

# Forest Health Technology Enterprise Team

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TECHNOLOGY  
TRANSFER

*Emerald Ash Borer*

## EMERALD ASH BORER RESEARCH AND TECHNOLOGY DEVELOPMENT MEETING

Pittsburgh, Pennsylvania  
October 23-24, 2007

Victor Mastro, David Lance,  
Richard Reardon, and Gregory Parra, Compilers



Forest Health Technology Enterprise Team—Morgantown, West Virginia

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October 23-24, 2007  
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## FOREWORD

Damage caused by the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, has increased dramatically since it was first discovered in June of 2002 near Detroit, Michigan. Parts of seven states now have known infestations: Michigan, Ohio, Indiana, Illinois, Maryland, Pennsylvania, and, most recently, West Virginia. The infested area also extends into Ontario, Canada, where EAB has been found as far east as Toronto. Tens of millions of ash trees are either now infested or have already been killed. According to a USDA Forest Service report, projected impacts on urban areas and forest settings are estimated to be in the billions of dollars. The EAB program currently is doing a more in-depth economic assessment.

When the EAB was first discovered in North America, there was little information on this insect. Within its native range, when feeding on indigenous species of ash, EAB behaves similar to many of the North American buprestid species, attacking only weakened or dying trees. Its natural range is still not completely known, and there was a general lack of knowledge about the behavior and biology of this species in particular and of the other *Agrilus* species and buprestids in general.

The current management approach of the USDA and affected states is to limit artificial movement of EAB with focused, aggressive regulatory and public-outreach programs. Given the limitation of monitoring tools and the size of the area that needs to be surveyed, the limits of the infestation were only beginning to be understood in 2006. In 2007, new populations were discovered in Pennsylvania and West Virginia. Active management has attempted to eradicate outlying infestations, focusing on those farthest from the core. The attempts to survey and delimit infestations have clearly pointed out the critical need for more effective survey tools. The program moved from visual survey to the use of trap trees in 2005. In 2008, the program will adopt a trap and lure based on visual cues and compounds released by stressed trees. Traps will be deployed in a systematic survey covering a 100-mile-wide band encircling the area known to be infested. In addition, most of the remainder of the United States will be surveyed using traps placed at sites that pose a risk of introduction. Control options have also been limited; to date, only ash tree removal has been applied on a large scale as a management tool. This approach is expensive, and its success is compromised by the limitations of past survey techniques.

Research support has been provided by federal and state agencies from the time EAB was discovered. The urgency of the need and the size of the mountain that had to be scaled, however, were brought into focus in 2005 at the annual EAB research meeting in Pittsburgh, Pennsylvania. At that meeting, a large number of scientists from many disciplines were brought together to produce a compilation of researchable topics that could provide products useful to support the control program.

The Deputy Administrator of APHIS-PPQ provided \$1.3 million in additional funding to support eight of the highest priority EAB projects. In the current report, we can see the first fruits of this expanded research program as well as preliminary products of ongoing research. These include better characterization of the insect's host-finding, mate-finding, and dispersal behaviors; discovery and evaluation of natural enemies and efficacious pesticides; design of a first-generation attractant and trap; and development of effective regulatory treatments. The program is adopting several of these findings. As noted above, a comprehensive survey will be carried out using an attractant-baited trap. After experimental releases of three species of parasitoids in 2007, the program is constructing a parasite production facility. In 2008, both the USDA forest Service and APHIS are adding an additional \$1 million to the existing funds to further accelerate the process. Useful products of these combined efforts will follow in the years to come; meanwhile, EAB continues its onslaught on *Fraxinus* in our forest and urban areas.

Although research on the Asian longhorned beetle and other invasive forest pests was not included in the most recent review, we plan to take advantage of this gathering of researchers and managers to exchange information as appropriate in the future. It is only by this type of exchange, the adoption of new techniques, and innovation that we can hope to keep pace with the introductions threatening our forests.

Vic Mastro

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# ABSTRACTS

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## EMERALD ASH BORER IN PENNSYLVANIA

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### ABSTRACT

Confirmation of adult specimens of emerald ash borer was received by the Pennsylvania Emerald Ash Borer Task Force June 26, 2007. The specimens were collected by USDA-APHIS inspectors as part of the state's cooperative emerald ash borer (EAB) survey activities. The initial detection site is located in Cranberry Township, Butler County. The Task Force responded with a one-mile by one-mile survey grid extending in a five-mile radius from the initial detection. Additional detections were made in multiple grids, with a total of 28 of 98 grids containing positive detections. Several of the positive grids were located in Allegheny County, south of the initial detection. Detection in Allegheny County was confirmed June 29, 2007.

The Task Force received excellent media cooperation, which spawned numerous public referrals from across the state. The Task Force divided up public referrals and investigated these reports, resulting in 10 additional positive detections. Two of the positive detections were outside of the original delimiting grid, but both were in counties already known to be infested.

In addition to regularly scheduled surveillance, the Task Force implemented a survey of major highways in western Pennsylvania and performed visual and destructive sampling in one-mile intervals. Two additional positive detections were recorded, but both were in the original delimiting grids.

Task Force cooperators continued planned survey activities, including trap tree removal, visual sampling, and destructive sampling at high-risk sites. As of October 19, 2007, Task Force cooperators inspected 1,062 sites and recorded 114 positive detections.

Regulatory actions included a quarantine of four western Pennsylvania counties, (Allegheny, Butler, Beaver, and Lawrence counties). Beaver and Lawrence counties were included due to their proximity to the Allegheny and Butler counties' infestations and a Mahoning County, Ohio, infestation. Simultaneously, a quarantine banning transportation of firewood out-of-state was also implemented.

Emergency funding was received to support survey and outreach efforts. Increased survey and outreach activities are planned for 2008.

## MANAGING THE EMERALD ASH BORER IN CANADA

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### ABSTRACT

The emerald ash borer (EAB) continues to pose an extreme risk to Canada's environment and valuable ash resource, with heavy mortality being observed throughout southwestern Ontario in 2007. With the recent confirmation of EAB in Norfolk County, six Ontario counties are now regulated for EAB in Canada by the Canadian Food Inspection Agency (CFIA).

Canada's strategy is to slow the spread of EAB through effective quarantine, regulatory, and communications strategies. Tree removal at and around infested outliers is no longer considered a cost-effective, sustainable strategy and has been discontinued other than for research purposes. The CFIA still considers the primary obstacle to effective control to be the relative paucity of accurate surveillance tools, and until such time as EAB can be detected at low population levels around outliers and the leading edge can be accurately determined, large-scale control actions cannot be considered as a viable strategy.

Canada's survey strategy is focused on the detection of outlier EAB populations and, to a lesser extent, the leading edge of infestation. This is accomplished by conducting detection and delimitation surveys. Throughout 2007, detection surveys were conducted at high-risk sites such as campgrounds, nurseries, parks, and sawmills across Ontario and in other areas of Canada to provide assurance that EAB does not occur in these areas. While the CFIA continues to focus its survey efforts on the detection of signs and symptoms and does not currently use girdled trap trees, wounded and banded sentinel trees were established for research purposes in infested areas in 2007 to assess the potential effectiveness of these as survey tools. Results from these trials are pending.

Quarantine of infested and high risk areas, in conjunction with public outreach and communications initiatives, are key elements of Canada's slow-the-spread strategy for EAB. As outlined in its EAB Management Plan, Canada establishes regulated areas in two ways: through the declaration of an area (usually at the county level) as infested by way of a federal Ministerial Order and through the issuance of legal notices to all property owners within a 5-km radius of a known positive detection. Both methods of quarantine may be in place in the same area concurrently where it is deemed desirable to slow intra-county spread. Quarantines are seen as instrumental to the CFIA in meeting its domestic and international regulatory obligations.

## MODELING EMERALD ASH BORER SPREAD IN OHIO AND MICHIGAN

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### ABSTRACT

Our group has been modelling the spread of emerald ash borer (EAB) in Ohio using a spatially explicit cell-based model that takes into account the insect's flight characteristics (Insect Flight Model) as well as external factors that enable the insects to travel passively (Insect Ride Model).

To accomplish this, we calculated the available ash from Forest Inventory Analysis data and created estimates for an EAB infestation "front" and years since colonization. The Insect Flight Model calculates the probability of colonization in each cell based on the basal area of ash (ash abundance) and EAB abundance by assuming an 11-year cycle starting with initial colonization of a site and ending when all ash at the site are dead. The Insect Ride Model weights the road network, wood products, population density, and campground information in a GIS and calculates an ash abundance multiplier that alters the ash abundance input to the Insect Flight Model. The modelled EAB colonization probability yields a map of colonization potential.

When the actual EAB finds were overlaid to determine the accuracy of our predicted spread, we found that 83% of the infections fell within a zone of high probability of colonization. In addition, 69% of the EAB finds (2004-2007) in the outlier zone (i.e., the zone beyond the immediate infestation front) occurred within 2 km of major Ohio roads. For campgrounds and wood products that are located farther from major roads, we are seeing more EAB positive detections beyond the immediate vicinity (2-10 km) of the major roads. This shows that these potential sources of infection are more likely to contribute to EAB finds as we move away from the roads. We found no significant relationship between ash basal area and EAB positive detections in either the occupied or the outlier zones. This analysis may contribute to more reasoned placement of detection trees.

We are currently applying the model to test EAB spread in Michigan, where campgrounds rather than roads are implicated as the major spread factor.

## DENDROCHRONOLOGICAL RECONSTRUCTION OF THE ESTABLISHMENT AND SPREAD OF EMERALD ASH BORER

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### ABSTRACT

Since emerald ash borer was discovered in southeastern lower Michigan in July 2002, it has been found to be responsible for the death or decline of several million ash trees. We used dendrochronological analyses to reconstruct where emerald ash borer originally became established and how it spread throughout southeastern lower Michigan. The area sampled was approximately 15,000 km<sup>2</sup> in size and encompassed the original six-county emerald ash borer quarantine area established in 2002. Two to four increment cores and/or cross-sections from emerald ash borer-killed green ash were preferentially collected over declining or non-stressed ash trees on a sampling grid of at least 4.8 × 4.8 km and on a sampling grid of 2.4 × 2.4 km throughout the heart of the core infestation. Samples were dried, mounted, and surfaced in the laboratory prior to measuring annual growth rings to the nearest 0.01 mm using a Velmex measuring system. Skeleton-plots depicting annual relative growth rates for each sample were generated and used to visually cross-date samples to a known master chronology compiled from ash trees surrounding the sample area.

Preliminary cross-dating analyses of ash trees in the sample area suggest that emerald ash borer initially became established and began to kill trees in the greater Westland-Garden City vicinity by 1997-1998. Additional analyses are currently in progress to verify the accuracy of the preliminary cross-dating analyses. In related research conducted at several emerald ash borer outlier sites, we have found that an area is typically infested for three to four years before tree mortality occurs. In turn, this suggests that emerald ash borer was introduced and became established in southeastern lower Michigan in the early to mid-1990s.



Preliminary measurements of the reconstructed spread of emerald ash borer in southeastern lower Michigan indicate that the emerald ash borer population exhibited a biphasic expansion following an initial establishment phase. This type of expansion is fairly characteristic of invasive species in which nearby expanding satellite colonies coalesce with their primary core infestation. The core emerald ash borer infestation initially radiated from the epicenter by about 6.5 km each year, then increased to 30 km per year as nearby satellite emerald ash borer colonies started to coalesce. Jump distances of new satellite colonies of emerald ash borer averaged 20 km from the nearest edge of the core infestation (95% core infestation = 15 to 24 km). In five years (1998 to 2003), the area occupied by the core emerald ash borer infestation increased 170-fold.

## MODELING THE SPATIAL AND TEMPORAL DYNAMICS OF ISOLATED EMERALD ASH BORER POPULATIONS

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### ABSTRACT

The ability to predict the distance and rate of emerald ash borer (EAB) spread in outlier populations is needed to continue development of effective management strategies for improved EAB control. We have developed a coupled map lattice model to estimate the spread and dispersal of isolated emerald ash borer populations. This model creates an artificial environment in which several iterations of emerald ash borer dynamics may be performed to represent the spatial spread of EAB over time.

The general spread model involves initial dispersal of adult beetles (e.g., from infested firewood), population growth (potentially constrained by the availability of suitable ash phloem), loss of the ash phloem resource by pre-reproductive emerald ash borer numbers (i.e., larval feeding), and subsequent dispersal of the next generation of emerald ash borer adults. The shift from two-year to one-year development of emerald ash borer larvae with increasing tree stress has been incorporated into the model. Also, the density and distribution of ash and initial emerald ash borer infestation levels can be varied to estimate emerald ash borer spread and dispersal according to site-specific conditions. To develop realistic estimates of emerald ash borer spread and dispersal, model parameters have been fit to match EAB dynamics observed at several outlier sites. In addition, model calibration via large-scale field sampling in forested areas is currently in progress.

Potential applications of this model include: 1) evaluating management techniques and strategies at distinctly different sites (e.g., forest, urban, and riparian sites); 2) determining ash removal zones at emerald ash borer eradication sites given ash distribution, infestation levels, and number of years infested; 3) predicting emerald ash borer dynamics following varying degrees of ash removal; and 4) evaluating the effectiveness of biological control agents. Implications of this research were discussed in relation to future management guidelines.

