. When plot data were pooled by county, examples of the average percent of dead ash in 2004 are: Arenac County—1%, Midland County—3%, Gladwin County—5%, Lapeer County—6%, Cheboygan County—7%, Oakland County—15%, Washtenaw County—31%, and Wayne County—61%.

The second ash monitoring system examines ash at over 262 sites in Lower Michigan that are in or near recreational areas (parks, picnic areas, rest areas, boat landings, campsites, etc.). A variety of tree health variables are measured at these sites along with recording presence of EAB and other types of tree damage. The major results from Summer 2004 are: 1) green ash and white ash were by far the most common ash species at our sites, 2) the origin of ash (natural, planted, or both) differed by site, with 50% of the sites containing all natural ash or almost all natural ash, 3) size of ash trees varied greatly by site, but over 50% of all sites had mean diameters above 25 cm, 4) mean percent ash dieback by site ranged from 1% to 30% both for sites with and without EAB, 5) percent mortality of ash trees varied from 0% to 100%, and 6) the potential EAB risk of sites may vary considerably due to presence or absence of ash and percent of ash dieback.

THE IMPACT OF EMERALD ASH BORER ON FORESTS WITHIN THE HURON RIVER WATERSHED

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ABSTRACT

The ability of exotic species to invade and alter the structure and function of native landscapes may be influenced by community structure and composition. The invasive insect emerald ash borer (EAB) (*Agrilus planipennis*) has already caused widespread ash (*Fraxinus* spp.) mortality in Michigan and Ohio. The objectives of the project are to: 1) characterize invaded stands to determine the effect of community composition on susceptibility of forests to invasion by EAB and 2) determine the effects of ash mortality on successional trajectories of invaded forest communities.

Thirty invaded stands within the Huron River watershed in southeastern Michigan were characterized by quantifying ash density, EAB-induced ash dieback and composition of the associated woody plant community along a landscape gradient that varies from dry upland sites to low wetland sites. Three replicated ¼-acre circular plots were established within each stand. The degree of EAB infestation was quantified by estimating ash canopy dieback and counting D-shaped emergence holes and woodpecker attacks on the boles of infested trees. Community response to ash decline and death is focused on replacement of ash in the canopy by established saplings and seedlings.

Sampled stands vary in ash density, ash basal area, stand density, and stand basal area. None of these variables are correlated with the degree of EAB-induced ash dieback. However, EAB-induced ash dieback of invaded stands is negatively correlated with stand distance from the putative epicenter of the invasion ($r^2 = 0.18$, $P = 0.017$). This suggests that all stands are susceptible regardless of community composition, and that all stands will ultimately experience

significant EAB-induced dieback and subsequent ash mortality as EAB continues to spread. Dieback of black ash (*F. nigra*) is greater than that of white ash (*F. americana*) and green ash (*F. pennsylvanica*) across all sampled stands (ANOVA, $P < 0.001$). Possible explanations for this observation include: 1) EAB may prefer to oviposit on black ash, 2) EAB may prefer the more open riparian and marshy habitats of black ash, and 3) black ash is inherently less resistant or tolerant to EAB than are green and white ash.

Fraxinus is the most common genus in the canopy, sapling and seedling layers of sampled stands. High ash density in the sapling and seedling layers may sustain EAB populations after the overstory succumbs, complicating eradication efforts. *Ulmus* (elm), *Acer* (maple) and *Prunus* (cherry) were also common in the understory of sampled stands; the relative dominance of these three genera will increase as it is likely that established saplings will replace mature ash that are killed by EAB. All plots are permanently mapped and future studies will document the progression of ash dieback over time, and monitor successional trajectory of invaded stands while evaluating the long-term ecological impacts of the emerald ash borer invasion.

DISTRIBUTION OF ^{14}C-IMIDACLOPRID IN *FRAXINUS* SPP. AND EFFECTS OF IMIDACLOPRID ON THE EMERALD ASH BORER

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ABSTRACT

Imidacloprid applied as a trunk injection is often effective in protecting ash trees from emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). The effectiveness of trunk injection treatments varies, however, suggesting that the chemical may not be completely or uniformly distributed within the tree. In June 2004, we applied radiolabeled ^{14}C-imidacloprid via trunk injection to assess the distribution, persistence and movement of imidacloprid in 40 green ash and white ash trees over two years. We also conducted bioassays to determine the response of EAB adults that were fed leaves from injected trees

METHODS

On June 14, 2004 we injected twenty container-grown green ash (*Fraxinus pennsylvanica*) trees (7.6 cm DBH) and twenty white ash (*F. americana*) trees (10.1 cm DBH) with 6 ml of imidacloprid (10% a.i.). The trunks of the trees were injected at 15 cm above ground level via two injection ports on opposite sides of the tree. Each tree received 25 µCi of ^{14}C-imidicloprid in a ratio of 1:2400 (labeled:non-labeled imidacloprid). After injection, half of the trees were kept well watered (3.8 cm irrigation week^{-1}) and half were subjected to water stress (1 cm irrigation week^{-1}). Imposition of water stress was verified with periodic measurements of soil water content and leaf gas exchange. We collected leaf, twig, trunk, and root samples 0, 2, 7, 21, 60, 105, and 150 days after treatments (DAT). After 105 DAT, five trees from each species were covered with netting to collect litterfall samples. All samples were brought to the lab; oven-dried, ground, weighed, and oxidized in biological tissue oxidizer. The resultant $^{14}CO_2$ was trapped in scintillation cocktail and radioactivity was determined by scintillation counting. A subsample of fresh leaves was collected 21 and 60 DAT for bioassays on adult EAB. In 2005, we sampled leaves, roots, stem, and trunk tissue for the trees that were injected the previous year. Samples were collected in June, July, and August. We destructively sampled eight trees (two replicates) for biomass analysis to develop a whole-tree ^{14}C budget.

RESULTS – 2004

Radioactivity in leaves collected from injected trees increased steadily from two days after treatment (DAT) to 105 DAT, reaching peak levels at the end of the season. There was little radioactivity in twigs and roots, where ^{14}C-imidacloprid levels did not significantly differ from zero. In autumn, radioactivity in leaf litterfall was as high or higher than samples collected at 60 DAT, indicating little re-translocation of imidacloprid or imidacloprid metabolites from leaves before leaf-fall. Specific activity (cpm g^{-1}) was somewhat lower in white ash than green ash, probably reflecting dilution in the white ash trees, which were slightly larger than the green ash. Mortality and knock-down of EAB adults that were fed leaves from the injected trees exceeded 70% at 21 and 45 DAT. Topical assays indicated an LD_{50} value of 7 ng/beetle, which is relatively low compared to other beetle species.

RESULTS - 2005

We re-sampled trees injected in 2004 to determine the persistence of ^{14}C-imidacloprid in the spring and summer of 2005. We detected radioactivity in the leaves of the treated trees in June and July 2005, but ^{14}C activity (indicating presence of imidacloprid and metabolites) was less than 10% of the activity observed during the injection year. Similarly, bioassays indicated that adult beetle survival varied little between leaves from treated and untreated trees. This indicates imidacloprid treatments would need to be repeated annually in order to be effective against EAB adults. We also began preliminary work to assess toxicity of imidacloprid metabolites on EAB adults. Results from topical assays indicate that the most toxic compound was imidacloprid, followed by ^{5}OH-imidacloprid and the olefine metabolites. We are continuing analysis to develop whole-tree ^{14}C budgets and developing phosphor screen autoradiographs from trunk segments to produce a graphic image of ^{14}C-imidacloprid distribution.

TIMING OF IMIDACLOPRID SOIL DRENCHES FOR EMERALD ASH BORER CONTROL

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ABSTRACT

BACKGROUND

Many arborists and homeowners are concerned about the appropriate time of year to apply imidacloprid as a soil drench to protect landscape ash trees from emerald ash borer (EAB), *Agrilus planipennis*. Current recommendations are to start treating ash trees as soon as the beetle is discovered infesting a new area. Depending on when infestations are reported, imidacloprid may be applied as a soil drench in the fall, spring, or summer. We conducted a two-year study to test the efficacy of imidacloprid applied as a soil drench around the base of ash trees at three different times of year. Our objective was to determine the best time of year to apply an imidacloprid soil drench to offer maximum protection from EAB.

METHODS

In spring 2003, we planted 130, 1-inch-caliper trees at the Michigan State University Tollgate Conference Center in Novi, Michigan. This area has had large populations of EAB since the pest was first discovered in summer 2002. We included four cultivars of North American ash, one cultivar of Asian ash, and a hybrid cultivar of native black ash crossed with an Asian ash species. The six cultivars and their production method are given in Table 1.

Table 1. The six cultivars of ash trees planted at Tollgate Conference Center in Novi, Michigan.

Fraxinus sp.	Cultivar	Code	Production Method
F. pennsylvanica	Patmore	FpP	container
F. americana	Autumn Blaze†	FaAB	container
F. mandshurica	Mancana	FmM	container
F. americana	Autumn Purple†	FaAP	container
F. pennsylvanica	Marshall Seedless	FpMS	bare root
F. americana	Autumn Purple	FaAP	bare root
F.nigra x *F. mandshurica*	Northern Treasure	FNT	bare root

† 'Autumn Blaze' and 'Autumn Purple' container-grown trees combined into one group.

All trees were planted in a randomized complete block design, blocked by cultivar and production method. We randomly assigned eight blocks (n = 24 trees) to one of three application dates: November, April, and early June. An additional 16 blocks (n = 48 trees) were assigned to the control group and received no insecticide treatment. The six treatment

dates were: 7 November, 2003; 23 April, 8 June, and 11 November, 2004; 28 April and 9 June, 2005. We applied Merit® 75WP to each tree at a constant rate of 1.7 g a.i. per 2.5 cm of trunk diameter because trunk girth was not highly variable among trees. For each application date, we mixed 60 g Merit® 75WP in 6,000 ml of water to create a stock solution. We then added 225 ml of stock solution to a 3.8-L pail of water, mixed well, and poured the entire solution evenly around the base of each tree.

All trees were exposed to EAB during the summers of 2003 and 2004. To assess levels of attack, we counted the number of D-shaped exit holes (formed by adult beetles as they emerge from host trees) in September 2004 and 2005. These counts quantified the level of EAB attack in 2003 and 2004, respectively. Trees that died in 2004 from attack during the previous year did not produce any additional adult beetles, so these trees were not included in the exit hole counts for 2005. We observed that container-grown and bare-root trees differed in root size and overall vigor, so we analyzed trees within each group separately. Data on the density of exit holes were not normal after log and square-root transformations, so we used the non-parametric Kruskal-Wallis test to separate application treatments. In June 2004 and 2005, we assessed canopy dieback for each tree to determine the level of damage inflicted by EAB. Trees were rated on a scale of 0 to 5, with 0 being 100% dieback (dead) and 5 being 0% dieback observed.

RESULTS AND DISCUSSION

Tree mortality due to EAB is shown for each treatment group in Figure 1. First-year mortality is attributed to EAB attack in 2003 prior to any soil drench treatments, whereas second-year mortality is due to EAB attack in 2004 after treatments were implemented. Overall, percent mortality was lowest for ash trees treated with Merit® 75WP in June of each year. Also, no additional mortality was observed for June-treated trees from 2004 to 2005. During the same time frame, mortality increased by 9% (n = 2 trees) in November-treated trees, 12% (n = 3 trees) in April-treated trees, and 21% (n = 10 trees) in untreated trees.

In general, exit hole density was greater in 2004 than 2005 (Figs. 2a, b). In 2004, there were no significant differences in mean exit hole density among trees treated with Merit® 75WP in November 2003, April 2004, or June 2004, and these were not significantly different from untreated trees (Figs. 2a, b). These results are expected given that EAB larvae were feeding within these trees prior to any soil applications of Merit® 75WP. In 2005, untreated container-grown trees had significantly higher densities of exit holes than all three groups of trees under treatment (Fig. 2a). For trees under treatment, November-treated trees had the lowest mean density of exit holes, but were not significantly different from the April- and June-treated trees. For bare root trees, there were no significant differences among treatment groups in mean density of exit holes in 2005 (Fig. 2b).

Canopy dieback was generally higher in 2004 than 2005 for both container-grown and bare root trees. The decrease in dieback in 2005 may be the result of removing trees that had died in 2004. Nevertheless, container-grown trees that received Merit® 75WP showed less dieback than trees that were left untreated. For bare root trees, only June-treated trees showed less dieback than untreated trees.