# MODELING EMERALD ASH BORER DISPERSAL USING PERCOLATION THEORY: ESTIMATING THE RATE OF RANGE EXPANSION IN A FRAGMENTED LANDSCAPE

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## ABSTRACT

The dispersal of organisms is rarely random, although diffusion processes can be useful models for movement in approximately homogeneous environments. However, the environments through which all organisms disperse are far from uniform at all scales. The emerald ash borer (EAB), *Agrilus planipennis*, is obligate on ash (*Fraxinus* spp.) in a widespread but slightly aggregated and spatially correlated pattern at landscape scales. Figure 1 shows the distribution of ash in Southeast Michigan and North Ohio. Modeling random dispersal through a landscape with pattern at a range of scales cannot be done with diffusion processes alone: a mechanism is required to model the spatial structure of the habitat itself.

Whereas *diffusion* processes ascribe the random, “drunkard’s” walk or ‘Polya process’ to the quantity moving, in *percolation* processes, the random movement is determined by the structure of the medium—for example, water “percolating” through ground coffee in a percolator or filter.

The structure of square lattices, such as the 1-km<sup>2</sup> cell map of ash density in Figure 1, are defined by the probability,  $p$ , that adjacent cells are connected. This probability is a good measure of the degree of fragmentation of the landscape: at low  $p$ , the landscape is made up of a large number of disconnected clusters, while at high  $p$ , the landscape is nearly uniform, with a small number of small voids. A characteristic of square lattices is that, at the critical value of  $p = p_c \approx 0.6$ , a large number of clusters coalesce into a single “super-cluster” that spans the lattice. The appearance of the spanning cluster occurs very rapidly near  $p = 0.6$  in a “phase transition” (Figure 2) that permits percolation across the lattice.

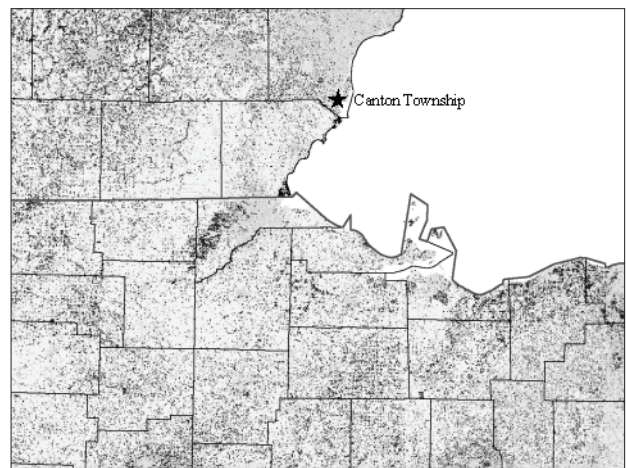


Figure 1. Percolation on a 320 x 400 quadratic lattice representing ash distribution in 1-square-kilometer cells.

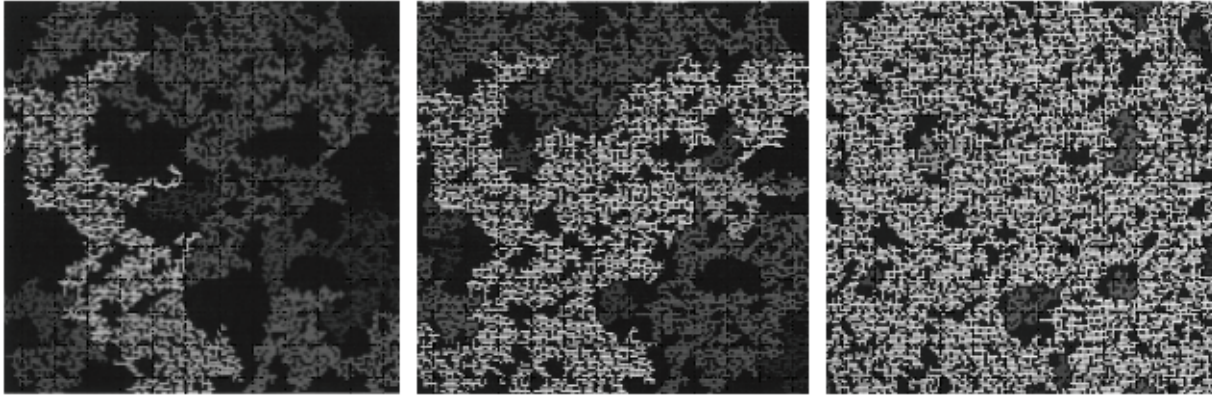


Figure 2. Increasing the occupation probability  $p$  from 0.58 (left) to 0.62 (right), many clusters combine in a “phase transition” at  $p = p_c \approx 0.6$  (center) to span the lattice. The parameter  $p$  is a useful variable to quantify landscape fragmentation.

For organisms capable of moving between clusters, a population can span lattices with  $p$  well below the critical value of 0.6. Thus, the speed with which organisms can cross landscapes will depend on their ability to cross the gaps in the landscape as well as the degree of fragmentation of the landscape. Their ability to cross the gaps is determined by the shape of the relationship between dispersal distance ( $D$ ) and numbers dispersing ( $N$ ). A general function relating  $N$  and  $D$  is  $N = \exp(a + b \cdot D^c)$ , where  $a$  specifies the source population,  $b$  defines the distance scale, and  $c$  defines the curvature. When  $c = 2$ , dispersal is pure diffusion by the Polya or drunkard’s walk, and as  $c \rightarrow 0$ , the dispersal curves become increasingly steep, with very long tails representing a small number traveling extreme distances. The diffusion function was fitted to flight endurance data of gravid female EAB obtained from flight mills (Figure 3), and an estimate of  $c = 1.7$  was obtained.

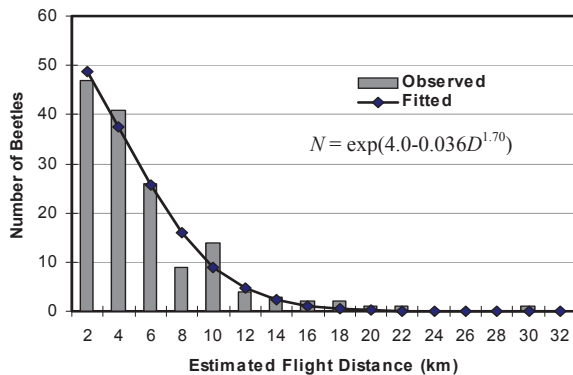


Figure 3. Observed and fitted flight range of gravid female emerald ash borer on flight mills.

reproduction rate of  $r = 10$  and an offspring sex ratio of 50%. Flight mill data show that mated females are most likely to fly long distances and thereby found new populations; thus, the net production of migrants  $r_m = 5$ . To reduce the intense computational requirements of the simulation, only the flights of females were modelled. Ash trees were assumed dead four generations following invasion. Simulations were run until EAB had spanned the map either north-south or east-west. The time in generations taken to span the map and the proportion of ash-populated cells invaded were then recorded.

To model EAB dispersal through the fragmented ash landscape of southeast Michigan and north Ohio, we have combined transport between cells defined by the diffusion function with percolation processes in a cellular automaton model that “flies” EAB between  $1 \text{ km}^2$  cells with ash basal area represented in Figure 1. Population growth was proportional to the ash basal area in each cell. Simulations were run for 90 combinations of  $c$  and  $p$  with the population origin at Canton Township, Michigan, for one gravid female having a net

In general, the longer it took to span the map, the more cells were occupied (Figure 4). In particular, the lower the habitat fragmentation (high  $p$ ) at high  $c$ , the more rapidly spanning occurred, while at high  $p$  and low  $c$ , the longer it took to span the map. Unfortunately, the most rapid spanning occurred at  $c = 1.7$ , the empirical estimate for EAB (Figures 3 and 4a). Most ash cells are invaded at low  $c$  and low  $p$  (high fragmentation), suggesting that increasing fragmentation by clear-cutting ash is unlikely to slow the spread and may result in higher proportion of unoccupied cells being invaded per generation. This does not mean that manipulation of the distribution of ash at the landscape scale cannot help slow the spread, but the strategy will require new approaches.

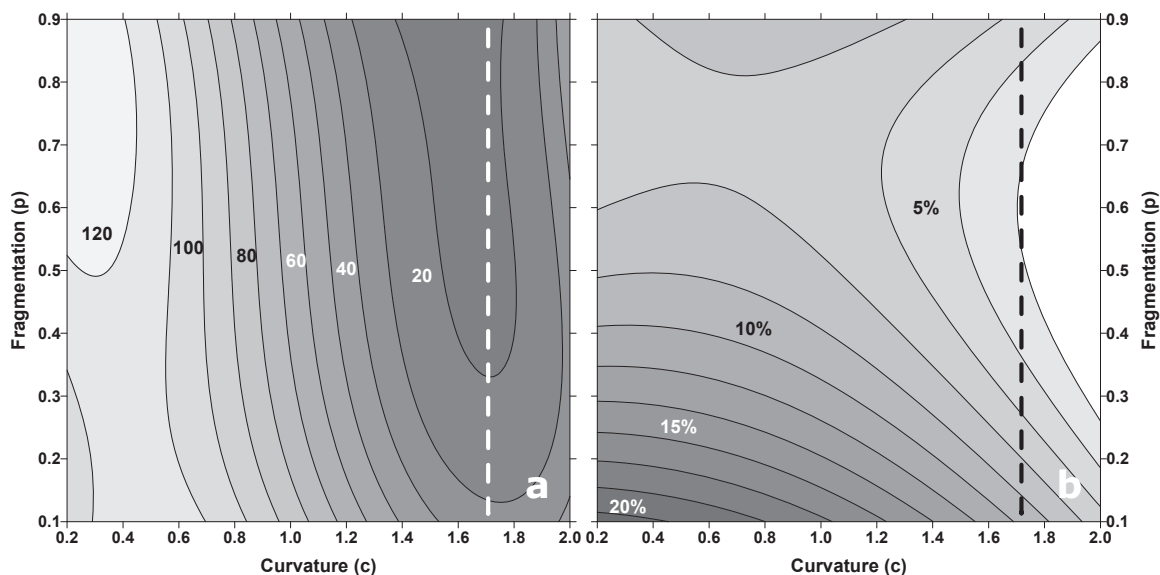


Figure 4. The rate of map spanning. a) The number of EAB generations required to span the ash map in Figure 1 depends strongly on  $c$ , with most rapid population expansion occurring at  $c = 1.7$ ; b) the percentage of cells invaded at spanning is highest at low  $p$  and low  $c$ .

It must be noted that spread in this model is strictly due to the beetles' flight; it does not take into account automobile phoresy or transport by other human agencies. Also, the results presented should not be interpreted quantitatively, although the qualitative results are useful and agree broadly with experience. To develop a quantitatively predictive model, EAB's reproductive rate and relationship between EAB density and ash morbidity must be better quantified and modeled.

## IMPACTS OF EMERALD ASH BORER-INDUCED GAP FORMATION ON FOREST COMMUNITIES

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### ABSTRACT

We have initiated a series of studies to investigate effects of ash mortality and associated gap formation on forest community structure, composition, and succession. To do so, we established 99 plots within the Huron River watershed in southeast Michigan across a gradient of emerald ash borer (EAB) impact ranging from zero to 100% ash mortality. Objectives include quantifying: 1) patterns and rates of ash mortality in relation to tree community composition, 2) successional trajectories in relation to gap size and tree community composition, 3) ash seed bank and seedling regeneration, 4) response of native understory vegetation, 5) spread and establishment of invasive plants, and 6) responses of native arthropod fauna, including ground beetle (Carabidae) assemblages. Once infested, ash decline and mortality progressed rapidly in all stands regardless of basal area, density, species composition, or other stand variables. Ash was the most common woody seedling, which could facilitate ash regeneration but also provide a continued host for EAB. The ash seedbank was limited, which suggests that long-term perpetuation of ash is precarious. Preliminary data suggests that EAB-induced gap formation will facilitate the spread of invasive plants. Ground beetle species richness was reduced in stands impacted by EAB, at least initially. These studies suggest that EAB will have substantial, long-term effects on forest communities.

## EMERALD ASH BORER GENETICS: AN UPDATE

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### ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, samples were collected from introduced sites in Michigan, Ohio, Indiana, Pennsylvania, Illinois, and Ontario, Canada, as well as native sites in China, Japan, and South Korea with the help of a network of collaborators. The beetles were analyzed using DNA sequences from mitochondrial cytochrome oxidase I (COI) and amplified fragment length polymorphism (AFLP) DNA fingerprinting.

EAB individuals from introduced sites in North America all had a mitochondrial COI haplotype identical in more than 450 nucleotides to the haplotype found in most individuals from our collection sites in China and in six individuals from three sites in South Korea. However, haplotypes from individuals from two collection sites in Liaoning Province, China, differed from this main haplotype by one base pair; five individuals from two populations in South Korea differed from the main haplotype by two to four base pairs; and a Japanese sample differed from the main haplotype by 22 base pairs. Interestingly, one individual from Ontario, Canada, differed from all other North American samples by three base pairs.

Two individuals collected in Moscow, Russia, were also analyzed by mtDNA COI and were found to be identical to the main haplotype. EAB is only native to Russia in the Far East north of North Korea; therefore, the population in Moscow was the result of an introduction from the beetles' native range.

AFLP analyses have been carried out using four selective primer pairs, which yielded 139 scoreable bands (loci). In neighbor-joining analysis, samples from throughout the introduced range in North America grouped more often with individuals from China than with individuals from South Korea and the individual from Japan. However, support for hypothesized AFLP relationships was weak. Therefore, microsatellite markers are being developed that we hope will provide the information necessary to identify the geographic origin of the North American EAB populations (and possibly the Moscow, Russia, population) and enabled us to reconstruct the invasion history of EAB in North America (including the separate introductions).

EAB from Lansing, Michigan, were used to develop 96 clones for evaluation. Forty-one primer pairs have been designed and tested for amplification, with 32 of these providing successful amplification. These primers are currently being tested for variability in EAB populations.

## PUTATIVE PHEROMONE FOR THE EMERALD ASH BORER\*

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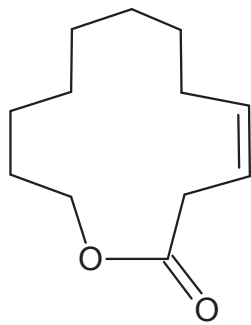
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### ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive beetle species from Asia that has caused extensive mortality of ash trees (*Fraxinus* spp.) since arriving in the United States in 2002. Monitoring its rapid spread has been difficult, and improved monitoring methods are urgently needed. Pheromones have been used to detect pest insects; however, none have so far been identified in the Buprestidae. We analyzed volatiles from adult EAB feeding on ash foliage to identify compounds with pheromonal characteristics that are sex-specific, consistently present, and readily sensed by antennae.

The macrocyclic lactone (3Z)-dodecen-12-olide (stereochemistry at left) was identified from the emissions of the emerald ash borer feeding on ash foliage. The lactone was observed in 129 of the 135 volatile collections from females and in 52 of the 119 collections from males (the compound was near the limit of detection in male samples). The lactone was never detected in volatile collections from ash foliage controls. The amount of lactone from females exceeded that from males by a factor of at least 10. It was consistently present in the initial collections when beetles were received from Michigan. There was a decrease in amount over time, but this may have been related to a general decline in beetle health; mortality was 60% at five days after adult emergence. Both male and female antennae readily sensed the compound. The identification of the lactone was verified by synthesis.



EAB reaches sexual maturity approximately seven days after emergence. However, our initial mating observations showed two-day-old (two out of six) and three-day-old (four out of 10) females readily mating with seven- to eight-day-old males, a time window which coincides with the observed higher emissions of the lactone by younger females.

The behavioral effects of the lactone were tested at ground level using purple sticky traps, but no pheromonal function of the lactone could be verified in this field test. Future field studies will test the behavioral activity of the lactone when placed in the tree canopy, well-exposed to sunlight and closer to the area of mating activity.

\* Full paper published as Bartelt et al. (2007), *Journal of Chemical Ecology* 33:1299-1302.



## EFFECTS OF CUTICULAR CHEMISTRY ON MALE MATING BEHAVIOR IN THE EMERALD ASH BORER

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### ABSTRACT

The invasive emerald ash borer (EAB), *Agilus planipennis*, was examined for behaviors related to mate location and identification. Previous research by our group indicates that EAB males locate conspecifics first visually and then discriminate between the sexes after contact based on some unknown chemical cue. Our laboratory focuses on behavior and chemical ecology, and one of our major objectives is to understand the contact cue that feral male emerald ash borers use to identify a female conspecific.

Currently, we have isolated at least one major and consistent sex difference in the cuticular compounds present on the beetle. In field experiments with EAB, dead female beetles were used to assess behavior involving this potential contact cue. These dead females were either unwashed, washed in solvent, or coated with varying concentrations (including less than one beetle-equivalent, one beetle-equivalent, and greater than one beetle-equivalent) of a twenty-three carbon compound that we isolated from the cuticle of female EAB. Feral male EAB spent the most time examining unwashed dead females, as shown previously. However, males spent significantly more time examining dead females to which the highest concentration of cuticular compound had been applied than those merely washed in solvent.

Future work will focus on isolating and identifying other cuticular substances differing between the sexes in an effort to re-create a precise 'blend' of cuticular compounds that feral males use to identify beetles that they have mounted as female conspecifics. Furthermore, this refined 'blend' will be field tested to determine what, if any, increases in trap catch it may generate when applied to a variety of currently used trapping regimes for EAB.

## HOW EMERALD ASH BORER FACILITATES A SECONDARY SPREAD OF INVASIVE PLANT SPECIES: IMPACTS OF EMERALD ASH BORER ERADICATION AND TREE MORTALITY

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### ABSTRACT

Ever since the discovery of the emerald ash borer (EAB) in 2002, eradication efforts have been implemented in an attempt to eliminate or contain the spread of this beetle. At the time, the eradication protocol called for the removal of every ash tree within a half-mile radius around an infested tree. In 2005, this study was established to identify the environmental changes attributed to the eradication program and measure subsequent shifts in forest community composition and structure.

The project design compares areas that received the eradication treatment (all ash trees cut down) to areas that were left uncut (control: ash trees still standing). This study is conducted in Pearson Metropark, located in Lucas County, Ohio. Emerald ash borer was not completely eradicated from this park, and therefore, those areas that were uncut in 2005 now represent the impact of the natural spread of EAB. These “uncut-control” areas are now experiencing EAB-induced ash tree dieback. The goal of this project is to identify how the plant community is responding in these two areas. Specifically, it is designed to describe the successional stages of plant colonization after a disturbance event, such as the eradication protocol, and to determine the potential for a secondary spread of invasive plant species.

Fourteen 20m x 25m plots (eight uncut and six cut) were established within Pearson Metropark. The plant community assessment included measurements of the canopy composition and structure and herbaceous understory. The light environment was assessed using hemispherical photographs, and soil compaction was measured with a soil penetrometer. Environmental changes in light and soil compaction were immediately detected in 2005 and were similar in 2006.

The eradication protocol accelerated the formation and size of gaps within the forest and thus increased the duration and intensity of light penetrating through to the forest floor. In addition, the vehicles used during the eradication efforts caused significant soil compaction. The degree of soil compaction in 2005 and 2006 was greater in the cut versus uncut plots, with those results detectable at each depth of the soil profile ( $P < 0.0001$ ).

These abiotic changes to the environment have impacted the composition of the plant community. Shannon's Diversity Index ( $H'$ ) was used to detect differences between cut and uncut plots ( $P < 0.001$ ). Cut plots in the West forest had greater diversity ( $H' = 0.58$ ) than the uncut plots ( $H' = 0.30$ ); however, no differences were detected between treatments in the East forest (uncut plots  $H' = 0.47$ , cut plots  $H' = 0.56$ ). In 2006, a Canonical Correspondence Analysis (CCA) was performed to identify species-specific distribution patterns. In Figure 1, 89 species are graphed based on their percent cover. The following non-natives *Alliaria petiolata*, *Cirsium arvense*, *Rhamnus cathartica*, and two *Lonicera* spp. (black stars) are found in cut plots.

One conclusion was that the spread of EAB increases the light environment. However, do eradication efforts cause a further compounding negative effect by increasing the light environment and creating physical disturbance? In 2007, an additional treatment was added to isolate the impact of an increased light environment without the effects of soil compaction. Six new plots, designated as 'cut without soil compaction', had all ash trees removed within them without the use of vehicles: by cutting these trees by hand and removing them with minimal impact, we eliminated soil compaction. In addition, soil cores were collected to determine the composition of species in the seed bank available for revegetation.

The treatments in this study represent three possible scenarios for the spread of EAB, and results will be measured over time. The cut plots in the eradication zone reflect an intense level of anthropogenic disturbance resulting in the greatest sensitivity to a secondary spread of invasive plant species. The 'cut without compaction' plots are designed to reflect a moderate level of anthropogenic disturbance with less susceptibility to invasive plant species predicted. The uncut plots exhibit signs of infestation with some ash tree dieback from the continued spread of EAB and, therefore, reflect a natural disturbance with successional stages of plant colonization. The seed bank study will tell us whether species in the seed bank are the same as the species in the existing plant community and allow us to determine whether, and at what proportion, invasive species exist in the seed bank.

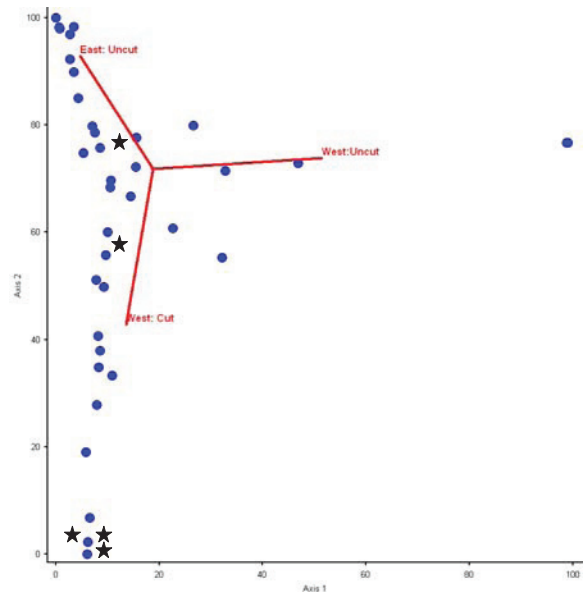


Figure 1. A CCA detected differences between the uncut and cut plots of the West forest. The West uncut plots are correlated with Axis 1 ( $R^2 = 0.989$ ) and the West cut plots were strongly correlated with Axis 2 ( $R^2 = 0.806$ ).

## GENETIC STRUCTURE OF GREEN ASH: IMPLICATIONS FOR THE ESTABLISHMENT OF EX SITU CONSERVATION PROTOCOLS

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### ABSTRACT

*Ex situ* conservation strategies—in the form of seed banks, greenhouses, or botanical gardens—can be used to successfully preserve the genetic diversity of populations under threat. Understanding the genetic structure of such populations is vital to maximizing the amount of genetic diversity collected and preserved by these strategies.

Since its identification in 2002, the emerald ash borer (EAB) has been determined to be a severe threat to native ash tree populations. Once a tree is infested with EAB larvae, there is a 100% mortality rate within 3-5 years. Due to the spread and efficiency with which this beetle can attack, there is great concern about the future of ash trees and thus, the need for *ex situ* conservation. In this study, we describe the genetic structure of nine populations of green ash (*Fraxinus pennsylvanica*) located in the Toledo Metroparks of Lucas County, Ohio.

Leaf tissue was collected from fourteen to fifteen trees from nine metroparks in northwest Ohio. Genetic structure was analyzed using five microsatellite loci. These markers are specific to the genus *Fraxinus* and were developed for the European ash (*Fraxinus excelsior*). Genetic diversity was quantified by calculating the number of alleles per locus ( $A$ ), the effective number of alleles per locus ( $A_E$ ), observed heterozygosity ( $H_O$ ), and Nei's expected heterozygosity ( $H_E$ ) (Nei 1987) for each locus and averaged over all loci. In addition, genetic differentiation was determined using the infinite allele model ( $F_{st}$ ).

Our data revealed that the observed number of alleles was quite high from 12 to 22 per locus, but the effective number of alleles was comparatively smaller: 3.3-6.7 per locus. Therefore, even though common alleles are shared among all parks, there are also rare alleles unique to each of the parks. Based on the high number of alleles per locus, one would expect a higher level of heterozygosity; however, this is not the case (Figure 1). The observed heterozygosity was actually lower than expected according to Hardy-Weinberg equilibrium, suggesting that there is a significant deficiency in heterozygosity. Genetic differentiation among the parks was also detected by F-statistics. The average  $F_{is}$  (genetic inbreeding within parks) was 0.475. The average  $F_{it}$  (reduction in heterozygosity of an individual relative to whole population) was 0.529. These results indicate a deficiency in heterozygosity and support the notion that

inbreeding has occurred in these parks. Furthermore, an  $F_{st}$  measurement of 0.1015 indicates that there is a moderate level of genetic differentiation between the parks.

**Figure 1.** Relationship between expected (Exp) and observed (Obs) heterozygosity for five micro-satellite loci.

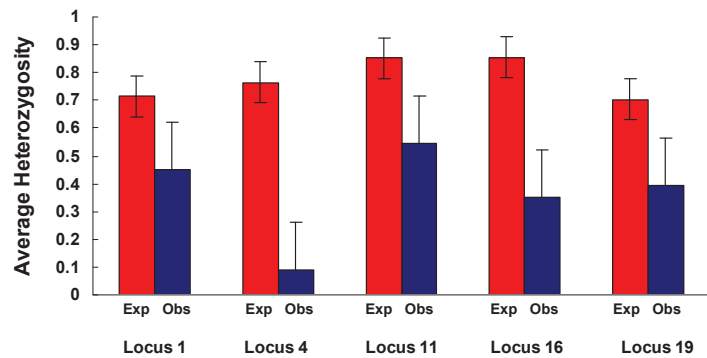
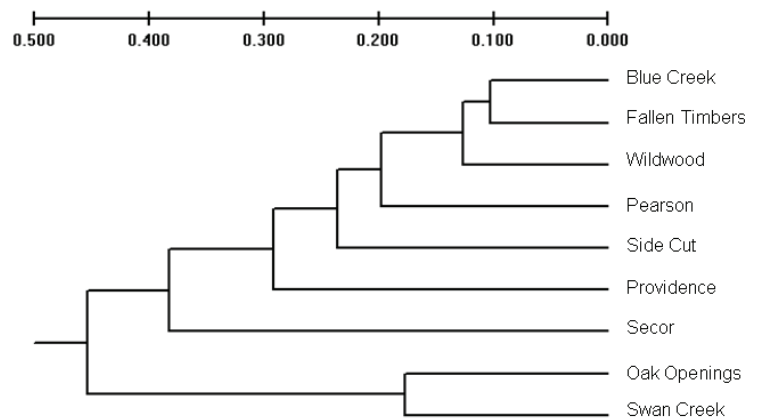


Figure 2 is a cladogram based on Nei's genetic distance that shows how closely related the parks are to each other. While there are distinct groups of parks that are more closely related than others, there is no correlation between genetic distance and geographic distance between the parks (Table 1, next page). Therefore, a conservation protocol cannot be constructed based on geographic distance alone. Further analyses of individual park characteristics are required to determine patterns of genetic diversity.