Because container-grown trees were generally healthier than bare root trees initially, we felt it necessary to analyze these two groups separately. Our results suggest that treating small trees (i.e., less than 6 inches DBH) with Merit® 75WP during the fall, spring, or early summer can reduce tree mortality due to EAB, reduce dieback symptoms due to EAB attack, and significantly reduce the number of adult beetles that successfully develop in trees. However, trees treated in early June may benefit the most in terms of mortality, canopy dieback, and number of beetles developing successfully. Moreover, it is important to keep trees healthy to maximize the benefit of drenching trees with Merit® 75WP.

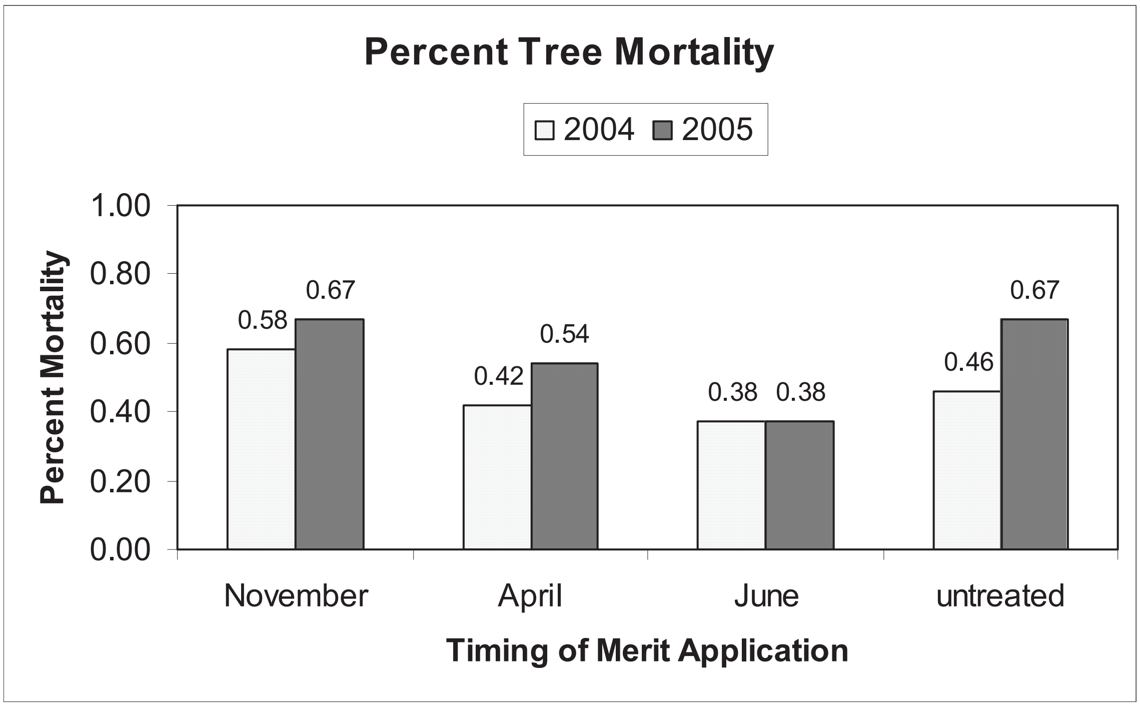


Figure 1. Percent mortality due to emerald ash borer for ash trees under three different Merit soil drench treatments and no treatment as assessed in 2004 and 2005.

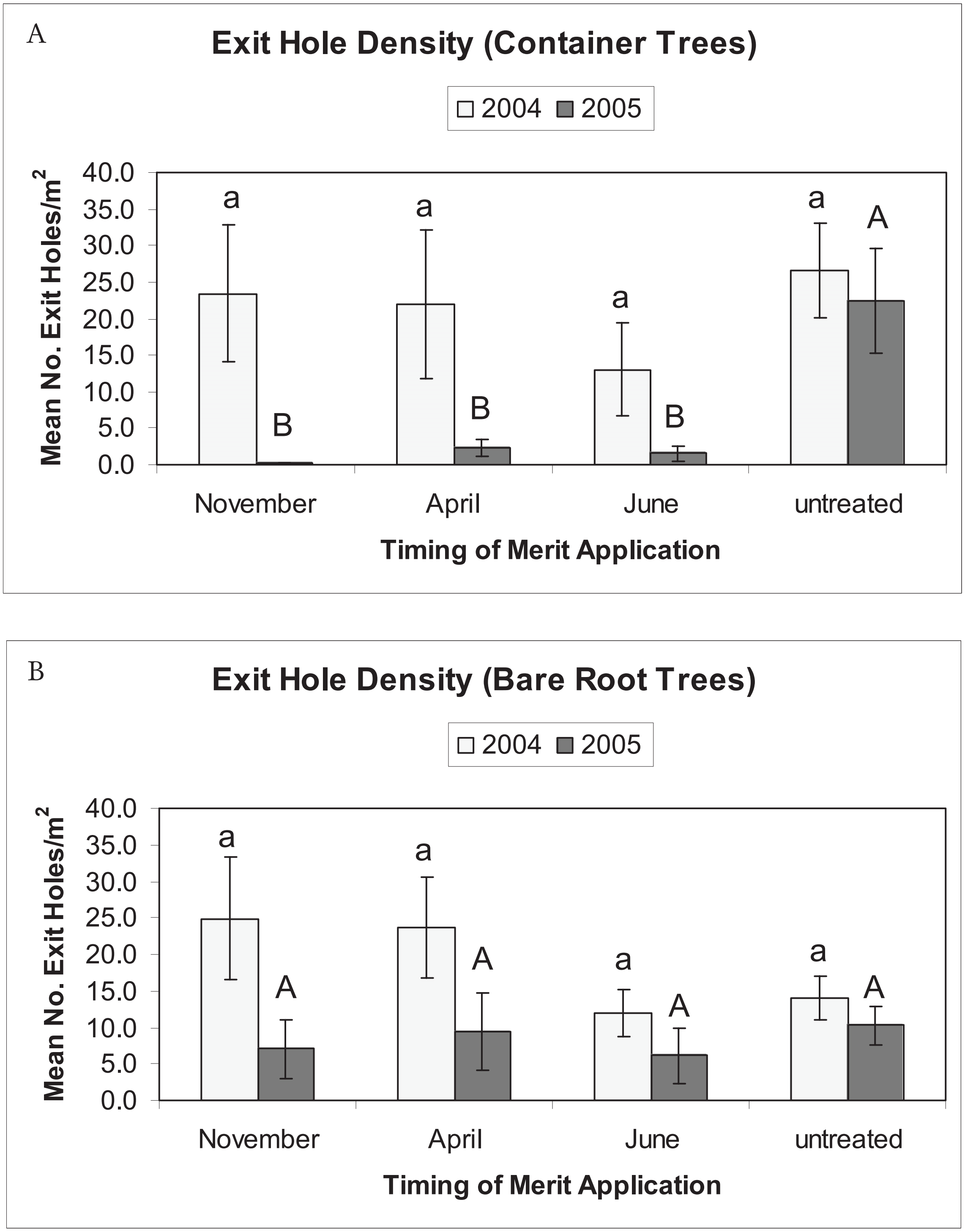
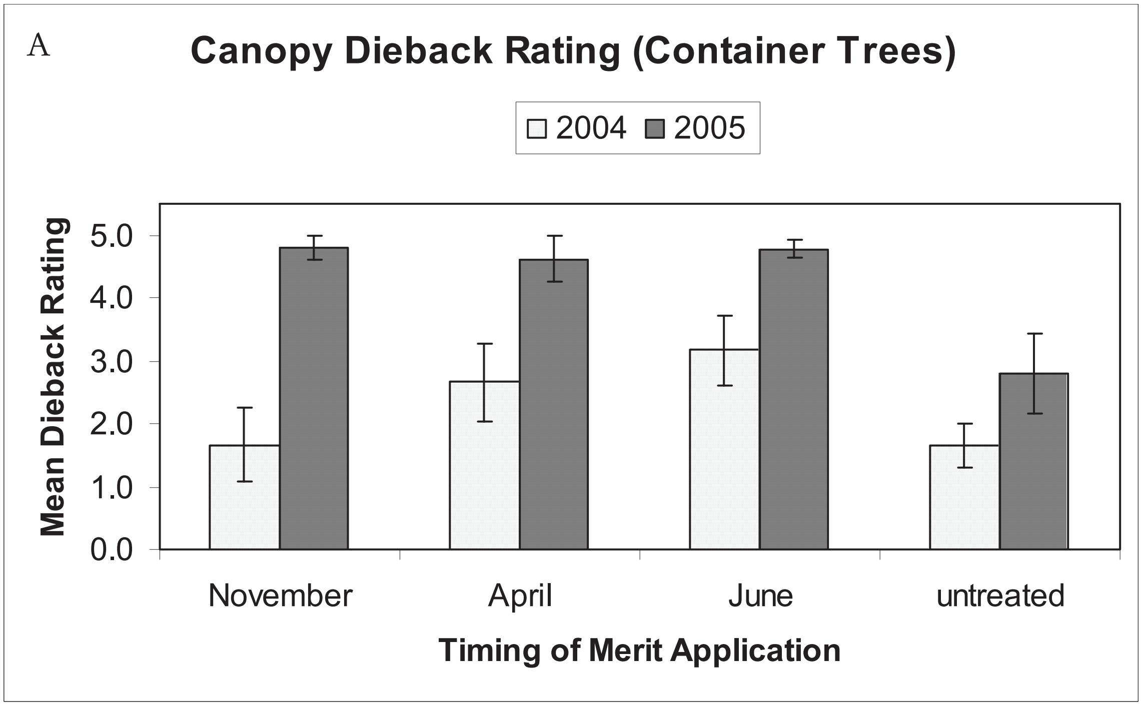


Figure 2. Mean number of D-shaped exit holes (± S.E.) per square meter of trunk for (A) container-grown and (B) bare-root ash trees under three different Merit soil drench treatments and no treatment as assessed in 2004 and 2005. Trees that died from EAB attack in 2004 were not included in the assessment of exit hole density in 2005. Means with different letters within a year are significantly different (Kruskal-Wallis test, $P < 0.05$).



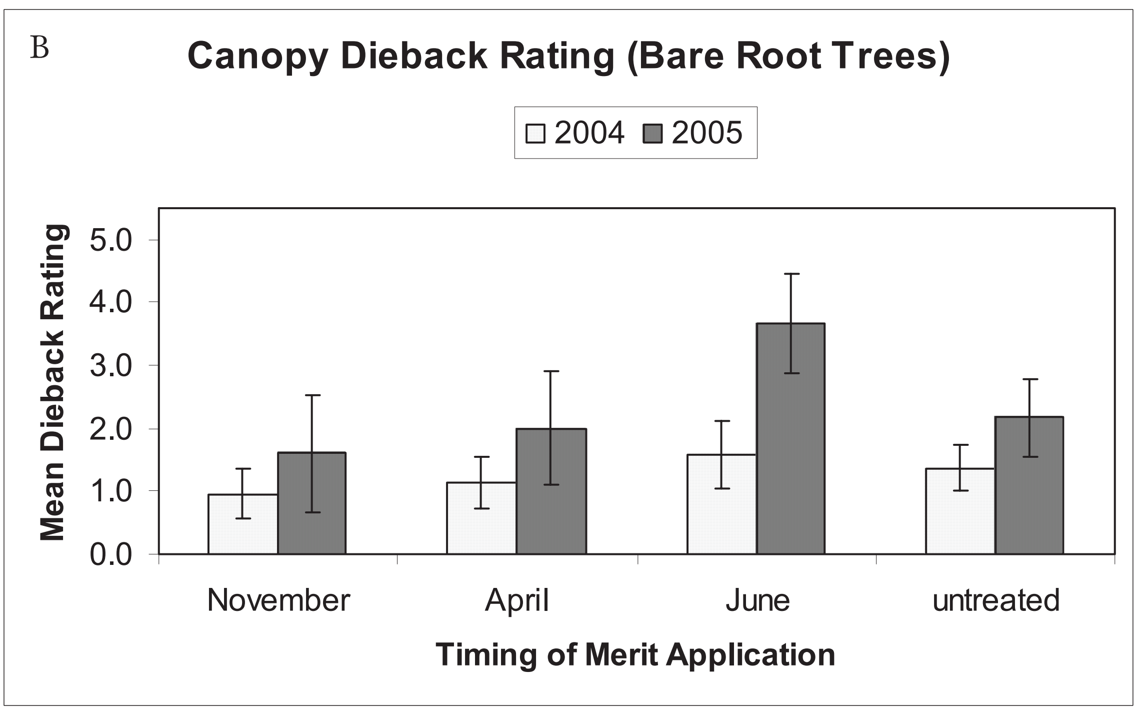


Figure 3. Mean canopy dieback rating (± S.E.) for (A) container-grown and (B) bare root ash trees under three different Merit soil drench treatments and no treatment as assessed in 2004 and 2005. Trees were rated on a scale of 0 to 5, with 0 being 100% dieback (dead) and 5 being 0% dieback observed. Trees that died from EAB attack in 2004 were not included in the assessment of canopy dieback in 2005.