**Figure 2.** Degree of relatedness between ash populations based on Nei's genetic distance.



Ultimately, these results will be used to create a conservation protocol that includes the establishment of an ash seed bank collection that maximizes the amount of genetic diversity preserved. The potential implications for this work include future ash tree replantings based on genetic diversity and the survival of ash populations.

**REFERENCE**

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**Table 1.** Pairwise comparison of all nine parks based on Nei’s genetic distance (below line) and their geographic distance in miles (above line).

Population	Blue Creek	Fallen Timbers	Oak Openings	Pearson	Providence	Side Cut	Secor	Swan Creek	Wildwood
Blue Creek		6.81	5.11	21.17	3.37	8.21	11.81	11.72	14.76
Fallen Timbers	0.10		8.47	14.39	8.27	1.54	9.54	5.23	9.45
Oak Openings	0.59	0.40		22.11	8.44	9.98	8.16	11.75	12.95
Pearson	0.24	0.18	0.46		21.97	12.95	18.02	10.42	12.19
Providence	0.28	0.24	0.35	0.32		9.35	14.98	13.49	17.28
Side Cut	0.24	0.21	0.44	0.34	0.36		10.3	4.47	9.22
Secor	0.55	0.32	0.42	0.39	0.36	0.40		8.49	6.26
Swan Creek	0.59	0.41	0.18	0.47	0.25	0.47	0.41		5.17
Wildwood	0.14	0.12	0.63	0.17	0.27	0.17	0.31	0.53	

## SURVEY OF EMERALD ASH BORER DISTRIBUTION IN CHINA

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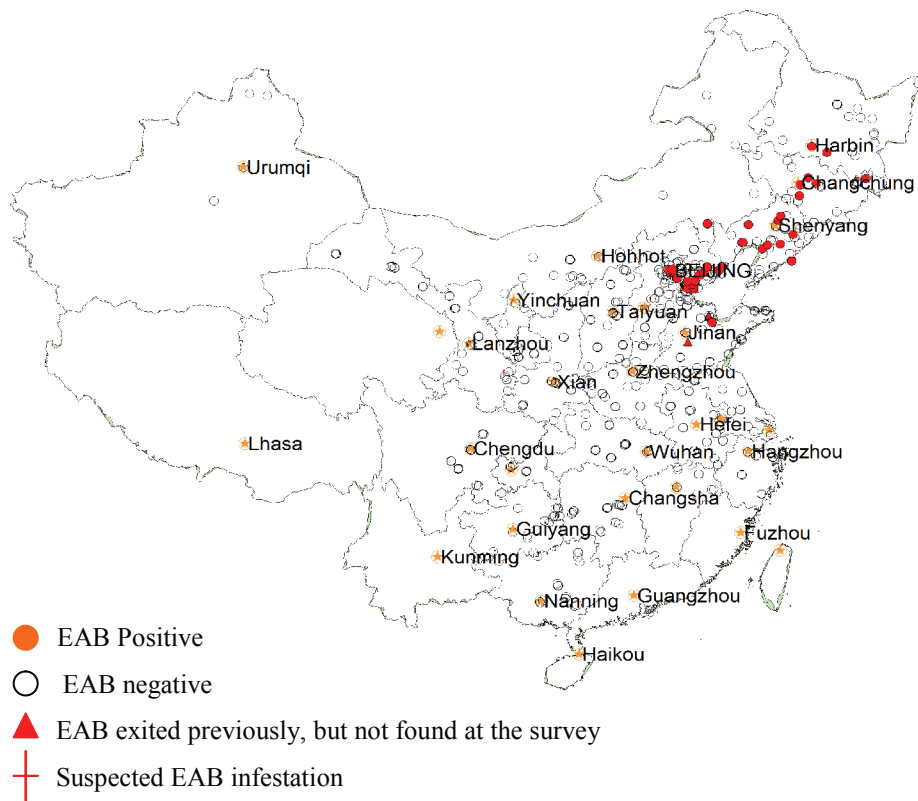
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### ABSTRACT

The goal of the project is to determine the distribution areas of emerald ash borer (EAB) in China in order to help: 1) identify other possible infested areas, 2) characterize the possible impacts in areas that are not currently infested, 3) prevent further spread of the beetle, 4) identify locations for collecting EAB natural enemies, 5) determine potential pathways, and 6) provide insights on how to control EAB in both countries.

Emerald ash borer distribution was recorded in areas outside of North America: in China, Japan, Korea, Mongolia, Russia (Far East), and Taiwan. Host tree species included in this survey were primarily ash, *Fraxinus* spp., but also included *Ulmus davidiana* var. *japonica*, *Juglans mandshurica* var. *sieboldiana*, *Pterocarya rhoifolia*, and *Ulmus propinqua*.

The project involves a literature review and numerous site surveys performed using girdle trees, visual traps, visual inspection, and dissection of infested trees. The locations of all visited trees were recorded via GPS coordinates. In provinces where EAB infestation has been reported, all counties in the affected prefecture or city were surveyed; in provinces adjacent to those where EAB host tree species occur, 10 evenly distributed sites were established for survey. If subsequent EAB or EAB infestations were found at those sites, five to 10 additional sites would be surveyed in each county of the prefecture or city where the infestation occurred and in all counties adjacent to the prefecture. For all other provinces, at least five sites, evenly distributed in the province, will be surveyed for EAB occurrence or infestation. This is an ongoing project, and an updated summary of EAB distribution in China is shown in the following map (next page).





## EVALUATION OF HEAT TREATMENTS FOR QUARANTINE USE WITH EMERALD ASH BORER IN FIREWOOD

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### ABSTRACT

The goal of this project was to determine the thermo-tolerance of emerald ash borer (EAB) larvae and develop a quarantine heat treatment that will allow ash logs, firewood, and pallet material to be safely moved from EAB-infested areas.

Separate experiments were conducted at the USDA APHIS Emerald Ash Borer Laboratory in Brighton, Michigan, to evaluate the thermo-tolerance of EAB using both infested firewood and prepupae removed from infested wood.

Three laboratory heat treatment experiments were conducted during the fall and winter of 2006-2007 with EAB-infested firewood. The initial two experiments used standard laboratory drying ovens (Precision Econotherm, 70 L capacity) and the third used a Blue-M Environmental Chamber using moist heat (0° C wet bulb depression).

Heat treatments were established based on the internal wood temperature at a depth of 3.5 cm from the outer bark surface. In the drying oven experiments, the treatments were 50, 55, 60, and 65° C for 30 minutes. In the environment chamber experiment, treatments were 50 and 55° C for 30 and 60 minutes, each. Six pieces of firewood (cut to ~40 cm lengths and split) were heated together with a thermocouple in each piece to monitor temperatures.

All treatments were replicated a minimum of four times, and wood was placed in emergence barrels after heating. Treatments were evaluated based on emergence of adult beetles. Adult emergence was standardized among treatments by measuring the bark surface area of each piece of firewood and calculating the number of adults per meter of bark surface.

To evaluate the effect of heat treatments on naked larvae, EAB prepupae were removed from logs and subjected to 12 time-temperature combinations. EAB prepupae were obtained from freshly cut (<7 days) green ash logs. Prepupae were acclimated to 23° C for 24 hours prior to heat treatment. Heat treatments were 50, 55, and 60° C for 15, 30, 45, and 60 minutes, each, plus an untreated control. Ten prepupae were used for each treatment, and each test was replicated four times. Prepupae were heated on wet filter paper in a closed Petri dish to avoid desiccation. A calibrated T-type thermocouple was placed in the Petri dish to monitor temperature. After prepupae were removed from the ovens they were allowed to cool and then stored undisturbed at 23° C, 80% relative humidity (RH) for 10 weeks. The number of hatched adults was then counted in each Petri dish to determine survival to adulthood.

Results from the firewood experiments were fairly consistent between the first two experiments (Figure 1). Emergence data indicates that EAB prepupae are capable of surviving a time-temperatures combination up to 60° C for 30 minutes in wood. The 65° C for 30 minutes treatment was, however, effective in preventing EAB emergence on both experiments. The third experiment, using moist heat, showed no EAB survival at either of the 50 or 55 °C treatments (Table 1). Results from the prepupae experiment showed EAB survivorship in time-temperature combinations up to 55° C for 30 minutes or 50° C for 60 minutes (Table 2). The treatments at 60 °C, which did not prevent EAB adult emergence in firewood, were effective in preventing pupation in naked prepupae across all four time durations. Overall, results suggest that EAB survival is variable depending on heating conditions, and that an internal wood temperature of 60° C for 60 minutes should be considered the minimum for safe treatment for EAB in firewood.

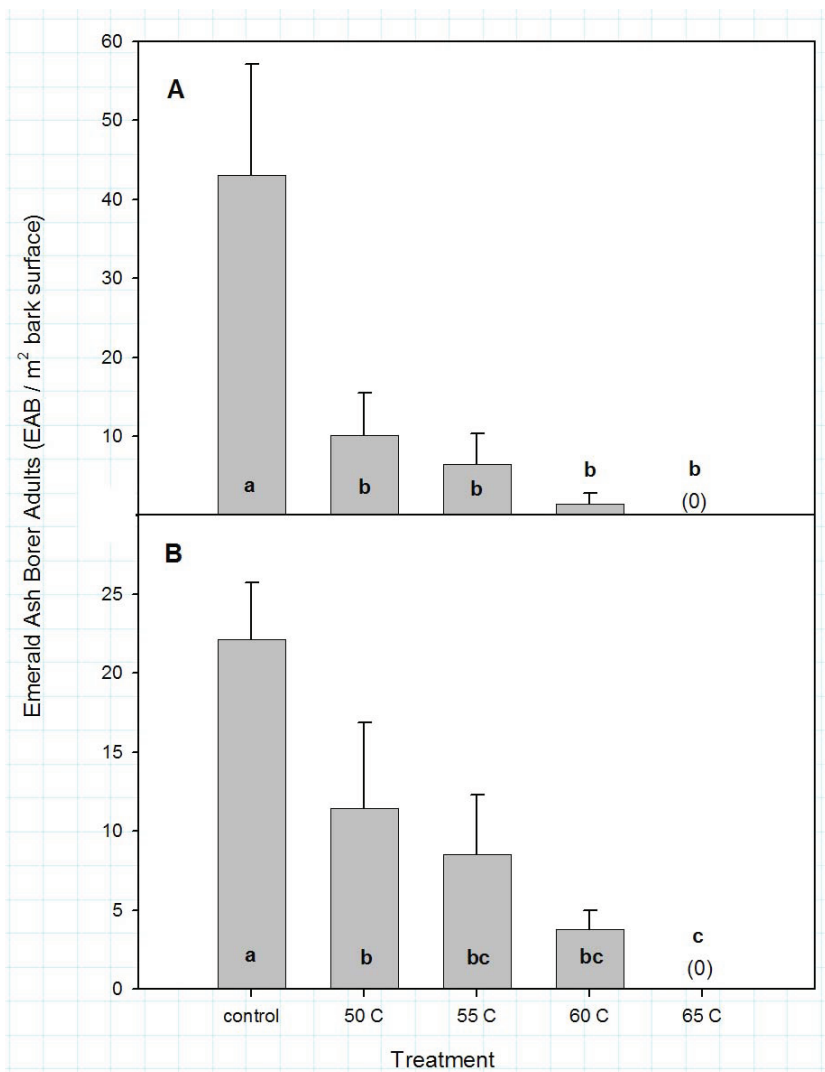


Figure 1. Adult emerald ash borer emergence in firewood heated to four temperatures at a depth of 3.50 cm for 30 minutes in two identical studies conducted in December 2006 (A) and January 2007 (B). Treatments within the same letter did not differ significantly (Tukey HSD,  $\alpha = 0.05$ ).

Table 1. Comparison of heat treatment parameters and adult EAB emergence ( $n = 4$ , mean  $\pm$  SEM) following heat treatment at four time–temperature combinations during June 2007.

Target Temperature (°C)	Time at Target (Min)	Adult EAB emerged	EAB/m <sup>2</sup> bark surface
50	30	0	0
50	60	0	0
55	30	0	0
55	60	0	0
Control		18.5 $\pm$ 5.1	42.0 $\pm$ 11.8

Table 2. Mortality of 10 EAB prepupae following heat treatments at 12 time–temperature combinations ( $n = 4$ , mean  $\pm$  SEM ) during March 2007.

Temperature (°C)	Time (Min)	Mortality (%)	Schneider-Orelli Adjusted Mortality (%)
60	60	100 $\pm$ 0.0 a	100 $\pm$ 0.0 a
60	45	100 $\pm$ 0.0 a	100 $\pm$ 0.0 a
60	30	100 $\pm$ 0.0 a	100 $\pm$ 0.0 a
60	15	100 $\pm$ 0.0 a	100 $\pm$ 0.0 a
55	60	100 $\pm$ 0.0 a	100 $\pm$ 0.0 a
55	45	100 $\pm$ 0.0 a	100 $\pm$ 0.0 a
55	30	90.0 $\pm$ 4.1 ab	87.5 $\pm$ 5.1 ab
55	15	92.5 $\pm$ 2.5 ab	90.6 $\pm$ 3.1 ab
50	60	72.5 $\pm$ 11.1 abc	65.6 $\pm$ 13.9 abc
50	45	55.0 $\pm$ 6.5 cd	43.8 $\pm$ 43.8 cd
50	30	37.5 $\pm$ 12.5 de	25.0 $\pm$ 13.5 de
50	15	67.5 $\pm$ 13.8 bcd	59.4 $\pm$ 17.2 bcd
Control		20 $\pm$ 10.0	-

Means with the same letter do not differ significantly (LSMeans,  $\alpha = 0.05$ , Tukey HSD).