LONG-TERM (THREE-YEAR) RESULTS OF TRUCK INJECTIONS FOR EMERALD ASH BORER CONTROL IN LANDSCAPE ASH TREES

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ABSTRACT

In southeastern Michigan, many landscapers and arborists are treating ornamental trees with annual trunk injections of imidacloprid to protect them from emerald ash borer (EAB), *Agrilus planipennis* Fairmaire. The two most popular products are Imicide, which is injected using Mauget capsules, and Pointer, which is injected with a wedgle, an apparatus that resembles a modified hypodermic needle. Application methods differ: uptake of Imicide from Mauget capsules, which are only lightly pressurized, is largely passive and can require several hours to be completed; in contrast, wedgle injections can be completed quickly but involve much higher injection pressure. Another product, Injecticide-B (bidrin), is used occasionally for EAB control: this organophophate insecticide is translocated more rapidly through the tree than imidacloprid products, which can be advantageous in some situations.

Results from our previous studies have shown that efficacy of trunk injections varies depending on injection timing and method, tree size and vigor, and EAB pressure. Because EAB is a relative newcomer to North America, however, there have been few long-term evaluations of insecticide effectiveness. Here we report results from an ongoing study designed to monitor EAB density and tree condition over time.

STUDY SITES AND TREATMENTS

We are testing Imicide, Pointer, and Injecticide-B in street trees growing in two subdivisions in Ann Arbor, Michigan. In May 2003, we randomly assigned 30 green ash trees (average of 42 cm DBH) in the Dartmoor neighborhood to one of five treatments: untreated Control, Imicide (10%, one 3 ml Mauget capsule per inch DBH/2), Pointer (12% in 2003, 5% in 2004 and 2005, 1 ml per 10.2 cm basal circumference injected with a wedgle), an "early" Bidrin treatment (12%, one 2 ml Mauget capsule per inch DBH/2), or a "late" Bidrin treatment (same specification). At the second site in the Forsythe neighborhood, we are monitoring 18 green ash trees (16 cm DBH) and 18 white ash trees (13 cm DBH). These trees were randomly assigned to be treated with either Imicide or Pointer, or left as untreated controls.

TREATMENT TIMING

Treatments have been repeated annually; 2005 represents the third year of injection. Imidacloprid (Imicide or Pointer) injections at both sites occurred on 21 May 2003, 19 May 2004, and 16 May 2005. "Early" injections of Injecticide-B were made in June to target adult beetles and occurred on 2 June 2003, 15 June 2004, and 16 June 2005. "Late" injections of Injecticide-B were made in mid July to target young larvae and occurred on 14 July 2003, 19 July 2004, and 21 July 2005.

EVALUATION

Canopy condition of each tree was estimated periodically in all three years. Number of exit holes per m^2 was determined in September 2004 and again in September 2005 on five sections (each 3800 cm^2) of each tree to estimate the cumulative production of EAB adults. Density of larval EAB was quantified in three to four bark windows (each ca. 300 cm^2) excavated on each tree in September of 2004 and 2005.

RESULTS – DARTMOOR

At the Dartmoor site, canopy dieback on the untreated Control trees averaged roughly 15% in June 2003 and remained at approximately 16% in July 2004. Dieback on Control trees, however, increased dramatically to an average of 50% in July 2005. Pre-treatment canopy dieback on all injected trees ranged from 15-19% in June 2003 and remained below 20% in all treatments in 2004. In July 2005, canopy dieback on treated trees ranged from an average of roughly 15% (Imicide) to 28% (Early Injecticide-B) and was significantly lower than dieback on Control trees.

Increased canopy dieback observed in 2005 was associated with increases in EAB attacks, particularly on the Control trees. Number of EAB exit holes on the untreated Control trees, which averaged roughly 6 exits per m^2 of phloem prior to EAB emergence in 2003, increased to 11 per m^2 in 2004 and was up to 30 per m^2 of phloem in 2005. In 2003, pre-treatment exit hole density on all injected trees ranged from 1 to 5 holes per m^2 of phloem. Density of exit holes remained low in 2004, averaging no more than 10 holes per m^2 of phloem in any treatment. In 2005, exit hole density on treated trees ranged from 11 to 15 holes per m^2 of phloem. Density of EAB in all Dartmoor trees may be increasing, however; larval density, quantified in September 2005, averaged 72 EAB per m^2 of phloem in Control trees and 51 to 70 EAB per m^2 in treated trees.

RESULTS – FORSYTHE

At the Forsythe site, green ash trees have consistently sustained higher EAB attack rates than white ash trees. Canopy dieback on untreated green ash Control trees has increased from an average of about 13% in 2003 to 63% in 2004 and 80% in 2005. One Control tree died and was removed during the 2005 summer. Canopy dieback on Control trees was significantly higher than dieback on treated trees. Dieback of green ash injected with Imicide remained at 20-25% in 2005, while Pointer-injected trees averaged 40 to 45% dieback in 2005. One green ash injected with Pointer died in 2005. White ash trees were substantially healthier than green ash trees in 2004 and 2005. Dieback on control and treated white ash trees increased slightly from 2004 but ranged from roughly 15 to 25%.

Density of EAB in the green ash Control trees had reached very high levels in 2005, averaging 117 holes per m^2 of phloem. Differences in exit hole density among all three treatments were significant in 2005. Imicide appears to be providing effective EAB control; the Imicide trees averaged only 12 exit holes per m^2 of phloem in 2005. In contrast, exit holes in green ash injected with Pointer averaged 55 per m^2 of phloem in 2005, about 30% more than in 2004. On the white ash trees, density of exit holes on all trees was roughly twice as high as in 2004. Average exit hole density on the white ash trees ranged from about 20 to 32 holes per m^2 of phloem, and differences were not significant. White ash trees are expected to experience increasing EAB pressure as green ash trees decline and succumb.

We hope to continue to treat and monitor the Dartmoor and Forsythe trees to assess EAB attack rates and the long-term efficacy of these products in green and white ash. Over time, EAB pressure may decline as ash trees in these neighborhoods and surrounding areas are killed. This situation could perhaps reduce the need for annual treatment and potential damage to trees caused by repeated wounding from trunk injections.

NON-INVASIVE NEONICOTINOIDS: TREATMENTS FOR ASH LOGS AND TREES

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ABSTRACT

"Invasive" application of neonicotinoids via trunk injection has become a popular method for protecting landscape ash trees from emerald ash borer (EAB) in the infested area of southeast Michigan. Trunk injection, primarily with imidacloprid products, is relatively safe for applicators, reduces effects on non-target organisms, and can provide effective control in some settings. However, trunk injections are relatively labor-intensive, and the repeated annual wounding over a long time period may threaten tree health.

Our first study explored the use of a new approach to systemic application of neonicotinoids: surface application of neonicotinoids in Pentra, a surfactant that functions as a bark penetrant carrier. We compared a basal application of Pentra mixed with imidacloprid (Merit 75WP) at two rates (0.39 and 0.78 g AI per inch DBH) to high pressure soil injection of Merit 75WP (0.55 g AI per cm DBH), a standard trunk injection of Imicide via Mauget capsules (0.06 g AI per inch DBH), and untreated controls. Soil injections were made on 5-6 May 2005; Pentra and Mauget treatments were made 24-25 May. Treatments were applied at three sites: HM (six replicates of green ash, 30 cm DBH, 24% June dieback), SL (eight replicates of white ash, 38 cm DBH, 24% June dieback), and HW (10 replicates of green ash, 20 cm DBH, <10% June dieback). Evaluations conducted in June, July and August included measurement of imidacloprid residues (ELISA) in composite samples of xylem sap and leaves collected from each tree. Bioassays were also conducted each month to assess adult EAB survival and leaf consumption. Larval density was determined for each tree at HM and SL sites in September 2005 by removing bark from two opposing 15x30cm windows on the trunk (1.5m) and 6 10x30cm windows distributed between the first branch and the highest 8cm diameter branch in the canopy. (The HW trees sustained very light attack by EAB in 2005 and will be used in related studies in 2006).

Imidacloprid residues in xylem sap collected in July were significantly higher for trees treated with the Pentra/Merit application than the Control trees at SL, although differences were not pronounced (< 30 ppb). At the SL site, July xylem sap values were significantly higher for the trees treated with Imicide or the soil injection compared with controls, but again, differences were relatively low. Effects of treatments on EAB adults were negligible: no differences in mortality were observed at either site. Leaf consumption was slightly lower for beetles caged with foliage from trees treated with the soil injection than the controls at the SL site. There were no effects of treatment on larval density at either the HM or SL sites ($P>0.10$).

The failure of any treatment to effectively control EAB in the HM and SL sites is probably attributable to several factors. Efficacy of Imicide delivered via trunk injection may have been impaired by poor uptake from capsules at both sites; we noted that over 20% of the capsules were empty when they were removed 48 hours post-application. Uptake or translocation may have also been problematic for trees treated with the high pressure soil injections. We observed similarly poor efficacy in other studies involving large trees that were treated with soil injection. Previous EAB damage to transport tissues can, of course, impair imidacloprid uptake or translocation. The pre-treatment density of EAB at the SL site, however, was five-fold lower than the EAB density at the HM site, indicating that other factors must have been involved. It is possible that beetles that oviposited on our study trees did most of their feeding on untreated trees outside the study areas. Our trees at both the SL and HM sites were surrounded by forested areas with high numbers of ash trees. In that case, our systemic treatments would have little effect on adult beetles, and data from previous studies suggest that spring injections of imidacloprid rarely have substantial effects on larvae. The Pentra treatments may have failed for any number of reasons; lacking experience with this novel treatment, our selection of application rates, formulation, and timing was based on studies conducted with different tree species and different products. However, the detection of elevated imidacloprid residues in xylem sap in Pentra-treated trees at one site demonstrates the potential use of this approach, and additional studies are planned for 2006.

We also investigated the use of non-invasive applications of neonicotinoids for control of EAB in felled ash trees or ash logs, particularly at outlier sites. Such a treatment could provide an alternative to current practices, which require intensive labor and mechanical processing (chipping, burning) to eliminate potential EAB emergence from cut material. We examined the effectiveness of surface applications of Pentra combined with neonicotinoid insecticides for control of pre-emergent adult EAB in spring and control of feeding larvae in fall.

In the first trial, we sprayed sections of seven freshly cut ash logs (ca. . 15 cm diameter) with Pentra and Merit 75WP (ca. 0.35 g AI/cm diameter) on 26 May 2005, before EAB emergence began. Adjacent logs from the same trees were used as controls. There were no exit holes on the logs at the time of treatment. On June 22, logs were examined for EAB exit, holes and then debarked. On average, 7.25 EAB adults emerged from the unsprayed logs while no adults emerged from sprayed logs. We did find a few live EAB adults or pupae under the bark; whether these would have successfully emerged or died while chewing through the bark is unknown.

In the second trial, we cut five 30-cm diameter logs from infested ash trees on 6 August 2005. Each log was sprayed with one of three neonicotinoid compounds at ca.. 0.35 g AI/cm diameter: Merit 75WP, Merit 2F, and Safari. Each product was applied alone, or in combination with 1.5 oz/gal Pentra, or was left as an untreated control (water spray only). Half of the logs were debarked on 9 September, and the number of larvae was counted. None of the spray treatments reduced larval density compared to the untreated controls. The remaining logs will be held outdoors until spring to assess adult emergence.

Further studies planned for 2006 will address the efficacy and reliability of Pentra/ neonicotinoid treatment of logs in relation to the size and condition of ash material. We will also evaluate timing and refine application methods.

EFFICACY OF SPINOSAD ON ADULT EMERALD ASH BORER

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ABSTRACT

Spinosad was tested for toxicity to emerald ash borer (EAB) adults by topical application as well as exposure to treated foliage. Adults exhibited mortality in a dose-dependent manner when technical spinosad was applied topically. EAB adults ingesting or coming into contact with treated foliage were similarly impacted; the lowest labeled rate retained activity 12 days after application. Quick knock-down after exposure to treated foliage was observed at the rates tested, and one of these rates was within the range of concentrations tested topically (375 ppm). There was no apparent adult repellency, even up to 10 times the maximum labeled rate.

BACILLUS THURINGENIENSIS: POTENTIAL FOR MANAGEMENT OF EMERALD ASH BORER

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ABSTRACT

The active ingredients of microbial insecticides are live microorganisms pathogenic to certain insects. One such insect pathogen is *Bacillus thuringiensis* (Bt), a bacterium found naturally in soil, on leaves, in places were insects are abundant (such as grain silos and insectaries), and in infected insects. Bt-based microbial insecticides are formulated with spores and materials produced during fermentation, including crystalline insecticidal proteins—also known as Cry toxins. After ingestion, these Cry toxins are responsible for the majority of Bt's insecticidal activity and narrow host range. Cry toxicity results in damage to the insect midgut, followed by bacterial septicemia and death. As of 1997, ca. 190 Bt-based microbial insecticides were registered in the United States for control of certain lepidopteran, dipteran, and coleopteran pests. There are also Bt strains active against species of Homoptera, Hymenoptera, Orthoptera, Mallophaga, nematodes, mites, and protozoa. Microbial insecticides formulated with Bt are the primary management tools used to control forest insect pests due to their narrow host range, superior safety record, public acceptance, and compatibility with other management strategies such as biological control (Reardon et al. 1994).

In 2003, we initiated research to identify Bt strains active against EAB. Initially, we screened emerald ash borer (EAB) adults with four Bt-based microbial insecticides registered for control of lepidopteran and/or coleopteran pests. Although the products showed some activity against adult EAB, rates 4 to 12 times maximum labeled rates were required to achieve 66 to 98% mortality six days after treatment (Bauer et al. 2004).

To identify fractions responsible for EAB mortality in the Bt products, we bioassayed EAB adults with crystalline Cry3Aa from coleopteran-active *B. thuringiensis* subsp. *tenebrionis* (Btt); spore/crystal complex from lepidopteran-active *B. thuringiensis* subsp. *kurstaki* HD-1 (Btk); solubilized protein from Btk; soluble Cry1Aa, Cry1Ab, or Cry1Ac toxins cloned from Btk and grown in *E. coli*; spores of Bt 4Q22, an acrystalliferous strain; and toxin mixtures.

These were bioassayed by allowing EAB adults to imbibe droplets containing known quantities of toxin and/or spores; daily mortality was assessed for up to seven days. Neither Cry3Aa (30 µg toxin/dose) nor the Cry1A toxins (3 µg toxin/dose), dosed separately or as mixtures, were toxic to EAB adults. Bioassay of Btk crystal/spore complex (ca. 3 µg protein + 1.3×10^4 spores/dose) in EAB adults resulted in ca. 78% mortality, whereas solubilized Btk proteins (3 µg protein/dose) caused 25% mortality. To ascertain the role of spores, we treated EAB adults with Bt 4Q22 (7.8×10^2 spores + 5 µg protein), a strain lacking Cry toxins; 63% of adults died within seven days. We then treated EAB adults with zwittermicin A (20 µg solubilized crude zwittermicin/dose), a spore-associated antibiotic produced by some Bt strains (including Btk HD-1); 100% of EAB adults died within six days. Although direct toxicity of zwittermicin in insects has not been reported, it is known to synergize Btk activity in gypsy moth (Broderick et al. 2000). Overall, these findings support the importance of Bt spores and/or other fermentation products in EAB mortality, as reported for several species including gypsy moth (Dubois and Dean 1998).

We are now focusing our research efforts on discovery of a Bt strain with high toxicity against EAB adults and a narrow host range. Initial bioassays will involve ca. 30 select strains, many with coleopteran toxicity, acquired from culture collections. We are also culturing Bt strains from EAB cadavers and ash leaves collected in southeastern Michigan in 2005. If we are successful, a new microbial insecticide will be developed and registered for management of EAB in forests and woodlots for ash tree preservation.

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DEVELOPMENT OF RESISTANT ASH TO FACILITATE THE ERADICATION OF EMERALD ASH BORER

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ABSTRACT

Two distinct processes are involved in the production of genetically engineered plants. First, the gene of interest (i.e., "transgene") needs to be stably integrated into the chromosome of a single plant cell. Second, this transformed cell must be induced to differentiate into a whole plant via a process known as regeneration. The latter is generally the most limiting of the two procedures. For transformation, we exploit the ability of *Agrobacterium tumefaciens*, the causative agent of "crown gall disease," to move genes into plant cells. *Agrobacterium tumefaciens* contains a tumor-inducing (Ti) plasmid, which is a closed circular piece of double-stranded DNA. A portion of this plasmid, the T-DNA, is inserted into a plant chromosome. The T-DNA is delimited by border sequences at its left- and right-hand ends. Transfer of the T-DNA is facilitated by virulence (*vir*) genes, which are located on the Ti plasmid, outside the T-DNA, and can act *in trans*. This T-DNA contains genes that encode enzymes that catalyze the synthesis of plant growth regulators. The resulting hormonal imbalance causes a proliferation of plant cells, leading to the formation of a gall (tumor). Other genes on the T-DNA encode enzymes that catalyze the synthesis of opines, unique amino acids, used as carbon and nitrogen sources by the bacterium. Strains of *A. tumefaciens* used to transform plant cells are "disarmed" by removing the T-DNA from their Ti plasmids. We then assemble a "binary vector", which contains the DNA to be inserted into the plant cell between border sequences. Generally, two genes are inserted: the gene of interest (e.g., an insect resistance gene) and a selectable marker gene. The latter allows for the isolation of transformed cells. The most common selectable marker is *NPT II*, which imparts resistance to the antibiotic kanamycin. The binary vector is transformed into a disarmed strain of *A. tumefaciens*, which is then used to transform plant cells.

Insect resistance genes are usually derived from *Bacillus thuringiensis* (Bt), a common bacterium pathogenic to various insects. Host specificity of different Bt strains results from various crystalline insecticidal proteins known as "Cry" toxins. Before using a Cry gene to genetically engineer a plant, however, it is necessary to identify a Cry protein toxic to the target insect. We bioassayed a range of cloned Cry toxins with known toxicity against certain beetles. Although we are in the process of replicating these assays, our preliminary results were promising. In parallel, we are optimizing transformation for white ash (*Fraxinus americana* L.) using a reporter-gene construct and an established *in vitro* regeneration system that utilizes embryo explants. We are also in the process of developing a regeneration system for green ash (*F. pennsylvanica* Marsh.) that utilizes explants from vegetative tissues. When an effective toxin is identified, the corresponding gene will be inserted into a binary vector downstream of a constitutive promoter. The resulting construct will be used to produce emerald ash borer (EAB)-resistant white and green ash.

Once transgenic trees are regenerated, they may be deployed in various ways. For example, they could be used as trap trees to attract and kill insects. While APHIS is, in general, opposed to transgene release from trees (much effort is being devoted to engineering reproductive sterility to develop a method of transgene confinement), it may be necessary to allow the spread of the *Bt* toxin gene if we are to prevent the extinction of *Fraxinus* spp. from North America. Allowing introgression of that *Bt* gene into wild stands will help maintain genetic diversity in the resulting offspring. The decision regarding transgene escape can be deferred for many years, though, because ash has a protracted juvenile period.

Recombinant DNA technology can be used in many other ways. For example, if we identify a resistance gene or genes in an Asian species of *Fraxinus*, we can insert it/them into species native to North America. Trying to develop resistant varieties via conventional breeding would take decades to accomplish. Once we have a resistance gene(s) from an Asian species, we can find the homologous gene(s) in American species. It is possible that the gene(s) is/are either expressed at very low levels or has/have been mutated. Using biotechnological tools, we can either up-regulate expression or repair the lesion. Finally, once we know the volatile organic compound(s) that attract emerald ash borer to ash, we can either up-regulate the expression of the gene(s) encoding the enzyme(s) responsible for its/their synthesis, thereby producing a better trap tree, or down-regulate the same gene(s) so the tree is no longer capable of attracting emerald ash borer to ash.

POTENTIAL OF *BEAUVERIA BASSIANA* STRAIN GHA FOR MANAGEMENT OF EMERALD ASH BORER: SUMMARY OF FIELD TRIALS

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ABSTRACT

Insect-pathogenic fungi were the most diverse and prevalent natural enemy of *Agrilus planipennis* dissected from infested ash trees (*Fraxinus* spp.) in a southeastern Michigan woodlot during 2003. To evaluate the potential of entomopathogenic fungi for management of *A. planipennis*, we compared the virulence of several fungal species and isolates against *A. planipennis* prepupae and adults. The most virulent strain was *B. bassiana* strain GHA, the active ingredient of BotaniGard®, a commercially available biopesticide registered in the United States since 1999. We reported previously the results from research on formulated *B. bassiana* strain GHA, including laboratory and greenhouse bioassays; effect of pre-emergence trunk sprays on adult infections in the field; impact of fall trunk sprays on larval and prepupal infections in the field; and field persistence of *B. bassiana* strain GHA conidia after foliar application (Bauer et al. 2004, Liu et al. 2005).

The efficacy of BotaniGard® ES against *A. planipennis* was evaluated in a 20-year-old ash plantation in Ann Arbor, Michigan, infested with ca. 140 *A. planipennis* larvae/m^2. A commercial applicator applied 6 qts BotaniGard® ES/100 gallons of water to 73 trees every two weeks from June 23 to August 3, 2004. To achieve good coverage, 2-3 gallons of BotaniGard® suspension was applied to drip on the crown and trunk of each tree; the trees were ca. 20 feet tall. Crown die-back for each tree was ranked as low, moderate, or high after leaf flush in both 2004 and 2005. In fall 2004, a sample of the sprayed and control trees were felled and dissected to determine numbers of *A. planipennis* and *B. bassiana* infection prevalence. Prior to adult emergence in 2005, another sample of sprayed and control trees were felled and held to determine numbers of emerging adults.

In fall 2004, we found 36% fewer *A. planipennis* larvae in the sprayed vs. control trees (62 vs. 96 larvae/m^2). Of the larvae dissected from sprayed trees, 20.6% later died from *B. bassiana* infection compared with 0.6% from control trees. In the spring of 2005, 68% fewer

A. planipennis adults emerged from sprayed vs. control trees (30 vs. 94 adults/m^2). Between 2004 and 2005, the sprayed trees had ca. 50% less crown dieback than did the control trees.

Our results support the efficacy of *B. bassiana* strain GHA, formulated as BotaniGard®, in reducing populations of *A. planipennis* and slowing ash decline, and possibly slowing its spread rate. This biopesticide may also prove useful in gateway areas by facilitating eradication of outlier populations. Further research is needed, however, to optimize application methods, rates, and frequency, as well as evaluating impacts on nontarget organisms.

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SIMULATED AERIAL APPLICATION OF *BEAUVERIA BASSIANA* FOR SUPPRESSION OF EMERALD ASH BORER

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PURPOSE

Determine the impact of *Beauveria bassiana* on emerald ash borer (EAB) infestation of individual street trees when it is applied from above the trees at the same rate and volume as in an aerial application.

INTRODUCTION

Recent studies by Bauer and Liu have shown that spraying ash trees with Botanigard (active ingredient: *B. bassiana*) causes a high level of mortality of emerald ash borer adults fed the foliage of sprayed trees up to 10 days after application. These results suggest that the persistence of *B. bassiana* spores is adequate for use in an aerial spray program. Laboratory studies indicate that emerald ash borer is extremely susceptible to the GH strain of *B. bassiana* used in the Botanigard product. Bauer and Liu have determined the LD50 to be less than 10 spores/ml. This makes emerald ash borer more susceptible to *B. bassiana* than almost any insect tested.

The only efficacy data available at this time is from a 2004 street tree test in Troy, Michigan (Troy-South test site), where individual ash trees were sprayed twice by a professional arborist (June 3 and June 24, 2004), with 6 quarts/100 gal of Botanigard. In fall of 2004, upper canopy branches were removed. The bark was peeled and emerald ash borer larvae counted. Ash trees sprayed with Botanigard averaged 4.7 larvae/m^2 compared with 10.1 larvae/m^2 in the control trees. At this time, Botanigard looks promising enough to be considered as a candidate for use in aerial applications to suppress emerald ash borer. However, aerial application is very different from hand-gun spraying of street trees, and the amount of Botanigard we can put on each tree by air is only about one-third of the amount applied from the ground with a hand-gun. Also, aerial application tests are expensive. Therefore, in order to determine if Botanigard has potential as an aerial application for emerald ash borer, we simulated an aerial application as accurately as possible in a street tree test.