## EVALUATION OF FIREWOOD BAGGING AND VACUUM TREATMENT FOR REGULATORY CONTROL OF EMERALD ASH BORER

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### ABSTRACT

Since its discovery in Detroit, Michigan, in 2002, the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), has caused extensive mortality of ash (*Fraxinus* spp.) as it has spread across southeast Michigan, Ohio, and Ontario, Canada (Haack et al. 2002, Poland and McCullough 2006). In addition to this core infested area, numerous outlier populations have been found throughout Michigan's Lower Peninsula, Ohio, Indiana, and Ontario, Canada, as well as isolated infestations in Maryland, Illinois, Pennsylvania, and West Virginia. Spread of *A. planipennis* is a result of natural dispersal and human-assisted movement of infested materials, including movement of ash nursery stock, logs, and firewood. *Agrilus planipennis* can survive and emerge from logs cut from infested trees; therefore, movement of ash logs from infested to uninfested counties is regulated by a federal quarantine (USDA APHIS 2003). In the case of firewood, all hardwood species are regulated because inspectors cannot easily identify the species of tree that was cut. Nevertheless, *A. planipennis* has continued to spread, and new outlier infestations, possibly due to human-assisted movement of infested material prior to enactment of the quarantine, have been detected each year.

Movement of firewood is extremely difficult to regulate and enforce. Unlike nursery trees and wood products that are produced and moved by licensed businesses, firewood is often moved by the general public. Despite extensive outreach efforts, many individuals are unaware of regulations prohibiting movement of firewood from infested areas. It is estimated that, of outlying infestations in Michigan with known origins, approximately 80% originated

in campgrounds, state parks, lakes and recreational areas, or cottage communities, suggesting they were the result of firewood movement. In order to prevent the spread of *A. planipennis* through movement of firewood, state regulatory and natural resource agencies are enforcing quarantine regulations by conducting inspections for firewood at campgrounds, rest areas, and key transportation gateways. Guidelines for treating and certifying wood to allow safe movement and for storage and handling of confiscated firewood are urgently needed to prevent new establishments of *A. planipennis*. We evaluated two regulatory treatments for infested logs: 1) bagging firewood to prevent emergence and escape of *A. planipennis*, and 2) vacuum treatment to kill *A. planipennis* inside infested wood and logs.

We conducted two experiments to evaluate the efficacy of bagging firewood to prevent emergence of *A. planipennis*. In the first experiment, 30 infested logs were randomly assigned to one of three treatments: 1) unbagged control logs placed in horizontal rearing tubes, 2) logs sealed inside two plastic bags and then placed in horizontal rearing tubes, and 3) logs sealed inside two plastic bags and then placed in vertical rearing cans. For the second experiment, 16 infested logs were randomly assigned to one of two treatments: 1) unbagged control logs in horizontal rearing tubes, or 2) logs that were sealed inside two plastic bags and held in the open on a bench top in the laboratory. Plastic bags used were clear, 24 × 48-inch, 4-ml-thick poly bags (BrownCor, Milwaukee, Wisconsin). For both experiments, logs were held in the laboratory and checked every other day for emerging adults. The bagged logs were examined by carefully inspecting the bag for holes and looking through the transparent bag wall to note any beetles inside.

Once beetle emergence was complete (i.e., no new beetles were collected for six days), the experiments were ended and the results noted. Logs were removed from their rearing containers and/or bags. All dead adults found inside the rearing containers and bags were tallied. The number of new emergence holes on each log was also tallied. A subset of logs were dissected to determine the number of dead adults and larvae that remained inside (N = 4 replicates for Experiment 1, and N = 2 replicates for Experiment 2). In both experiments, several new emergence holes were found and many live *A. planipennis* adults emerged from the control logs; however, no adults escaped from the double-bagged logs.

There were no significant differences among treatments in log length, log diameter, number of exit holes at the start, number of new exit holes at the end, and number of dead adults and larvae found inside the logs at the end of the experiment. All of the *A. planipennis* that emerged from the unbagged control logs were collected live in the rearing tube or collection jar, whereas all of the *A. planipennis* that emerged from the double-bagged logs held in rearing tubes, rearing cans, or in the open on laboratory benches were found dead in the bags by the end of the experiment. There was no evidence of beetles attempting to chew through the inner or outer bags.

We evaluated vacuum treatment for efficacy in killing *A. planipennis* larvae either exposed or inserted into wood. Exposed larvae were placed individually in open Petri dishes inside the vacuum oven. Ten larvae were treated in the vacuum oven at a time and all larvae were weighed before and after treatment. One larva from each treatment group was placed on the load cell of an analytical balance inside the vacuum oven that was connected to a datalogger and recorded weight every 5 seconds. Larvae were subjected to different temperatures and

pressures to determine desiccation rates and lethal percentage weight loss. Some *A. planipennis* larvae died at 26% weight loss, and all were dead at approximately 40% weight loss, the latter of which required at least 15 hours at 20 mmHg and 20° C. The desiccation rate of *A. planipennis* larvae under vacuum at 20 mmHg and 20° C was 2.395% weight loss per hour.

Temperature, pressure, and relative humidity affected desiccation rate. Larvae desiccated slower at cold temperatures; no larvae had died after 36 hours of vacuum treatment at -10° C and 20 mmHg, and weight loss was approximately 5%. Desiccation and mortality were also lower for larvae when inserted into blocks of wood (10 cm wide by 10 cm long by 2.5 cm thick) made from 5.1 cm × 10.2 cm (2" × 4") Douglas fir lumber with a moisture content of 16.6%. After 28 hours of vacuum treatment at 20 mmHg and 20° C, mortality of larvae inside wooden blocks was only 13% and weight loss was only 26%. We also evaluated vacuum treatment of naturally-infested logs with greater than 30% moisture content. Infested logs were approximately 15 cm in diameter and 60 cm long. For each replicate, five infested logs were placed inside a vacuum treatment bag at 20 mmHg and 20° C, and one log was held in the laboratory at ambient conditions. The experiment was replicated four times. Logs were checked every day, and a small area was dissected to determine if larvae inside were dead or alive. If a live larva was found, the logs were returned to the vacuum bag for continued treatment.

After 10 days, the treatment was ended, and all logs were completely dissected to determine the total number of live and dead larvae remaining. After 10 days of vacuum treatment, mortality of *A. planipennis* larvae inside the logs was greater than 98%. The final moisture content of logs following treatment was 18.7%. The conclusion was that at least 10 days of vacuum treatment would be required to kill *A. planipennis* in infested logs.

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## THREE-YEAR PROGRESSION OF EMERALD ASH BORER- INDUCED DECLINE AND MORTALITY IN SOUTHEASTERN MICHIGAN

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### ABSTRACT

We monitored the progression of ash (*Fraxinus* spp.) decline and mortality due to emerald ash borer (EAB), *Agrilus planipennis*, in 38 forest stands in the upper Huron River watershed region of southeastern Michigan from 2004–2007. Black ash (*F. nigra*), green ash (*F. pennsylvanica*), and white ash (*F. americana*) were most common species in hydric, mesic, and xeric stands, respectively. A transect was established within each forest stand consisting of three 0.1-ha circular plots (114 plots total). Within each plot, all ash trees were identified to species and assigned a crown dieback rating on a scale of 1–5, with ‘1’ representing ‘no decline’ and ‘5’ indicating a dead tree.

Ash decline significantly increased over time, from a mean dieback rating of 3.5 in 2004–2005 to 4.8 in 2007. Although black ash initially experienced greater decline and mortality than white or green ash in 2004–2005, this trend was absent in 2006 and 2007, indicating that all species were declining at equal rates. A significant negative relationship was detected between percent ash tree mortality and distance from the epicenter of the infestation in the township of Canton, Michigan, from 2004 to 2006, with mortality decreasing 2% with each kilometer away from the epicenter. On average, percent mortality of ash increased 30% over the three year study period; however, the slope of line describing this relationship (2% decrease in mortality per kilometer away from the epicenter) remained unchanged. This relationship, however, was not significant in 2007, as stands farther away from the epicenter of infestation were approaching 100% ash mortality.

Cumulative survival distributions calculated for a subset of individual trees from 2003–2007 showed that white ash had the highest survival rate of the three species (~three times greater) followed by green and black ash, respectively. Over three years, survival for all species decreased 30–50%. The life expectancy of surviving white ash in summer 2007 was found to be  $1.3 \pm 0.01$  (mean  $\pm$  SE) years,  $0.92 \pm 0.04$  years for green ash, and  $0.79 \pm 0.05$  years for black ash.

Overall, our results indicate that as EAB is causing large-scale mortality of the three major ash species in the upper Huron River watershed region. Thus, EAB has the potential to drastically and irreversibly alter the structure and composition of these North American central hardwood forests.

## HOW FAST WILL TREES DIE? A TRANSITION MATRIX MODEL OF ASH DECLINE IN FOREST STANDS INFESTED BY EMERALD ASH BORER

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### ABSTRACT

We recorded *Fraxinus* spp. tree health and other forest stand characteristics for 68 plots in 21 EAB-infested forest stands in Michigan and Ohio in 2005 and 2007. *Fraxinus* spp. were a dominant component of these stands, with more than 900 ash trees (including *Fraxinus americana*, *Fraxinus pennsylvanica*, *Fraxinus profunda*, and *Fraxinus nigra*) monitored at different sites. Ash condition was rated on a scale of 1 to 5, where ‘1’ represented a healthy tree, ‘5’ represented a dead tree, and ‘2’ to ‘4’ indicated stages of dieback. Individual trees were tracked through time by matching tree diameter and position in the plot.

A general linear multivariate mixed model was used to test the effect of ash condition in 2005 (ordinal), tree diameter, ash species, stand condition in 2005 (average ash condition), habitat, ash density, stand average ash diameter, and ash composition on ash condition in 2007 (ordinal), with individual ash trees as the unit of replication. Ash condition in 2005 was correlated with ash condition in 2007, which showed that trees that were in poor condition in 2005 were likely to be in poor condition or dead in 2007. Smaller-diameter trees underwent more rapid changes in ash condition within the two-year period than did larger-diameter trees. Stand condition in 2005, the average of the ash condition for all ash trees in the stand, was a strong predictor of ash condition in 2007. As the average condition of the stand declined, individual ash trees declined more rapidly.

Stands were separated into four groups based on stand condition in 2005, and these data were used to create four transition matrix models of ash decline, which show the probability of a tree transitioning from each ash condition in 2005 to each ash condition in 2007. In newly infested stands with a stand condition between 1.5 and 1.8, most of the healthy trees remained healthy over the two-year period. In slightly stressed stands and declining stands with stand condition of 2.0–2.5 and 2.6–3.3, mortality and decline increased, but some healthy trees remained healthy. In dying stands with stand condition between 3.7 and 4.1, almost all



trees died within the two-year period. Trees that were healthy in 2005 were either dead or in severe stages of decline by 2007.

A test of the transition matrix model, using 13 stands in Michigan that had not been used to create the model, showed that the model accurately predicts the future conditions of stands. The model can be used to forecast the future conditions of forest stands in newly infested areas. For one hypothetical stand that begins with mostly healthy trees in 2007, the model predicts a 5% increase in mortality by 2009, 50% mortality by 2011, and 98% mortality by 2013. This shows that a healthy stand can be nearly completely killed within six years. We plan to improve and further test the model before making it available to forest managers.

## REGENERATIVE CAPACITY OF ASH FOLLOWING MORTALITY OF CANOPY TREES CAUSED BY EMERALD ASH BORER

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### ABSTRACT

In an era in which the rate of ecological change is unprecedented, understanding the effects of introduced species is critical for predicting the consequences for native biodiversity. Introduced in the Detroit area in the late 1990s, emerald ash borer (EAB) has spread to Ohio, Illinois, Indiana, Maryland, Pennsylvania, West Virginia, and Ontario, Canada, and killed over 98% of healthy ash trees greater than 2.5 cm in diameter. Despite aggressive quarantine and eradication efforts, extensive tree mortality suggests that EAB impact on North American ash species may be comparable to the devastation of chestnut blight on chestnut trees and Dutch elm disease on American elm.

Ecological research on this exotic insect has emphasized monitoring ash mortality and modelling EAB dispersal. Little information exists, however, to describe the regenerative capacity of ash following EAB outbreaks— notable because EAB do not attack ash saplings and seedlings. Our objectives were to describe the spatial variability of ash regeneration within the core area of the outbreak in southeastern lower Michigan, to assess the potential for ash trees to replace themselves following the outbreak, and to predict future changes in ash distribution within the outbreak area.

The first step was to resample previous study sites established to monitor ash mortality and the spread of EAB. We conducted extensive field surveys, employing long belt transects to sample ash regeneration and overstory composition in 45 natural areas in southeastern lower Michigan during the summer of 2007. Mortality of overstory ash trees is typically 97-99% within forty miles of an EAB introduction point; nevertheless, preliminary results suggest that, although ash seedling and sapling density varies greatly across the study area, there is sufficient ash regeneration to withstand competition from other woody species and to replace killed overstory trees. Regeneration was most common on mesic to dry-mesic sites, suggesting that site quality is an important explanatory variable in ash recovery. Seedlings and saplings of green ash and black ash in river floodplains and wetlands, respectively, were far less common than white ash regeneration on uplands, suggesting a strong reduction in overstory ash dominance in wetland and bottomland ecosystems resulting from EAB mortality. Ash regeneration on uplands was higher near forest edges than forest interiors, indicating a displacement of the ash component from interior forests towards the forest edge.

The current ash component has been limited to only a few relict overstory trees that have not yet been killed by EAB. Current levels of ash regeneration are promising for future replacement and recovery of ash as an overstory species, but its ability to reoccupy the overstory will ultimately determine whether ash will be restored to the forest canopy. The future of the ash component of midwestern hardwood forests will depend strongly on future EAB population dynamics—specifically, whether EAB will attack regeneration once those trees have grown to sufficient size for infestation.

## EFFECTS OF HOST STRESS ON EMERALD ASH BORER DEVELOPMENT: WHAT MAKE A GOOD HOME?

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### ABSTRACT

**Background.** Since 2003, we have documented a two-year larval development of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, at a number of newly infested outlier sites. For example, we debarked over 100 ash trees in February 2004 at an outlier site that was infested when nursery trees were planted in 2003. We found a total of 22 larvae in eight relatively healthy trees growing within 800 m of the origin of the infestation; of those 22 larvae, at least 75% were first, second, or third instars and would likely have required an additional summer of feeding to complete development (a 2-year life cycle). A ninth tree, which was highly stressed, contained a total of 36 larvae; all of the larvae on this tree were prepupae or fourth instars and would likely have emerged as adults the following summer (a 1-year life cycle). If the prolonged development of EAB larvae documented at this site is typical, it would strongly influence EAB spread, population dynamics, and survey activities of program managers.

**Methods.** In 2006, we began to examine the role of tree stress on the development, mortality and within-tree distribution of EAB larvae. Our study involved a randomized block design with 90 healthy green ash trees (average DBH of  $12.3 \pm 0.3$  cm) in a 15-year-old, well-stocked ash plantation. There were 30 trees receiving one of three treatments: 1) girdling, 2) exposure to methyl jasmonate (MeJa), a stress-elicitor, or 3) untreated controls. This site had a very low, nearly undetectable population of EAB. An average of  $0.9 \pm 1.8$  adult beetles per tree were trapped on sticky bands during the summer; nearly all of the 81 beetles were trapped on girdled trees. All 90 trees were felled between January and March 2007 and bucked into 1 m sections to height of 7 m. Each section was debarked and larvae identified to instar. Fourth instars and prepupae were recorded as one-year larvae, while first, second, or third instars were classified as 2-year larvae because they would likely have fed for an additional year to complete development. When dead larvae were encountered, they were assigned to one of three categories: predation by woodpeckers, cannibalism, or death from unknown causes (most likely a pathogen or desiccation).

**Larval density and within-tree distribution.** Larval density varied greatly among treatments. Girdled trees had an average of  $57.62 \pm 13.13$  larvae per  $m^2$ , while control and MeJa trees had an average of  $3.92 \pm 1.60$  larvae per  $m^2$  and  $5.76 \pm 5.06$  larvae per  $m^2$ , respectively. Larval density was consistently highest at 3 to 4 m aboveground regardless of treatment. Woodpecker predation was the most common cause of death, causing 61% of all larval mortality. We also found that 70% of woodpecker attacks occurred at least 4 m aboveground.

**Tree Stress and Larval Development.** A significantly greater proportion of larvae on girdled trees were 1-year larvae (57.3%) compared to larvae on control trees (18.9%) and MeJa-treated trees (11.9%). When calculated by height and treatment, about 90% of larvae below the girdle (0 – 1 m) were 1-year larvae, while about 50% of larvae above the girdle (1 – 7 m) were 1-year larvae. The pattern of larval feeding was also different below the girdle than above the girdle. The horizontal distance that a larva traveled (the furthest horizontal distance that the gallery extended) averaged 10 cm below the girdle compared with 3 cm above the girdle. The average horizontal distance traveled for larva on control and MeJa-treated trees was also 3 cm at all heights. Only galleries made by larvae that had reached the prepupal stage were measured.

We repeated the study in 2007 with 90 new trees and applied the same three treatments. The number of adult EAB captured on sticky bands during the summer increased by a factor of ten over the 2006 results. We expect to see a similar increase in larval density: debarking of these study trees is in progress.

## INTERSPECIFIC VARIATION IN RESISTANCE OF ASH TO EMERALD ASH BORER

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### ABSTRACT

We conducted a three-year study to compare the susceptibility of selected North American ash and an Asian ash species to emerald ash borer (EAB), *Agrilus planipennis*. We hypothesized that Manchurian ash, an Asian ash species, would be more resistant to the beetle than its North American congeners because of a coevolutionary relationship between Asian ash species and EAB. Consistent with our hypothesis, Manchurian ash exhibited far less mortality and yielded far fewer adult beetles than several cultivars of North American green and white ash. Surprisingly, a hybrid of North American black ash and Manchurian ash was highly susceptible to EAB, indicating that this cultivar did not inherit EAB resistance from its Asian parent. A corollary study examined the efficacy of soil-applied imidacloprid (a systemic, neonicotinoid insecticide) for controlling EAB in each of the five cultivars. Imidacloprid had no effect on EAB colonization of Manchurian ash, which exhibited low susceptibility in untreated as well as treated trees. In contrast, imidacloprid did enhance survival of the North American and hybrid cultivars and significantly reduced the number of EAB adults emerging from green and white ash cultivars.

Host phloem chemistry, both constitutive and induced, might partly explain this interspecific variation in resistance. We analyzed the constitutive phloem chemistry of Manchurian, white, and green ash trees. Analysis of the crude phloem extracts revealed the presence of an array of phenolic compounds, including hydroxycoumarins, a monolignol, lignans, phenylethanoids, and secoiridoids. Both qualitative and quantitative differences were observed among the three ash species. Hydroxycoumarins and the phenylethanoids, calceolariosides A

and B, were present only in the phloem of Manchurian ash and might represent a mechanism of resistance against EAB.

To further examine interspecific biogeographical variation in ash resistance/susceptibility to EAB, the experiment plantation was expanded in April 2004 to include the additional North American ash species *F. quadrangulata* (blue ash), *F. nigra* (black ash), and *F. latifolia* (Oregon ash), the Asian species, *F. chinensis*, and the European species, *F. ornus*, *F. excelsior*, and *F. oxycarpa*.

## DEVELOPMENT OF NOVEL ASH HYBRIDS TO INTROGRESS RESISTANCE TO EMERALD ASH BORER INTO NORTH AMERICAN ASH SPECIES

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### ABSTRACT

Currently, there is no evidence that any of the native North American ash species have any resistance to the emerald ash borer (EAB). This means that the entire ash resource of the eastern United States and Canada is at risk of loss due to EAB. In contrast, outbreaks of EAB in Asian ash species are rare and appear to be isolated responses to stress (Bauer et al. 2005, Schaefer 2005, Gould et al. 2005). Our work is based on the hypothesis that there are resistance mechanisms and thus resistance genes that have evolved within Asian ash species that allow them to coexist with the EAB. This hypothesis is supported by a common garden study (Rebek et al., in press) that demonstrated that a cultivar of the Asian species *Fraxinus mandshurica* exhibits a higher level of resistance to EAB than several cultivars of North American green ash and white ash. It is our long-term goal to introgress, or introduce, these genes through the development of novel ash hybrids and, through subsequent rounds of backcrossing, retain all of the characteristics of the native North American species in addition to EAB-resistance.

Over the past three years, 31 different combinations of ash species have been used to perform controlled cross-pollinations. Nine different species were used as the maternal parent; eight different species were used as pollen donors. A total of 1,619 seeds were produced, but only four different species combinations germinated, producing a total of 44 seedlings. Genetic markers such as AFLPs and SSRs are being used to confirm the parentage of the resulting hybrid progeny, which will be supplemented through grafting and tested for EAB resistance. An estimation of relatedness of the species based on a phylogram generated by comparison of ITS sequences (Wallander 2001) indicates that the successful hybridizations occurred between species that were closely related.

In addition to genetic relatedness, there are many other potential barriers to successful interspecies hybridization, including differences in ploidy, phenology, and breeding systems. Ploidy levels in some Asian and North American species can vary depending on the region of origin. Ploidy levels of a few species remain unknown, including *F. quadrangulata* (blue ash) and *F. mandshurica* (Manchurian ash). Ash cytogenetics experiments are being initiated to determine the ploidy level of these species as well as to confirm that parent pairs used in hybridizations have compatible ploidy levels.



Equally significant barriers to a successful breeding program are the lack of species with known resistance to EAB and the need for well-defined, genetically diverse resistant trees for use as parents. To address these issues, efforts are being made in conjunction with various arboreta throughout the United States to obtain accessions of Asian ash species with diverse geographic origins. Information on genetic variation has been limited by the use of clonal horticultural cultivars in recent studies (Rebek et al., in press). In order to allow estimation of genetic variation in EAB resistance both within and between species, plantings are being established. These plantings include four North American ash species, three Asian ash species, and three European ash species. All material included in this planting is of seed origin, with one to three provenances represented per species.

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## POTENTIAL OF SPINOSAD APPLICATIONS FOR EMERALD ASH BORER CONTROL

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### ABSTRACT

One of the pressing needs for effective management of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is an insecticide that can be used over large, wooded acreages where this insect has been identified as recently introduced or where it is found at low population densities. Spinosad, a biological insecticide produced by a soil bacterium, has been shown to be toxic to the adult life stage of the EAB and is a superior candidate for use in aerial applications.

Spinosad is in common use by organic growers and was granted a Green Chemistry award by the EPA, which classifies it as “reduced risk” because of its good environmental profile. On an acute basis, spinosad is slightly to moderately toxic to fish and toxic to aquatic invertebrates; however, spinosad’s half short-life in the environment mitigates this acute toxicity to a large degree. Spinosad is practically non-toxic to avian and mammalian species and exhibits large margins of safety to many beneficial insects. Spinosad has short half-lives in soil (9-17 days), on foliage (4-16 days), and in water (hours to 2 days), and has very low potential for run-off or leaching as it binds strongly to soil.

Technical spinosad and spinetoram were tested for toxicity to EAB adults by topical application. Adult insects were also exposed to foliage from field-grown plants treated with spinosad formulated as GF-976 (Tracer; 480SC) and SPLAT<sup>®</sup> 30M-1 to determine if the latter formulation could prolong foliar residue. Plant foliage was collected for residue analysis and adult EAB exposure at 0, 1, 2, 7, 14, 28, 56, and 84 days post-treatment. Residue analysis of spinosad will be performed using an ELISA kit manufactured by Strategic Diagnostics.

Adults exhibited mortality in a dose-dependent manner when technical spinosads were applied topically. LC<sub>50</sub> and LC<sub>90</sub> estimates generated at 24 and 48 hours after exposure were similar to those previously reported; LC<sub>50</sub> = 59 ppm and LC<sub>90</sub> = 373 ppm at 24 hours post-exposure and LC<sub>50</sub> = 20 ppm and LC<sub>90</sub> = 82 ppm at 48 hours post-exposure. EAB adults ingesting or coming into contact with treated foliage were similarly impacted, with near 100% mortality for both formulations tested at 7 days after application (DAA) and 2 days of exposure. Mortality declined slightly to between 60% and 70% in foliage aged 14 DAA. Activity was apparently retained beyond 60 DAA, with 30% mortality observed for GF-976 and 60% for the SPLAT formulation.

The retention of insecticide activity of the field applications is in contrast to previous observations during an aerial application in 2006, when leaf residues rapidly declined 2 DAA and were almost non-existent 13 DAA. Foliage residue analysis should provide additional information on actual levels of field degradation of spinosad.

## EVALUATION OF NON-INVASIVE TRUNK SPRAYS AND TRUNK-INJECTED EMAMECTIC BENZOATE

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### ABSTRACT

In 2007, we continued to evaluate two neo-nicotinoid insecticides, imidacloprid and dinotefuron, applied as non-invasive trunk sprays to control emerald ash borer (EAB), *Agrilus planipennis* Fairmaire. Neo-nicotinoid products are widely used to protect landscape ash trees because they are relatively safe for humans and non-target species. These systemic products generally applied annually to the soil or via trunk injection and the insecticide is translocated to the canopy. Adult EAB, which must feed on ash leaves for at least two weeks and often longer before oviposition occurs, encounter the insecticide in the foliage. Although these products can be effective, concerns have arisen about the long-term effects of repeated wounding associated with trunk injection, the time required to apply or monitor trunk-injected products, and possible negative consequences of applying insecticides to soil at some sites.

**Background.** In 2006, we evaluated a non-invasive, efficient, and simple method of applying imidacloprid and dinotefuron to the trunk of ash shade trees. This application method involves mixing the insecticide with PentraBark™, a non-toxic, bark-penetrating surfactant (Agrichem, Medina, Ohio). The formulated solution was applied directly to the bark on the lower trunk of a tree with a common garden sprayer. PentraBark™, originally developed as an agricultural surfactant, has recently been used to carry fungicide products through the bark and into the xylem tissue of trees, through which the product is then translocated to the canopy. We sprayed the bark on the trunk of the trees from 20 cm to 1.6 m aboveground until it was wet.