PROCEDURE

All applications of Botanigard were made through a mist blower from above the tree using a bucket truck. This mist blower has been modified with a Micron Air nozzle that produces droplets in the same range as expected from flat fan nozzles in aerial application. Botanigard was applied at a rate of 12 qt/acre. The ash trees at our test site have crowns about 20 feet across. We have calculated the amount of Botanigard to be sprayed on each tree by assuming the tree canopy covers the area of a circle with a 20-foot diameter. This comes out to 139 trees per acre, and 82 ml of Botanigard per tree. We diluted the Botanigard 3-fold to apply 246 ml of spray solution per tree. The treatments are:

1. Botanigard in early May (leaves are very small) – designed to spray trunk and branches
2. Botanigard in early June – designed for foliage application when beetles are most active
3. Botanigard in early June and late June
4. Botanigard in early May and early June
5. Tempo in early June
6. Control

Each treatment is replicated 10 times with a single tree as a replicate. Treatments are blocked in a RCB design. In September 2005, three large branches were removed from the upper canopy of each tree. The bark was peeled to count live larvae.

RESULTS

ARBORIST GROUND SPRAY, TROY-SOUTH

In 2005 the ground spray described in the introduction was repeated. Applications were made by a professional arborist with a volume of spray similar to what they typically use. Botanigard was sprayed twice in 2005 at the same rate as in 2004. Branches from Botanigard-treated trees and control trees were sampled in September 2005. From 2004 to 2005, the mean larvae/m^2 in the control treatment increased from 10.1 to 65.9, while the larvae/m^2 in the Botanigard treatment increased from 4.7 to 73.7. Under the intense pressure from EAB in 2005, the Botanigard treatment was not different from the control treatment.

SIMULATED AERIAL APPLICATION OF BOTANIGARD, TROY-NORTH

Under intense pressure from EAB in 2005, all of the trees that received Botanigard treatments and the Tempo treatment averaged approximately 15% less live larvae/m^2 than was found in the control trees. A complete data analysis is still pending, but a preliminary analysis indicates that none of the treatments were significantly different from the control treatment.

CONCLUSIONS

Apparently the intense pressure from EAB at this location made it very difficult to obtain adequate control, as a single application of Tempo—which usually provides adequate control as a ground spray—did not work well in this simulated aerial application test. The failure of Botanigard to control EAB in the second year of the Troy-South ground spray test and failure of Botanigard to reduce the density of EAB larvae in this simulated aerial application test to individual trees makes it seem unlikely that Botanigard will provide adequate suppression of EAB as an aerial application to a heavily infested area. However, the simulated aerial application of Botanigard did appear to have some effect on EAB, and area-wide applications should be more effective than treating individual trees (individual trees were treated in this test). Although the results of this test makes it look less promising, the impact of an aerial application of Botanigard to a low density of EAB will not be known until it is tested in a full-scale experiment.

SURVIVAL OF EMERALD ASH BORER IN CHIPPED AND GROUND ASH

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ABSTRACT

Effective methods to destroy emerald ash borer (EAB), *Agrilus planipennis*, life stages in infested ash material are needed for the EAB regulatory program. Chipping and grinding infested ash wood are destructive methods that may eliminate EAB viability and thus assure no adult emergence from the processed wood material. McCullough et al. compared EAB survival in chips produced by passing infested ash through a grinder fit with either a 1" or 4" mesh screen. No intact EAB were found in the 1" chips; however, intact prepupae were found in the 4" chips. In 2005, we evaluated chipping and grinding to assess the size distribution and survival of EAB in ash material processed by a chipper or by a grinder fit with a 2", 4", or 5" screen.

In February 2005, heavily infested ash logs were transported to the marshalling yard in Plymouth, Michigan. These logs were separated into five relatively equal piles of 50 logs each. The log piles were randomly assigned to one of five treatments: 1) chipping with a Morbark Model 12 Tornado wood chipper, 2) grinding using a grinder fit with a 5" screen, 3) grinding with a 4" screen, 4) grinding with a 2" screen, and 5) "control" logs. The control logs were used to determine the total volume and weight of material and to estimate EAB density. Density of prepupae was estimated by counting EAB in a peeled bark window on one-third of the control logs; adult density was estimated by counting emerged EAB from the remaining control logs placed in barrels. After the logs in each pile were subjected to their respective chipping or grinding treatments the material was separated into different size classes by sieving through a 1", then a 2", and lastly a 4" sieve. For each treatment, all of the sieved material of each size class was collected in 18-gallon plastic bins. The amount of material in each size class was determined by counting and weighing the filled bins. We observed that several unusually large pieces were produced by the grinder regardless of screen size. Further

investigation revealed the presence of a 6-8”-wide gap above the top of the screen, which allowed large pieces of material to come out without passing through the screen. This gap occurred when the interior guides for the screen were modified by the operator, which caused the screen to drop too low. The modification was recent, and the grinder operator was unaware of the problem until we called it to his attention. The large pieces were collected separately and dissected to look for EAB larvae and prepupae. Two bins of material from each chipping and grinding size class, for each of the four treatments, were held in the laboratory for several weeks and monitored for any adult emergence. One bin for each size class and treatment was completely filled and the other bin was partially filled (3 inches deep). After seven weeks, the material in each bin was sorted to look for live EAB and EAB remains.

The total volume of the control log pile was 0.39 m^3; the total surface area was calculated to be 11.94 m^2. The average estimated weight of wood for each treatment pile was 249 kg. The average beetle density for the control pile was 146 $\pm$ 21 total individuals/m^2 and 131 $\pm$ 19 prepupae/m^2. Based on the calculated surface area of the pile, we estimated that 1,565 prepupal EAB were present in each pile before processing. None of the bins containing chipped and ground ash material yielded adult EAB.

The large, anomalous chips resulting from the poor-fitting screens did contain live EAB prepupae, which presumably would have emerged. Nine intact prepupae were found from both the 5” screen treatment and the 4” screen treatment and 3 intact prepupae were found in the large pieces from the 2” screen treatment. No EAB were found in any of the chips produced by the chipper. The improperly fitting screens are probably a fairly rare occurrence, but these results do point out the need to frequently inspect equipment used for regulatory treatments. Grinding with a 4” or 5” screen was not 100% effective in destroying all EAB, although no adult EAB emerged from these chips. Had the grinder screens fit properly, it is possible that no EAB would have been found in material from the 2” grinder screen treatment. Previous results (McCullough et al.) indicate that no EAB were found in material produced with a 1” grinder screen.

The chipper consistently produced a high proportion of small (< 1 inch) chips. Moreover, no EAB were found in chips of any size class that were processed by the chipper. Chippers, which have the advantage of being portable, warrant more consideration for regulatory treatment of infested ash.

EGG AND LARVAL PARASITOIDS OF EMERALD ASH BORER FROM CHINA: POTENTIAL FOR BIOCONTROL IN NORTH AMERICA

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ABSTRACT

Results of an earlier study on *Agrilus planipennis* natural enemies in a southeastern Michigan woodlot demonstrated relatively few natural enemies attack its immature stages. Mortality factors included fewer than 2% larvae infected with insect pathogenic fungi, fewer than 1% of larvae and fewer than 0.5% of eggs parasitized by insect parasitoids, varying levels of predation by woodpeckers and beetles, cannibalism, starvation, and dehydration. Low mortality rates of *A. planipennis* apparently results from few natural enemies and high susceptibility of North American ash (*Fraxinus* spp.), providing ideal conditions for this invasive buprestid to achieve population densities lethal to our native ash species. The complexity of this problem suggests research on *A. planipennis* population dynamics in Asia may provide solutions for its management in North America.

To this end, we began studying populations of *A. planipennis* in China in 2003. We focused our efforts on forests and parks in northeastern China where *A. planipennis* is not considered a pest (Liu et al. 2003). Since our work began, we have found two new parasitoid species attacking *A. planipennis* in addition to the previously known larval ectoparasitoid, *Spathius agrili* (Braconidae) (Yang et al. 2005). The new species include a gregarious larval endoparasitoid, *Tetrastichus* sp. (Eulophidae) (Liu et al. 2003), and a solitary egg parasitoid, *Oobius agrili* (Encyrtidae) (Zhang et al. 2005). Results from 2005 seasonal sampling of *A. planipennis* in a Jilin Province forest showed *Tetrastichus* sp. parasitism rates of 40% and *O. agrili* rates of 27% by August. We are developing rearing methods and studying these parasitoids' biology and host ranges in our containment laboratory in Michigan. Depending on our findings, these parasitoids may be used for biological control of *A. planipennis* in North America.

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PROGRESS ON COLLECTING, STUDYING, AND REARING PARASITOIDS OF EMERALD ASH BORER FROM CHINA

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ABSTRACT

USDA-APHIS and USDA-Forest Service have contracted with Dr. Yang Zhong-qi of the Chinese Academy of Forestry to discover, collect, and study natural enemies of the emerald ash borer (EAB) in China. Dr. Yang conducted exploratory surveys throughout China several years ago and has found one site in Tianjin City and one site in Harbin, Heilongjing Province, to be most productive.

In Tianjin, collections were made from *Fraxinus velutina*, and two parasitoid species were found. A bethilid in the genus *Scleroderma* was found, but parasitism was low, 80% of the females are wingless, and some members of the genus bite humans. This species is not being considered for release in the U.S. *Spathius agrili* Yang (Hymenoptera: Braconidae) shows a great deal of promise: it is a gregarious ectoparasitoid that paralyzes third or fourth instar EAB larvae and then deposits 2-18$^+$ eggs on each host. One female will oviposit on 1-8 EAB in her lifetime. Adults are long-lived for parasitoids (29 days at room temperatures), are active searchers, and have a female-biased sex ratio (3.6:1). It is estimated that *S. agrili* has 3-4 generations per year in Tianjin. This parasitoid is also extremely well synchronized with the phenology of its host. EAB adults emerge in mid-May, but *S. agrili* adults delay emergence until late-June or early-July when EAB of the appropriate stage are available for oviposition.

Collections in Harbin revealed one parasitoid attacking EAB in that region. *Tetrastichus* species (Hymenoptera: Eulophidae) is a gregarious endoparasitoid that also has a female biased sex ratio (1.6:1) and multiple generations. Unlike *Spathius*, however, females emerge in late May, which is probably even sooner than the EAB in this Northern Province. Whereas the majority of the EAB spend the winter as mature larvae in overwintering chambers in Tianjin, the EAB larvae take two to three years to develop in Harbin. As a result, larvae of all instars can be found throughout the year, and larvae of the appropriate stage for oviposition are available in the spring when *Tetrastichus* emerges. This has important implications for biocontrol in the U.S.; only in situations where EAB has a two-year life cycle can we expect this parasitoid to establish and exert control.

Spathius agrili and *Tetrastichus* sp. have been imported into the quarantine facility at the USDA-APHIS laboratory in Bourne, Massachussetts. EAB larvae are presented to parasitoid females in chambers carved in ash twigs, and adults are fed honey. Three generations of *S. agrili* have been reared in the U.S. using this method. Methods for mass rearing this species are being developed, and we are able to rear the EAB host so that larvae are available for the parasitoid colonies.

Future priorities include: 1) development of efficient mass rearing techniques, 2) development of methods to stockpile parasitoids using cold storage, 3) conducting host-specificity testing to evaluate safety, 4) testing release methods in China (determining which stage to release, time of year, numbers, etc.), and 5) developing protocols to evaluate the success of biocontrol.

OVERVIEW OF HYMENOPTERA GENERA CURRENTLY CONSIDERED FOR EMERALD ASH BORER BIOCONTROL RELEASE

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ABSTRACT

Three wasp species reared from emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, in its native range in China are candidates for eventual release in North America. All three species have been recently described as new species: *Spathius agrili* Yang (Braconidae), *Oobius agrili* Zhang and Huang (Encyrtidae), and *Tetrastichus* n. sp. (Eulophidae) (in press with full specific name). Species of *Spathius* and *Oobius* typically attack only wood borers, where as species of *Tetrastichus* attack a wide array of insects and other arthropods. The genera these new species belong to include other members previously reared from *Agrilus* species. Two of the new species attack EAB larvae: *S. agrili* as an idiobiont ectoparasitoid and *Tetrastichus* n. sp. as a koinobiont endoparsitoid. *Oobius agrili* parasitizes EAB eggs. These three genera are currently being reviewed by specialists.

EXPLORATION FOR EMERALD ASH BORER AND ITS NATURAL ENEMIES IN SOUTH KOREA DURING MAY-JUNE 2005

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ABSTRACT

We spent four weeks in 2005 exploring the northern and central regions of South Korea for populations of *Agrilus marcopoli*. We continued as last year with a trapping scheme that included the use of trap trees and white plastic sticky traps. Having had no success in 2004 trapping adult beetles on Korean ash, *Fraxinus rhynchophylla*, we decided to focus this season's efforts on Manchurian ash, *F. mandshurica*, which has been recorded as an emerald ash borer host in China. We set up a total of 24 girdled trap trees and 12 plastic sticky traps among three mountainous locations: two in natural riparian habitats (Jeombongsan and Bangtesan) and one in a forest plantation (Pyongchang). Ultimately, as in 2004, we did not trap any EAB adults and conclude that simply wounding trees low on the trunk may not be attractive to adults in low-density endemic populations.

In addition to trapping, we investigated two sites with stressed Korean ash trees: a landscape planting of small trees along a country road near Daejeon and a road construction area near Inje where trees had been girdled to facilitate their removal. We did not observe any EAB adults during our initial visits to those sites from May 31 to June 1. On June 5, we acquired a single live EAB from a collector, who captured it at a pulp mill near Samcheok among piles of logs of mixed species. In the last week of the trip (June 20-22), we revisited the two sites with stressed Korean ash. We found live beetles at both sites, observed mating and oviposition behavior, and collected 14 specimens for taxonomic reference and DNA analysis. Note that Daejeon and Inje are about 200 km apart. The 15 specimens that we collected altogether exhibited interesting variability in coloration that seems not to occur in the Michigan population. We felled three of the landscape trees at Daejeon and peeled their bark. The trees contained serpentine galleries and D-shaped holes, and two galleries had associated immature stages of other species that may be parasitoids. We conclude that, despite its apparent rarity, EAB probably can be found throughout South Korea by girdling or otherwise stressing Korean and Manchurian ash trees.

RESEARCH ON PARASITOIDS OF BUPRESTIDS IN PROGRESS AT THE ARS BENEFICIAL INSECTS INTRODUCTION RESEARCH UNIT

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ABSTRACT

In 2003, we began research on natural enemies of emerald ash borer (EAB), *Agrilus planipennis,* in response to the impact of the then newly-discovered pest in Michigan. If efforts by federal and state regulatory agencies to eradicate EAB in the Great Lakes Region are not successful, biological control will be needed for suppression of this pest. There have been very few biological control projects directed at wood boring insects, and we know of none for buprestid beetles, so there are no precedents as to the prospects for success in biological control of this pest. In 2005, work on biological control of emerald ash borer at the Agricultural Research Station (ARS) Beneficial Insects Introduction Research Unit was expanded to include studies on natural enemies of the pest in both the Far East and North America. In addition, we have been cooperating with Michael Smith of this laboratory in studies on parasitoids of Asian longhorned beetle, *Anoplophora glabripennis*.

Our current program has two major objectives:

1. Exploration for and discovery of natural enemies of the emerald ash borer – this includes foreign exploration in the Far East and also a search for native natural enemies of indigenous buprestid beetles. Subobjectives include finding suitable investigation areas, constructing and inventory of natural enemies, investigations into the structure of the enemy complex, and field studies of their impact on the target pest.
2. Bioecological studies on promising natural enemies discovered. These include studies on life history and behavior, host specificity (Asiatic species), synchronization with the pest, physiological tolerance to different climatic factors, and establishment of priorities in utilization of promising species based upon their biological characteristics.

In 2003-2004, one of us (PWS) conducted explorations for natural enemies of EAB in Japan, South Korea, and Mongolia, but no suitable populations were found. Therefore, we intend to work in China this year and next. The most promising area to look for specialized parasitoids of both emerald ash borer and Asian longhorned beetle (ALB) in natural settings would be the region north of Korea straddling the 130th meridian. This region has deciduous and pine-deciduous forests containing substantial components of ash (*Fraxinus* spp.) and maple (*Acer* spp.), preferred host plants of EAB and ALB, respectively. Of course, EAB is not confined to a single region of China, but occurs in a number of provinces. Because man plants trees wherever he wants them, pests often take advantage of new host sources, ranging far outside their ancestral home to invade the new plantings. Usually, these secondary distribution areas are of limited interest to biocontrol workers because the spread of natural enemies tends to lag behind the spread of pests, resulting in an impoverished parasitoid complex at new sites. Such a situation exists in Shandong Province, which has substantial plantings of velvet ash, a North American species. Thus, we will not dismiss such areas out of hand because they might prove to be sources for parasitoids that would attack EAB on Nearctic species of ash.

Because some North American parasitoids might adapt to EAB or ALB, we have begun studies on parasitoids attacking buprestid and cerambycid beetles in Pennsylvania and Delaware. Two approaches are used: (1) girdling preferred host plants (white, green, and black ash for EAB and maples, poplars, willows, and others for ALB), then felling them at intervals thereafter, and (2) taking infested material from sites with recent logging debris. In the latter sites, we can capture foraging females of parasitic wasps with an insect net for laboratory study. Usually, bolts are taken from the field to an unheated insectary. Bolt ends are waxed to inhibit desiccation, and the bolts are then stored in ventilated trash cans or cardboard tubes for emergence of borers and their parasitoids. Containers are checked three times a day for emergence, and parasitoids are placed in mating cages if both genders are recovered. This line of research only started in July, so we have limited data on parasitoid presence. So far, we have recovered buprestids in the genera *Dicerca* and *Chrysobothris* from ash. With respect to parasitoids, we have recovered at least four species of parasitic wasps: a braconid in the genus *Atanycolus,* two ichneumonids, one chalcidoid, and one tachinid. Field-collected or mated females are exposed to the target pest in quarantine. We have only recently received a permit to culture EAB in quarantine, so all of our exposures to date have been against ALB. The *Atanycolus* has been observed probing a number of other tree species in the field, including hickory, red maple, and red oak, so we suspect that it is a generalist. We have made over 50 exposures of this species to ALB but have yet to observe any successful parasitism. Insufficient exposures of the other species have been made to support any conclusions. In any case, we are anxious to try exposing these parasitoids to EAB in quarantine.

Host specificity is a major consideration in the classical approach to biological control of insect pests because it determines whether unwanted shifts of biocontrol agents to attack non-target species are likely to occur after introduction. Differences in parasitoid biology profoundly influence host suitability. Koinobionts (endoparasitoids that develop in living hosts) have venoms, teratocytes, and symbionts that suppress the host's immune system, and host range is usually determined by host phylogeny; idiobionts (ectoparasitoids or endoparasitoids that attack eggs or any stage lacking an immune system) do not need to suppress the host's immune system, and habitat or host plant is more likely to determine host range than host phylogeny. Host specificity in parasitoids of woodborers and other insects living in concealed places is often determined ecologically rather than by host phylogeny. Because the emerald ash borer attacks *Fraxinus* spp. nearly exclusively, host plant species might be an important determinant of the host range of its natural enemies. Different approaches might need to be developed to test nontarget species selected for testing promising candidates found overseas. The overall goal is to use methods that prevent the release of any organism likely to have an unacceptable level of economic or environmental impact but minimize the likelihood that safe and potentially useful biological control agents will be rejected.

USING *CERCERIS FUMIPENNIS* WASPS TO MONITOR THE SPREAD OF EMERALD ASH BORER

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ABSTRACT

Cerceris fumipennis Say is a widespread beetle-hunting wasp (Crabronidae) that stocks its nests almost exclusively with adult Buprestidae. Beetles of the genus *Agrilus* account for 31% of the species and the majority of the specimens recorded as prey of *C. fumipennis*. Up to 51 *Agrilus* specimens have been recovered from a single wasp larval cell: each wasp burrow includes several cells, and each nest site usually includes multiple burrows.

The high number of *Agrilus* gathered by female *C. fumipennis* wasps is of current interest because these wasps have several properties that render them efficient tools for monitoring the spread of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, in eastern North America. *Cerceris fumipennis* forms perennial nesting aggregations ("colonies"), to which wasps carry hundreds of *Agrilus* per day throughout the EAB flight season. We have found that female wasps can be easily intercepted as they return prey-laden to their nests, and mark-recapture experiments have shown that individual wasps can be repeatedly robbed of beetles over a period of weeks. Preliminary studies of four colonies in southern Ontario confirms that *C. fumipennis* colonies in EAB areas do harvest EAB adults as well as other buprestids in the same size range (including three buprestid species not previously recorded in Ontario).

Many questions about *C. fumipennis* remain unanswered, and work is needed to assess the distribution of colonies around the edge of the current EAB range, to assess foraging range, to investigate the resilience of wasp colonies to other kinds of manipulations (such as colony splitting), and to answer related questions about the biology of the wasp and its potential as a practical EAB survey tool. Continued work on this system will also yield an extensive collection of wasp-captured beetles that will be of value in documenting the northeastern buprestid fauna.

EFFECTS OF TRAP DESIGN AND PLACEMENT ON CAPTURE OF EMERALD ASH BORER, *AGRILUS PLANIPENNIS*

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ABSTRACT

The ongoing objective of this research is to develop a trap that can improve the sensitivity and efficiency of emerald ash borer (EAB) surveys and aid the overall program in achieving its goals. As part of this work, we sought to determine the optimal location for trap placement. In 2004, we found that some traps placed along the edge of woodlots caught more EAB than those placed inside the woodlots or along adjacent fields. We continued this research in 2005, performing two studies. The first of these was a continuation of the previous year's work looking at paired purple corrugated plastic panels (0.15m x 0.90m) placed 0.90m and 2.1m above the ground. Traps were placed along the edge of woodlots in South Lyon and Ann Arbor, Michigan, 15.0 m inside the woodlots and 15.0 m in adjacent fields. Trap results from the two heights were not significantly different, but there was a significant site interaction, with more EAB caught in Ann Arbor than in South Lyon. In Ann Arbor, traps placed along the edge and in the field caught more beetles than those inside the woodlot. The lower trap catches in South Lyon did not yield any significant differences.

In a similar study, we looked at the interaction between trap location in relation to woodlots and trap color on EAB capture. We placed purple, white, and red box traps (four 35.0-cm x 60.0-cm corrugated plastic panels) along the edge of woodlots in Ann Arbor and South Lyon, Michigan, 15.0 m inside the woodlots and 15.0 m into adjacent fields. Purple traps in the field caught more than purple traps inside the woodlot, but neither was different from traps along the edge. Purple traps at all locations caught more than white or red traps, and there was no difference in catch among white or red traps.

In a third study we wanted to determine the role, if any, that height plays in EAB trap catch. We placed purple box traps at three heights: 1.5 m, 7.5 m and 15.0 m. The lower traps were hung from rebar poles at the base of ash trees, while the higher traps were hung from branches on the same tree. While more beetles were caught at the middle height, these data may not be statistically significant.

In addition to optimal trap location, we also hoped to determine the most effective trap design for EAB. We looked at four designs: 1) a girdled tree with a glue-covered stretch wrap band placed around it, 2) a purple box trap (see dimensions above), 3) a purple crossvane trap (45.0 cm x 60.0 cm panels) with a purple top, and 4) a purple crossvane trap with a translucent top. The traps were placed inside a woodlot to accommodate the stretch wrapped trees. Girdled, stretch wrapped trees caught significantly more EAB than the other three trap types inside the woodlot. There were no differences between catches from the other three trap types. In a separate study using small nursery (5 cm caliper) green ash, we tested four damage types: 1) a crown cut removing the top portion of the crown, 2) a root prune removing all but the main portion of the root, 3) a full girdle, and 4) a trunk scraping removing the outermost bark layers. We also tested two controls: a rebar pole and an undamaged tree. A 30.0cm x 15.0cm purple triangular trap was placed around the base of each tree (and pole). While there were no significant differences between the damage classes and the undamaged tree or among the damage classes, all treatments that included a host were significantly different from the rebar pole control.

From our results we can conclude that purple traps are relatively more effective in open areas than in wooded areas. We will be performing electroretinographic studies to further evaluate EAB responses to color. Girdled, stretch wrapped trees are more effective in wooded areas than other traps tested. From these results along with those from the damage type study, it would appear that the beetles are using olfactory cues to aid in finding a suitable host. Currently research is being performed to find potential volatile attractants.

EFFECTS OF TREE WOUNDING AND BANDING ON EMERALD ASH BORER CAPTURE

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ABSTRACT

Previous studies have demonstrated that emerald ash borer (EAB), *Agrilus planipennis*, is attracted to girdled ash trees. Other studies have demonstrated their attraction to color, especially purple. In the summer of 2005 we conducted three studies to explore these sources of attraction for EAB.