In our 2006 study, we used a randomized block design with 6 to 12 trees per treatment, replicated at four sites; average DBH of trees ranged from 5 to 15.5 inches. Each block consisted of five trees treated with: 1) a non-invasive trunk spray of Macho® 2F (imidacloprid) + PentraBark™; 2) a non-invasive trunk spray of Safari® (dinotefuron) + PentraBark™; 3) a soil application of Merit® 75WP (imidacloprid) applied at the base of the tree with a Davey wand; 4) a trunk injection of Imicide® (imidacloprid) applied with Mauget capsules (e.g., a positive control); or 5) left as an untreated control.

Data from 2006 showed that the trunk sprays of imidacloprid + PentraBark™ and dinotefuron + PentraBark™ effectively moved the insecticides into the vascular tissue of trees and that the insecticides were translocated to the canopy. Dinotefuron, which is highly soluble in water, appeared to translocate relatively rapidly into the canopy. Residue levels peaked in mid-June, and then declined by roughly 40-50% over the next three weeks, suggesting that the product may break down relatively quickly. Residue levels in imidacloprid trees from trunk sprays and soil applications continued to increase from mid-June to July to August, suggesting that the product moved relatively slowly into the canopy or foliage. In contrast, foliar imidacloprid residues peaked in mid June in trees treated with the trunk injection (Mauget capsules). In bioassays, beetle mortality (control-corrected) after four days of exposure ranged from 27-55% among imidacloprid-treated trees and 56-77% on dinotefuron-treated trees. Larval density varied considerably among treatments and sites, but was generally lower on treated than untreated trees. Differences among treatments were statistically significant at one site, where larval density was roughly 50 to 75% lower on treated trees than on control trees.

**2007 Study.** In 2007, we continued to work with neo-nicotinoids trunk sprays to assess the consistency of results, determine whether adjusted application timing would enhance EAB control, and evaluate whether the PentraBark™ product improved efficacy of the insecticide products. In addition, we evaluated the efficacy of emamectin benzoate applied via trunk injection. Although emamectin benzoate is used in a variety of pesticide products, it is not yet registered for use on ornamental trees.

We established a total of 25 blocks at three different sites, each consisting of seven trees with an average DBH of 6.6 to 13.2 inches. The seven treatments represented in each block included: 1) untreated control; 2) trunk injection with Imicide® (10%, 3 ml Mauget capsules); 3) trunk injection with emamectin benzoate; 4) a non-invasive trunk spray of Macho® 2F (imidacloprid) + PentraBark™; 5) a non-invasive spray of Macho® 2 without PentraBark™; 6) a non-invasive trunk spray of Safari® (dinotefuron) + PentraBark™; and 7) a non-invasive trunk spray of Safari® without PentraBark™. Application rates for the Mauget trunk injections and trunk sprays were the same as those used in 2006 (McCullough et al. 2007). The emamectin benzoate was applied as a 4% solution with an Arborjet micro-injector. Application dates were May 4 for trunk sprays of Macho® 2F, May 22 for trunk injections of Imicide® (Mauget capsules) and emamectin benzoate, and May 31 for trunk sprays with Safari®.

**Foliage residues.** Translocation of insecticides to leaves in the canopy was evaluated by collecting composite foliage samples from eight locations in each tree in mid-June, early July, late July, and mid-August. Foliage samples were individually bagged and frozen for eventual residue analysis with ELISA (imidacloprid, dinotefuron) or MS/HPLC (emamectin benzoate). Analysis of foliage samples is in progress.

**Adult EAB bioassays.** Bioassays were conducted in mid-June, early July and late July, to assess survival of EAB beetles caged with leaves from each study tree. On each date, two leaves were collected from opposite sides of each tree and three beetles were placed on each leaf for four days. In the 15 June bioassay, no EAB survived on leaves from emamectin benzoate-treated trees. Beetle survival on trees treated with Safari® (dinotefuron) dropped to less than 15% by Day 4. Beetle survival on leaves from trees treated with Mauget capsules and Macho® 2F (no PentraBark™) was also significantly lower than survival on control trees. In the early

July and late July bioassays, we again observed 100% mortality of EAB on the emamectin benzoate-bearing leaves. Beetle survival was generally lower on other treated trees than on controls in July bioassays; however, at least, 40% of the beetles survived on the imidacloprid- and dinotefuron-treated trees.

**Larval density.** We assessed larval density in late September by felling and debarking areas on the trunk and canopy of trees. We felled three blocks of trees at the 7-L site and four blocks of trees at the IS site (49 total trees) to estimate larval density in 2007. The remaining blocks of trees will be re-treated and monitored through 2008.

At the 7-L site, we removed bark from at least 9 to 12 bark windows (each  $\geq 500$  cm<sup>2</sup> in area) per tree on the 21 trees that were felled. At the IS site, we examined at least 32 windows per tree on the 28 trees that were felled. All seven trees treated with emamectin benzoate, however, were completely debarked. Larval density, stage, and viability were recorded and standardized per m<sup>2</sup> of phloem.

Larval density varied considerably within and among treatments, as expected. The untreated control trees at the 7-L site and the IS site averaged 132 and 68 EAB per m<sup>2</sup>, respectively. Trees treated with imidacloprid applied either as a trunk injection (Mauget capsules) or as a trunk spray had an average of 14 to 62 EAB per m<sup>2</sup> at the 7-L site and 14 to 75 EAB per m<sup>2</sup> at the IS site. The trees sprayed with dinotefuron had an average of 41 to 51 EAB per m<sup>2</sup> at both sites.

The efficacy of the emamectin benzoate, however, was striking. When we completely debarked the seven emamectin benzoate trees, we found only eight live larvae, equivalent to 0.19 larvae per m<sup>2</sup>. There were no more than three live larvae on any of the trees, and two of the trees had zero live larvae. Overall, emamectin benzoate provided greater than 99% control of EAB. We recovered a total of 81 dead EAB larvae on the seven trees, most of which were late instars. Results from the larval sampling and the adult bioassays indicate that emamectin benzoate probably acts primarily on adult EAB and/or neonate larvae; otherwise, we would have expected to find hundreds of dead late stage larvae on the trees. Moreover, because emamectin benzoate affected adults and neonate larvae, the trees sustained little injury.

We plan to continue this project to evaluate product persistence, application rates, application timing, and other factors. The remainder of the trees treated in 2007 will be held and either re-treated or monitored in 2008. The notable control provided by the emamectin benzoate may provide a new tool, both for urban ash tree protection and perhaps for use in an integrated EAB management strategy. We expect to work with industry representatives and state regulatory officials to consider special registration for emamectin benzoate, perhaps as early as 2008.

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## IMIDACLOPRID CONCENTRATION EFFECTS ON ADULT EMERALD ASH BORER: A GREENHOUSE STUDY

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### ABSTRACT

Imidacloprid is the active ingredient of many widely used products applied to control the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, in valuable urban trees. Systemic treatment with imidacloprid is typically made in the spring to reduce the number of larvae that would otherwise be generated by oviposition during the summer. Substantial evidence suggests that imidacloprid suppresses larval density indirectly by its effects on survival and/or reproductive performance of adults interacting with treated foliage. This conclusion is based on findings from five years of research, in which we have observed: 1) far greater toxicity of imidacloprid on adult EAB than on larvae, 2) little evidence of larval mortality or growth suppression when treated trees are debarked, and 3) significant reductions in survival and feeding of adults exposed to treated foliage. In this study, we sought to more precisely quantify the effects of foliar imidacloprid on adult EAB survival and feeding. The results will suggest the relative importance of lethal and sublethal effects and define the residue range at which these effects occur.

**The experiment.** We planted bare-root green ash whips in 1-gallon pots in a greenhouse. To create a range of imidacloprid residue levels, we treated the pots at leaf flush with a soil drench of Bayer Advanced™ (1.5%) applied at rates that varied from minimal to very high. In Trial 1, there were seven replicates for each of six treatments, ranging from 0.8 to 2500 mg AI of imidacloprid in 5X increments. In Trial 2, there were 11 replicates of eight treatments, with 6.25 to 800 mg imidacloprid applied in 2X increments. When leaves were fully expanded (6-8 weeks post-planting), two leaves from each plant were collected. Leaf area was measured, and each leaf was placed in a petri dish with three EAB adults. Beetle survival in each dish was monitored for four days. Leaf area was then re-measured, and foliage was transferred to the USDA APHIS PPQ CPHST Otis lab for ELISA analysis.

**Results.** We found a linear relationship between log(treatment dose) and log(imidacloprid residue) in both trials (Figure 1). For Trial 1, mean leaf residues ranged from 0.75 ppm (for 0.8 mg/pot) to 141 ppm (for 2500 mg/pot). In Trial 2, leaves contained far lower residues for a given treatment dose. Mean leaf residues ranged from 0.03 ppm (for 6 mg/pot) to 25 ppm (for 800 mg/pot).

For both trials, we determined thresholds for leaf consumption and beetle survival. We found that beetle leaf consumption was significantly affected at lower residue levels than was four-day survival (Figures 2 and 3). In Trial 1, no feeding occurred at residues >3.5 ppm, and no beetles survived exposure to >33 ppm imidacloprid. In Trial 2, thresholds for feeding cessation and survival were 3.8 ppm and 40 ppm, respectively.

In a related experiment, we evaluated the fate of individual beetles exposed to the 25 ppm treatment in Trial 2 to determine whether beetles receiving a sublethal dose sufficient to inhibit feeding recover if removed to clean foliage. We found that, 48 hours after being removed from leaves containing imidacloprid and placed on clean foliage, half of the initially intoxicated beetles (unable to walk or feed) recovered.

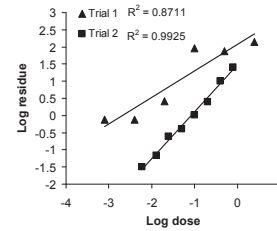


Figure 1. Relationship between treatment level (log(mg/pot) and leaf residue (log(ppm imidacloprid)).

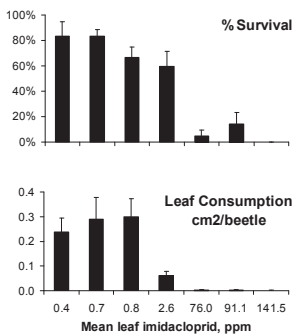


Figure 2. Emerald ash borer response to leaf residue, Trial 1. For treatments >2.6 ppm, survival differs from control. For treatments >0.8 ppm, leaf consumption differs from control.

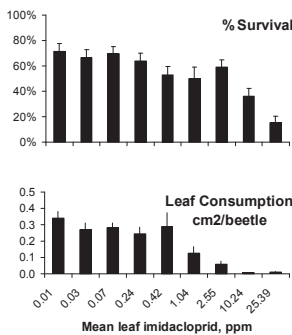


Figure 3. Emerald ash borer response to leaf residue, Trial 2. For treatments >10.2 ppm, survival differs from control. For treatments >0.42 ppm, leaf consumption differs from control.

To provide a basis for comparison between results of this greenhouse trial and actual imidacloprid treatment of trees, we compiled a summary of peak foliar imidacloprid residues observed in 2006 field trials. For two treatments (trunk injection of Imicide® via Mauget capsules and soil injection of Macho® 2F) used at three sites, mean residue levels in foliage ranged from 2.1 to 10.6 ppm (Table 1).

**Discussion.** In this experiment we found that imidacloprid strongly affects beetle feeding at levels well below those that cause mortality. Further, we established an approximate range of leaf imidacloprid concentration where lethal and sublethal effects are observed. In practice, the leaf residues that we found to inflict high EAB mortality are not typically achieved in field applications of imidacloprid. It appears likely that the benefits of treatments are primarily attributable to sublethal effects on EAB adults. Our experiment evaluates only one such effect: feeding inhibition. Other effects associated with feeding inhibition might be physiological (long-term mortality, reduced fecundity), behavioral (increased dispersal), and/or ecological (exposure of intoxicated beetles to predation or desiccation). A true understanding of the mechanism for imidacloprid efficacy will require investigation of these effects.

Table 1. Foliar imidacloprid residues. Values determined for July samples in 2006 MSU pesticide trials.

		Foliar Imidacloprid Residue, ppm	
Site	Treatment	Max	Mean
7L	Mauget	8.8	3.5
	Soil Merit	6.5	2.1
7s	Mauget	38.9	10.6
	Soil Merit	14.4	5.2
LA	Mauget	14.3	4.3
	Soil Merit	7.3	3.3



## DISTRIBUTION OF TRUNK-INJECTED <sup>14</sup>C IMIDACLOPRID IN ASH TREES: VARIATION BETWEEN SPRING AND FALL INJECTIONS

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### ABSTRACT

Trunk-injected applications of imidacloprid are an effective but variable means of control for emerald ash borer (EAB), *Agrilus planipennis* Fairmaire. There is some evidence that a single application of imidacloprid can provide two years of EAB control, but the mechanism and overall length of control remains unknown. Arborists usually perform trunk injections of imidacloprid in either the spring or fall of the year. Two questions remain: 1) Can fall imidacloprid trunk injections provide adequate EAB control the following spring? and 2) Can a single spring or fall trunk injection provide more than a single season of EAB control? This portion of the <sup>14</sup>C imidacloprid trunk injection experiment examines the differences in Imidacloprid equivalent concentrations between trees injected during the spring and trees injected during the fall.