GIRDLE/HALF GIRDLE STUDY

One of our objectives was to investigate the relationship of the degree of tree wounding to EAB capture and whether purple bands increased adult capture rates. Trees were wounded at breast height (approximately 1.4 m), and sticky bands were placed above the wound. There were five treatments: 1) a full girdle with a clear shrinkwrap band covered in Pestick insect trapping glue (Hummert International, Earth City, Missouri), 2) a half-girdle with a clear sticky band, 3) an unwounded tree with a clear sticky band, 4) a full girdle with a preglued purple elm bark beetle (EBB) (Pherotech, Inc. Vancouver, British Columbia, Canada) panel trap stapled to the tree, and 5) a half-girdle with a preglued purple EBB panel stapled around the tree. There were a total of 10 replicates of each treatment. We collected 1,066 males and 572 females for a total of 1,638 adult EAB. There were no significant differences in capture rates for any of the treatment types.

TREE BAND STUDY

Our next objective focused on tree wounding and different types of sticky bands. Trees were girdled at breast height (~1.4m) and sticky bands were placed directly above the girdle. There were six treatments: 1) an unwounded tree with a clear sticky band, 2) a girdled tree with a clear sticky band, 3) a girdled tree with a purple EBB panel stapled to the tree, 4) a girdled tree with a band covered in Pestick that had been dyed purple, 5) a tree with approximately 40% of it's crown removed and with a clear sticky band, and 6) a girdled tree with a sticky corrugated purple plastic panel (Coroplast, Dallas, Texas) attached. There were a total of six replicates of each treatment. This study was conducted at two sites, each containing three replications of each treatment type. We collected 3,082 males and 1679 females for a total of 4,761 adult EAB. The girdled tree with a purple Pestick band caught the most beetles, but there were no significant differences among any of the treatments when all six replicates were analyzed together. The purple Pestick caught significantly more beetles than the other treatment types at only one site.

AGE OF GIRDLE

Our final objective was to investigate the effects of timing and placement of girdling on EAB capture at both higher and lower EAB density sites. Eleven replicates of each treatment were placed in high beetle density sites and ten replications were placed in a low beetle density site. There were a total of five treatments: 1) an ungirdled tree, 2) a tree girdled at breast height on April 2nd (while trees were still dormant), 3) a tree girdled at breast height on May 3rd (before major leaf out), 4) a tree girdled at breast height on May 25th (after leaf out), and 5) another tree girdled 10 ft above the tree base on May 25th. The sticky bands were placed directly above the girdle, with the exception of the last treatment which was placed at the same height as the lower girdled trees. In the higher beetle density sites we collected 2,557 males and 1,300 females for a total of 3763 adult EAB, while in the lower density site we collected 1,018 males and 158 females for a total of 1,176 adult EAB. There were no significant differences in catches among treatment types in the higher EAB density sites. In the lower EAB density site the treatment with the girdle located 10 ft from the base of the tree caught significantly more EAB than the treatment with an ungirdled tree and a sticky band located at the same height.

ATTRACTION OF EMERALD ASH BORER TO TRAP TREES: EFFECTS OF STRESS AGENTS AND TRAP HEIGHT

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ABSTRACT

Effective methods to detect low density populations of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, remains a high priority for regulatory and resource management agencies. Regulatory personnel also need an effective means of delineating outlier sites and monitoring sites where emerald ash borer eradication activities have occurred. Visual surveys are often problematic because of the difficulty of finding EAB exit holes or external symptoms on newly infested trees.

Results from research we conducted in 2003 demonstrated that more EAB adults were captured in sticky bands applied to girdled ash trees than in similar bands on healthy trees or cut ash logs. Trees treated with Pathfinder herbicide were not badly stressed but were somewhat attractive and captured an intermediate number of beetles. In sites where EAB density was relatively low, girdled trees functioned as a “sink” for EAB eggs, and larval density per m^2 of phloem was significantly higher on the girdled trees than on the other trees and logs. This effect was not apparent at high density sites, where the phloem was completely utilized by EAB larvae.

Our research in 2004 was designed to evaluate whether EAB adults were attracted to girdled trees because of volatiles associated with the wound or volatiles resulting from stress imposed on the trees. Three sites were selected, all of which were densely stocked woodlots with closed canopies and low densities of EAB. We compared adult beetle capture and larval density on trees that were healthy (no stress or wound), girdled (stress and wound), treated with Garlon 4 herbicide (stress but no wound), or wounded (wound but little stress). On wounded trees, we removed a vertical strip of bark and phloem equal in area to that removed on trees with a standard girdle. Sticky bands on ash trees that were treated with Garlon captured significantly more adults than the healthy control trees, even though the Garlon trees died by late July. More beetles were captured on the girdled and wounded trees than on control trees, but differences were not significant. Trees were felled and debarked during the winter to evaluate larval density. Trees with the standard girdle had significantly more larvae per m^2 than all other trees. These results indicated that volatiles associated with stress, rather than wounding, were attracting EAB adults.

In 2005, we compared adult EAB capture on trees treated with different stress agents, including Garlon 4 herbicide (chemical stress), girdling (physical stress) and methyl jasmonate (stress-elicting plant hormone), or left as untreated controls. Methyl jasmonate was applied by suspending ten slow-release bubble caps in the tree canopy. Using a randomized block design, we established 18 replicates of each treatment in three sites. Half of the blocks were in densely wooded, closed-canopy settings, while the other blocks were comprised of exposed trees growing in fields (total of 72 trees). In addition, we assigned each tree a ranking based on the degree of exposure ranging from 1 (open-grown) to 5 (completely shaded). Sticky bands were applied 1.5 m aboveground on all trees. On trees in half of the blocks, we placed an additional sticky band 3 to 5 m aboveground and suspended two sticky purple panels in the canopy.

Traps were checked weekly through the summer and biweekly in autumn. Beetle activity was highest in late June and early July, consistent with results from our 2003 and 2004 studies. Significantly more EAB adults were captured on girdled trees than on the control trees or the methyl jasmonate trees; the Garlon trees, which again mostly succumbed by late summer, were intermediate. More than five times as many beetles were collected from the traps on girdled trees compared with the traps on control trees.

There were no significant differences among trap types in the average number of beetles captured for any treatment. This indicates that the low bands (1.5 m aboveground), which are much easier to apply and monitor, were as effective as bands higher on the trunk or the suspended panels.

Adult EAB capture was affected by shading and exposure. More beetles were captured on trees exposed to full sunlight than trees that were mostly or totally shaded. Relative rates of EAB capture on sticky bands and purple traps, however, varied between open-grown and shaded trees over the summer. For example, early in the summer, several adult EAB were captured on the purple panel traps on shaded trees, while relatively few were ever captured on the purple traps on open-grown trees.

All trees will be felled and sampled over the winter to quantify larval density among treatments. Results of these and related studies and ongoing efforts to learn more about the behavior of EAB adults should help program managers more effectively detect and survey EAB populations.

CHEMICAL ECOLOGY OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA: BUPRESTIDAE), IN RELATION TO TREE VOLATILES

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Buprestidae), a native of Asia, was discovered in the USA and Canada in 2002. This serious pest of ash trees (*Fraxinus sp.*) infests and quickly kills trees by mining the cambium area, disrupting the tree's transport system. As newly infested trees do not typically show distinctive external visual symptoms there is a growing need to be able to trap adult beetles so that accurate surveying can be done. In Michigan during the 2004 and 2005 flight seasons, we collected bark volatiles from healthy and girdled (stressed) trees using Super-Q cartridges. Ratios of bark emitted monoterpenes and sesquiterpenes were determined for both girdled and non-girdled trees. Bark samples taken from girdled trees were found to have a higher ratio of sesquiterpenes compared to non-girdled samples. Compounds were identified by gas chromatography (GC) and mass spectrometry (MS) and screened for EAB antennal activity using electrophysiological techniques. Several of the 'bark girdled' sesquiterpenes were seen to give consistently strong antennal responses on both male and female EAB adults.

Lures were then developed and field tested in both marshalling yards and along the edge of infested ash stands during 2005. Two prototype purple-panel trap designs were used to test potential attractive compounds; a cross vane and a four-sided box. Due to the high cost and low availability of some of the sesquiterpenes identified from bark aerations an alternative source was required for fieldwork trials. Manuka oil, a steam distillate from the New Zealand Manuka tree, *Leptospermum scoparium*, was chosen as a potential cheap lure because it contained high levels of three of our identified ash stress sesquiterpenes. Purple traps baited with manuka oil captured significantly more EAB than traps baited with other compounds. In accordance with their antennal responses, traps attracted both male and female adults in equal numbers. Other traps tested were a purple trap (control), two different ratios of pinene: limonene, 1 g of EAB frass, elm bark sesquiterpene lure, and an ash leaf stress blend developed by the USDA Forest Service. Box trap-shaped purple traps caught significantly more EAB adults than the cross-vane traps.

Sesquiterpenes from ash bark therefore appear to show good potential as a lure for attracting both male and female adult EAB. Work is now underway to develop a better 'ash like' fraction of manuka oil. We also hope to test some of these compounds individually in the field next year.

TRAPPING AND DETECTION OF EMERALD ASH BORER: IDENTIFICATION OF STRESS-INDUCED VOLATILES AND TESTS OF ATTRACTION IN THE LAB AND FIELD

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), has killed an estimated 15 million ash trees in southeastern Michigan and Windsor, Ontario, since its discovery in 2002. The regulated area in southeastern Michigan has continued to expand and as of April 2005 included 20 counties and at least 25 outlier populations in Michigan; additional outliers have been found in Indiana and Ohio. Accurate delimitation of the infested area and detection of new outlier infestations is critical for regulatory officials who must establish quarantine boundaries and implement eradication and control measures. Trapping and detection techniques would greatly enhance efforts to delineate the distribution of EAB and locate new infestations.

In 2003 and 2004, we identified potential attractants for EAB using coupled gas-chromatography electro-antennal detection (GC-EAD) of ash volatiles. Walking olfactometer bioassays were used to select the most attractive compounds for field testing. Trapping experiments using a prototype purple-panel trap and other purple trap designs were conducted to compare several potential attractants. Purple panel 'prototype' traps baited with a blend of host volatiles (α-humulene, pentadecane, *trans*-3-hexenol, and *trans*-caryophyllene) captured significantly more beetles than traps baited with individual compounds. Cross vane, triangular, and flat purple corrugated plastic traps baited with a blend of host volatiles (hexanal, *trans*-2-hexenol, and *cis*-3-hexenol) captured significantly more beetles than unbaited traps. We also found that EAB were attracted in significantly higher numbers to ash trees that were stressed by girdling or by treatment with the herbicide Garlon-4 compared to healthy ash trees. Our results suggested that EAB are attracted to stress-induced volatiles produced by the trees.

In 2005, we collected and analyzed volatiles from healthy and stressed ash seedlings. Ash seedlings were stressed by mechanical damage, insect feeding, or treatment with methyl jasmonate (MeJA), a volatile derivative of the stress-eliciting plant hormone, jasmonic acid. Feeding damage by adult *A. planipennis* and MeJA treatment increased volatile emissions from ash compared to controls. In olfactometer bioassays, adult females were attracted to volatiles from plants damaged by beetles and those treated with MeJA. At least ten compounds from ash were found to be antennally active by GC-EAD: hexanal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, 3-methyl butylaldoxime, (*Z*)-3-hexen-1-yl acetate, hexyl acetate, (*E*)-β-ocimene, linalool, 4,8-dimethyl-1,3,7-nonatriene, and *E*,*E*-α-farnesene.

We also collected volatiles from the canopies of mature trees that were part of a large trap tree study and had been girdled, treated with the herbicide Garlon-4, or with MeJA. Analysis of these compounds is underway. In addition, we conducted trapping experiments using the prototype purple panel traps baited with various combinations of stress-induced ash volatiles. Preliminary results suggest that the volatile profile of mature ash trees is similar to that of ash seedlings, and that the blends of stress-induced leaf volatiles were only modestly attractive when tested in the purple panel traps.

PROGRESS ON REMOTE SENSING APPLICATIONS FOR EMERALD ASH BORER SURVEY: ANALYSIS OF 2004 HYPERSPECTRAL IMAGERY

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ABSTRACT

Hyperspectral images were collected by the contractor, SpecTIR Inc., using their HyperspecTIR sensor during flights in July and August 2004 over areas in Michigan and Ohio. The sensor scans in whiskbroom fashion and consists of two cameras. One camera covers the spectral range from visible light bands through near infrared (the VNIR camera with bands 1-39), while the other covers the shortwave infrared bands (the SWIR camera with bands 40-227). Ground level data included spectrographs of the crowns of ash trees in various states of decline and other tree species collected using an ASD spectrometer, as well as GPS and sketch map locations of many tree specimens under the hyperspectral flight lines collected by USDA personnel and others and used as ground truth data.

Initial processing of the images and attempts to mosaic them into long continuous coverages of the flight lines revealed several problems. Some problems were due to the contractor, including geolocation distortions between individual scans and flight lines, spatial misregistration between the VNIR and SWIR cameras, and excessive noise in the SWIR images. These problems limited our ability, respectively, to mosaic the images, to analyze data from the two cameras together, and to glean much useful information from the SWIR camera. Two additional problems were inherent to hyperspectral imagery: the tangential scale distortion and bidirectional reflectance distribution function (BRDF). Of the two problems, BRDF is the most difficult to overcome before the imagery can be classified. BRDF is a variation in illumination across an individual scan that results from the low flight altitude needed to achieve high spatial resolution, different sun angles, and variability in the azimuth of flight lines.

In the ideal case, with clean data, images would be georeferenced, mosaicked, and then classified. Classification is the identification and mapping of features of interest in the

imagery (such as ash trees). We considered two basic approaches to classification: use of the ASD spectrographs and development of spectral signatures directly from the images using tree crowns identified in ground truth data. The first approach was not pursued because of the BRDF issue and because of inconsistencies between the ASD and the imagery-derived spectral signatures. The second approach was used in all subsequent analyses, and initial analyses showed that using ratios of the bands (rather than the bands themselves) reduced the BRDF effect. The possible combinations of ratios were then reduced to a minimum set that gave maximum information using Principal Components Analysis.

The MahalClass classifier developed at Clark Labs (and available in IDRISI Kilimanjaro) essentially gives the probability that an individual pixel in an image belongs to a particular class, such as an ash tree with 60% crown remaining or a silver maple tree. This classifier uniquely identified infested ash crowns in three levels of decline, as well as crowns of willows, Norway maples, and silver maples.

However, the classifier developed from a tree crown in one image did not work well in classifying the same tree in another overlapping image, a problem called the "non-transportability effect." Thus, a classifier for a feature of interest from one image cannot necessarily be applied to other images. This problem suggested residual BRDF in the images. We at Clark Labs are currently investigating various modeling approaches for removing the remaining BRDF and facilitating the development of transportable classifiers that can be used to map ash trees over wide areas.

ASH DIEBACK IN MICHIGAN, 2003–2005

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ABSTRACT

A visual survey of ash dieback in southeast Michigan was initiated in 2003. Because the core infestation of emerald ash borer in Michigan appears to be centered about 20 miles west of downtown Detroit, we used the freeway system to sample ash dieback. We drove all of the expressways coming out of Detroit, getting off at each exit to estimate dieback. The first 10 ash trees (> 6" dbh) found were compared with a standard set of ash dieback photographs to visually estimate the level of canopy dieback for each tree. If 10 ash trees were not found on the exit ramp, we turned right, then right again until 10 trees were found. Ash trees in all locations were surveyed, including freeway property, ditch banks, commercial property, yards, parks, and woodlots. All ash species (in the genus *Fraxinus*) were included. Dead ash trees were included, but stumps were not. The survey continued outwards from Detroit in all directions until at least three consecutive sites were found where trees averaged less than 25% dieback. The survey was repeated in 2004 and 2005. In 2005, emerald ash borer exit holes were also counted on ash trees at all sample sites in the western portion of the survey (from Jackson to Ann Arbor to Flint to Lansing).

In 2003, the most severely impacted area was along I-275 between Novi and I-94. Along this stretch of about 20 miles, most of the ash trees were dead or dying, and canopy dieback averaged greater than 80% (Figure 1). If the smallest possible circle is drawn to include 90% of the survey sites in 2003 that average over 80% dieback, the diameter of the circle is 18 miles and the area is 1,017 mi^2 (Table 1). In 2004, dead ash trees were found along the same stretch of I-275 but also west to Ann Arbor for another 10 miles south of I-94 (Figure 2). If the same method as described above is used to draw a circle around the area containing 90% of the sites with greater than 80% dieback in 2004, the diameter of the circle is 22.5 miles and the area is 1,589 mi^2. In 2005, dying ash trees were found all the way to Flint going north and to Monroe when going south (Figure 3). The circle containing 90% of the sites that average over 80% dieback now has a diameter of 38 miles and an area of 4,534 mi^2.

Table 1. Diameter and area of the smallest circle that contains 90% of the survey sites that average over 80% ash canopy dieback in 2003, 2004, and 2005.

Year	Diameter of circle where canopy dieback is >50%	Area of circle where canopy dieback is >50%
2003	18 miles	1,017 miles^2
2004	22.5 miles	1,589 miles^2
2005	38 miles	4,534 miles^2

In 2005, the number of emerald ash borer exit holes per 10 trees at each of 33 sample sites correlates well with the average level of canopy dieback at the same sites ($r^2 = 0.59$, Figure 4). No exit holes were found at any site that averaged less than 25% dieback, and at least one exit hole was found on all sites that averaged greater than 40% dieback.

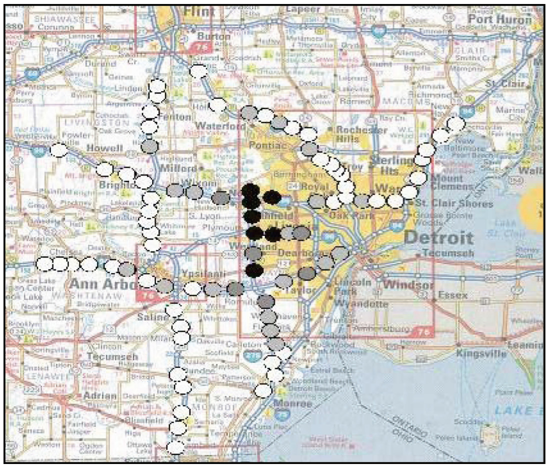

Figure 1. Ash dieback in southeast Michigan in 2003. Black dots indicate sites where canopy dieback averaged > 80%.

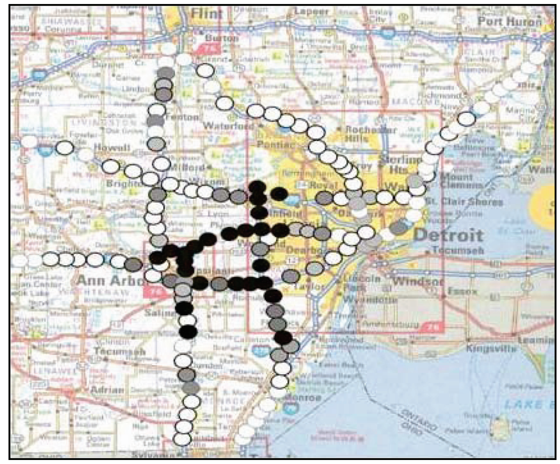

Figure 2. Ash dieback in southeast Michigan in 2004. Black dots indicate sites where canopy dieback averaged > 80%.

Figure 3. Ash dieback in southeast Michigan in 2005. Black dots indicate sites where canopy dieback averaged > 80%.

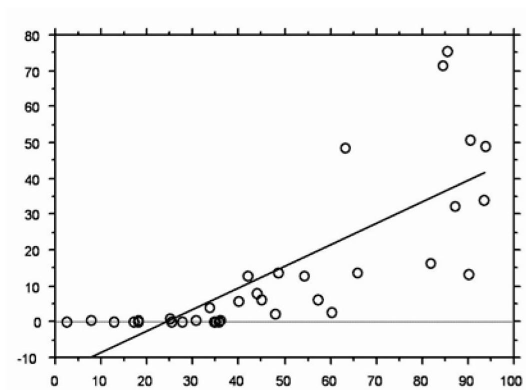

Figure 4. Relationship of the mean number of emerald ash borer exit holes per tree (n = 10) to the mean ash canopy dieback of of the same trees at 40 survey sites.

LIVING WITH EMERALD ASH BORER: DETECTION OF OUTLIER POPULATIONS

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ABSTRACT

In July 2002, beetles collected from ash trees in southeastern Michigan were identified as *Agrilus planipennis* Fairmaire (emerald ash borer). Since the initial discovery of this exotic pest, it has spread and established throughout portions of Lower Michigan and neighboring areas. It is capable of infesting and killing apparently healthy green, black and white ash. At low population densities, such as those in newly established outlier populations, it has proven difficult to detect this insect. The currently accepted methods for detectiion include the use of girdled trap trees with sticky bands to trap adult insects and subsequent bark peeling to detect larvae. There is an ongoing need to detect this insect throughout Michigan and in other states, either in support of local eradication programs or for prioritization of ash removal projects to limit the damage from this insect.

The 2004 Michigan trap tree survey has been expanded in 2005 to include Northern Wisconsin. This survey is risk-based and targets locations where infested ash firewood may have been moved. Trap trees have been established in state, county, private and federal campgrounds with ash resources that would be threatened by emerald ash borer. In addition to monitoring these trap trees, surveyors undertake evaluation of firewood piles and apparently stressed ash trees. In 2004, the response to detection of outlier populations was anticipated to be local eradication. In 2005, the response to detection is expanded to include ash removal from forested areas in proximity to outlier populations to reduce the population density of this insect.

In addition to the survey and detection activities, we have also carried out a number of studies to further refine current trapping technologies and to gain insight into the landing behaviors of emerald ash borer. These include:

- Comparison of landing rates on ungirdled ash trees, ash trees girdled in 2004, and ash trees girdled in 2005.
- Comparison of landing rates on purple box traps that were baited with leaves or scorched wood or were unbaited were compared with landing rates on girdled and ungirdled ash trees.
- Comparison of landing rates on different colored sticky traps.
- Characterization of landing rates on all trees in approximately 0.25 hectare plots where each tree greater than 2.5 cm diameter at breast height was used as an ungirdled trap regardless of species. All landing behavior studies were initiated in May 2005 and continued throughout the flight season of the adult beetles.

WHAT DO THE USDA FOREST SERVICE TECHNOLOGY AND DEVELOPMENT CENTERS DO?

Keith Windell

USDA Forest Service, Missoula Technology & Development Center

SYNOPSIS

The USDA Forest Service maintains a Technology and Development Program to assist the National Forest System and State and Private Forestry units with their specialized equipment and technology development needs. MTDC has already supplied Bob Heyd (Michigan Department of Natural Resources) with some aids (motion stabilized binoculars, rope saws) for field survey crews and an experimental camera-on-a pole for Therese Poland's Research group to look in tops of trees. We have also done a little bit of high speed video filming and analysis of emerald ash borer with weights glued on their backs (at the North Central Research Station) to try and determine how much a practical tracking harmonic transponder can weigh. (It is my understanding that the actual development of the transponder will be done by the Agricultural Research Service in Wooster, Ohio, and not by MTDC.)

To implement this Technology and Development (T&D) Program the Forest Service has established two National Service Centers: one in Missoula, Montana (MTDC), and one in San Dimas, California (SDTDC). Program areas serviced by the T&D Centers include: Forest Health Protection, Cooperative Forestry, Fire & Aviation, Residues, Wildland Fire Chemical Systems, Airtanker Base, Uniforms, Safety & Health, Reforestation & Nurseries, Forest Management, Range, Recreation, Engineering, Facilities, Global Positioning System, Inventory and Monitoring, Law Enforcement, Watershed/soil/air, Range, and Environmental Compliance and Protection.

Each of the T&D Centers employs about 50 people. The T&D Centers employ Engineers, Physical Scientists, Photographic Technologists, Sociologists, Foresters, Graphic Artists, website design Specialists, Writer/Editors, a Draftsperson, Equipment Specialists, Machinists, and others. The Centers have the ability to tackle every aspect of a equipment/technology development request which might include: market/literature searches, equipment selection of commercially available products, design and in-house fabrication of prototypes, equipment evaluations (in lab or field), creation of engineering drawings, and technology transfer by electronic or published hardcopy documents, websites, and video productions. Center Project Leaders occasionally enter into cooperative agreements or contracts with academia, other government agencies, or private industry when highly specialized knowledge or skills are needed to address a problem.

Forest Health Protection (FHP) projects are among those focused at MTDC. To view some of the current and past T&D projects go to: *http://www.fs.fed.us/eng/t-d.php*.

Administratively, the T&D Centers are part of their Washington DC Office organization. They derive the majority of their assigned work through requests from the field (project proposals) that have been prioritized and funded through various program sponsors, sometimes

with the help of a Steering Committee. Simple and quick technical assistance is sometimes handled without going through the project proposal route.

To request technical assistance from MTDC or to learn more about submitting a project proposal, contact either MTDC's FHP Program Sponsor (Harold Thistle, Morgantown, West Virginia, 304-285-1574) or MTDC's in-house Program Leader (Dick Karsky, Missoula, Montana, 406-329-3921).