On June 27, 2006 and September 5, 2006, we trunk injected 32 trees (16 *Fraxinus americana* and 16 *F. pennsylvanica*) and eight trees (4 *Fraxinus americana* and four *F. pennsylvanica*), respectively, with 25 µCi <sup>14</sup>C labeled imidacloprid and Imicide® imidacloprid at a ratio of 1:1300 labeled to unlabeled compound. The trees were 1.5 – 2.0” caliper bare root and were planted in pure sand in 25-gallon containers. The single injection point was determined by the first whorl of branches. We injected trees at either 0° to the first whorl of branches or 90° to the first whorl of branches. Preliminary analysis presented here examines trees injected at 0° to the first whorl of branches (Figure 1). Trees were injected at 10 cm above the graft union. Holes were drilled in the trunk using a 5/16” drill bit. Stem injection tubes were inserted into the holes, and the trees were injected at 30 PSI using a bicycle pump. Stem injection tubes were removed after the trees took up all fluid. Each branch of the first three whorls of the tree was labeled 0°/180° or L90°/R90° depending on the location of the branch in relation to the injection point. Each branch was sampled separately.

Leaf samples of trees injected in June were taken at 0, 2, 7, 21, 60, 98, 336, 350, and 429 days after treatment (DAT). Leaf samples of trees injected in September were taken at 0, 2,

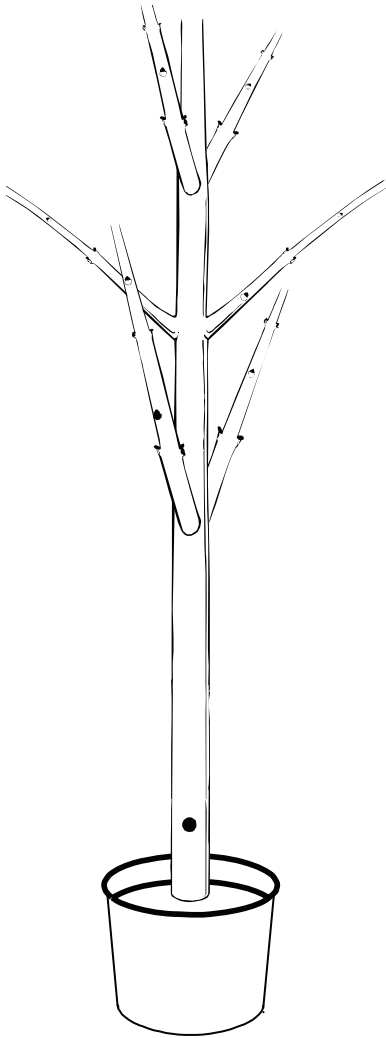


Figure 1. Tree injected at  $0^\circ$  to the first whorl of branches. Mean distance from injection point to the first whorl = 1.28 m. Mean distance between whorls = .18 m.

7, 21, 267, 280, and 360 DAT. Preliminary analysis presented here examines leaf samples 21 DAT for both the spring and fall injections and 350 and 280 DAT for spring and fall injected trees, respectively. Days 350 and 280 corresponded to peak flight of EAB adults in 2007.

All leaf samples were oven dried, ground with a mortar and pestle and oxidized in a biological tissue oxidizer. The resultant  $\text{CO}_2$  was trapped in scintillation cocktail and the amount of radioactivity was determined by using a scintillation counter. Counts per minute were recorded for each sample. Total “imidacloprid equivalents” per microgram of dry weight were calculated from activity counts after accounting for oxidizer efficiency (97%) and scintillation counter efficiency (97%).

Imidacloprid equivalent concentration in leaves varied with time, orientation to the injection point, and whorl height. Leaves on branches opposite the injection point ( $180^\circ$ ) had lower imidacloprid-equivalent concentrations than the leaves on branches in the same plane as the injection point ( $0^\circ$ ). Leaves on branches of trees injected during the fall had a lower imidacloprid-equivalent concentration 21 DAT when compared to leaves of trees injected during the spring 21 DAT (Figure 2.A): this could be a result of lower air temperatures and decreased rates of transpiration. Leaves of trees sampled 350 DAT for the spring injection and 280 DAT for the fall injection had greatly reduced imidacloprid-equivalent concentrations following 2006 litterfall and overwintering (Figure 2.B). However, there is still evidence of straight sector flow when leaves of branches  $0^\circ$  to the injection point are compared to leaves on branches  $180^\circ$  to the injection point. The results of the current study indicate decreased imidacloprid-equivalent concentrations in the leaves of trees injected in the fall when compared to the leaves of trees injected during the spring. In addition, imidacloprid-equivalent concentration in the leaves is greatly reduced following the first season’s litterfall.

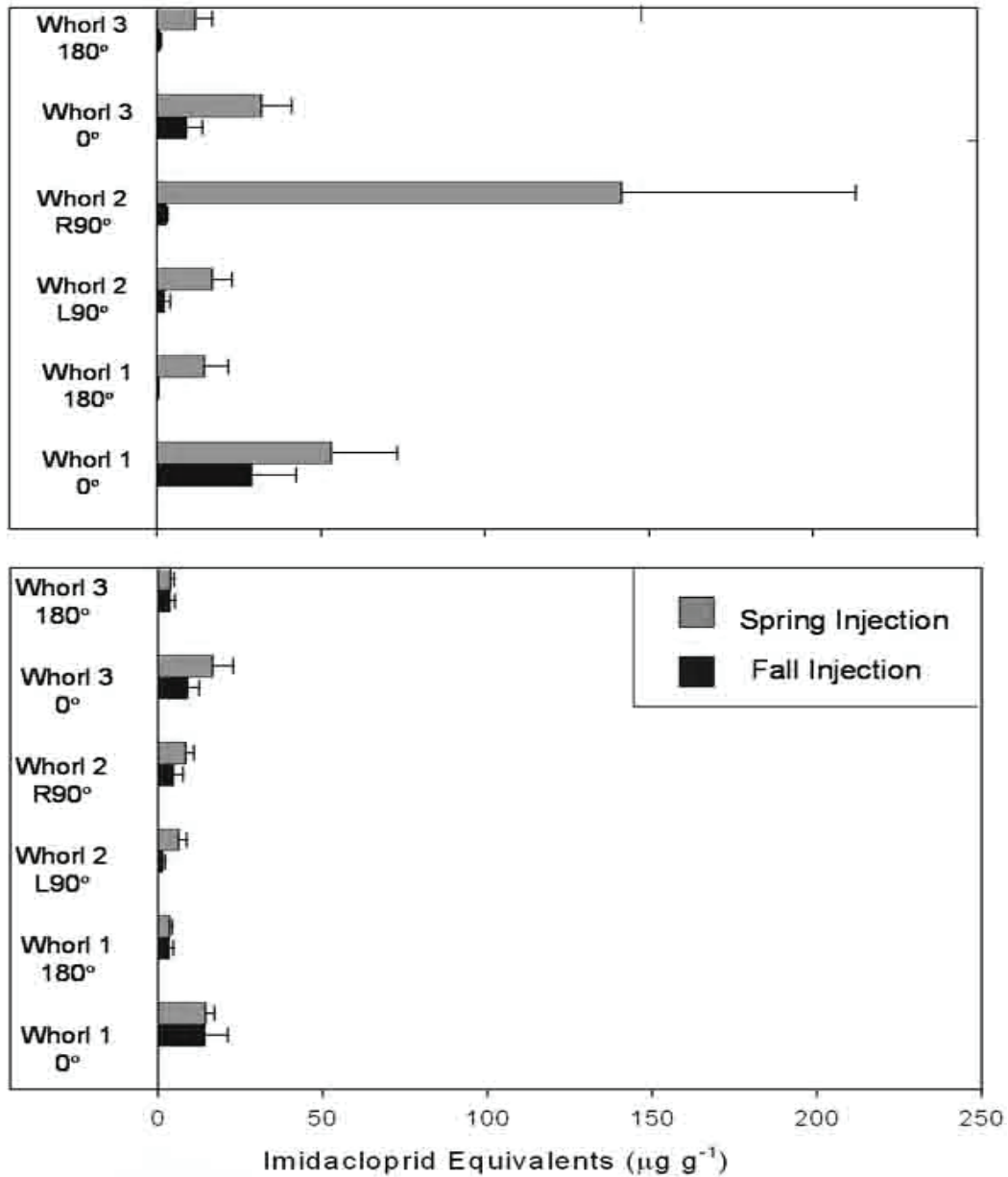


Figure 2.A Imidacloprid equivalent concentration in leaves of spring and fall <sup>14</sup>C imidacloprid trunk-injected *Fraxinus* trees injected at 0° to the first whorl of branches, 21 days after treatment (DAT) (July 18, 2006 and September 26, 2006).

Figure 2.B Imidacloprid equivalent concentration in leaves of <sup>14</sup>C imidacloprid trunk-injected *Fraxinus* trees on June 12, 2007 (350 DAT spring-injected trees and 280 DAT fall-injected trees).

## EMERALD ASH BORER MICROBIAL CONTROL WITH THE ENTOMOPATHOGEN *BEAUVERIA BASSIANA* GHA FORMULATED AS BOTANIGARD®

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### ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), a sporadic wood-boring pest native to northeastern Asia, was found attacking ash trees (*Fraxinus* spp.) in southeastern Michigan in 2002. Despite regulatory efforts to quarantine and eradicate EAB, this invasive beetle has continued to spread throughout Michigan, Ohio, Indiana, and Ontario, Canada, and caused the death of millions of ash trees. Infestations have also been found in Illinois, Maryland, Pennsylvania, and West Virginia. EAB adversely impacts our forest biodiversity, ash resources, and urban areas as ash species are widely distributed in forested ecosystems and planted as shade trees and ornamentals. While eradication may be a viable option for small, outlier infestations, effective and environmentally sound management measures are clearly needed for containment and suppression of dense EAB populations over large areas to help protect our ash resources in other areas in North America.

Entomopathogenic fungi were determined to be the major mortality factor of EAB in Michigan field populations during a survey of EAB natural enemies in 2002. We found localized fungal epizootics and isolated more than 100 strains from five fungal species. Fungal infections were most prevalent among mature larvae or prepupae, which are found near the bark surface during winter. From 2003–2005, we evaluated insect-pathogenic fungi for management of EAB by 1) screening potential fungal isolates for virulence using laboratory bioassays, 2) evaluating the most virulent strain for efficacy against EAB using different treatments in greenhouse and caged-field trials, and 3) expanding efficacy studies using fungal applications on infested ash trees in the field. The results of these studies are summarized below.

Laboratory bioassays showed adult EAB were susceptible to *Beauveria bassiana* and *Metarhizium anisopliae*. Significant time-mortality response was found for each isolate tested. Isolate *B. bassiana* GHA killed EAB adults at a faster rate when compared to other isolates tested, with the lowest average time-to-death values. Significant concentration-mortality responses were also observed for two registered *B. bassiana* GHA bioinsecticide formulations, BotaniGard® ES and Mycotrol® O, which were applied as foliar sprays using a spray tower. The LC<sub>50</sub> values ranged from 114.5 to 309.6 conidia/cm<sup>2</sup> and 18.4 to 797.3 conidia/cm<sup>2</sup> for BotaniGard® and Mycotrol®, respectively (Liu and Bauer 2006).

Subsequent greenhouse and field trials with formulated *B. bassiana* GHA demonstrated its lethal and sublethal effects on emerging EAB adults as well as active larvae. Adult infection rates ranged from 27.7 to 37.7% for the application rate of  $25\text{--}75 \times 10^{13}$  conidia/ha under greenhouse conditions and 58.5 to 83% for the application rate of 10 and  $100 \times 10^{13}$  conidia/ha in the field. The sublethal effects of *B. bassiana* GHA was observed on adult longevity, female fecundity, and larval development. The longevity of adult EAB surviving the fungus was reduced from approximately 22 days to 13 days in females and from approximately 28 days to 14 days in males. In addition, fewer eggs were produced by *B. bassiana* GHA-treated adults, and surviving EAB larvae took longer to develop than controls. We also evaluated the efficacy of *B. bassiana* GHA impregnated fungal bands, which were wrapped around the trunks of infested trees during the period of peak EAB emergence. Adult mortality was 31.6% from fungal infection on treated trees compared to 1.1% on control trees. During the fall, *B. bassiana* GHA was also found effective against EAB larvae overwintering under the bark when applied through trunk application. A total of 7.9% of the larvae were infected in the treated trunk sections compared with 1.6% in the controls. Larval infection rate was positively correlated with larval density in the field.

Finally, the effects of *B. bassiana* GHA on newly colonized and well-established EAB populations were evaluated in the field using foliar and trunk sprays. We found *B. bassiana* GHA applications on leaves and trunk reduced EAB colonization on relatively healthy green ash trees. Results from our trials carried out at one site showed a 40.7% reduction in new EAB colonization in fungal treated trees compared with that of untreated controls. *B. bassiana* GHA was also responsible for reducing the larval population from the previous year by 19.6%. For the well-established EAB population at the other site, larval density was reduced by 46.7% for trees treated with *B. bassiana* GHA compared to the controls, in which 20.9% hatched larvae of the current generation died of fungal infection. Fungal-treated ash trees also produced fewer adults the following year, with a 63.3% reduction in adult density observed from treated trees compared to the controls. As a result, fungal-treated trees sustained 41.5% less crown defoliation than the controls. *Beauveria bassiana* GHA conidia persisted well under field conditions, with EAB adult mortality of 100, 96, 88, and 78% observed on leaves collected at 0, 4, 7, and 11 days after the treatment, respectively.

Our results demonstrated that BotaniGard® was capable of reducing EAB populations and slowing ash decline in the field through trunk and foliar applications. Thus, this bioinsecticide may be useful for treatment of heavily infested ash trees in the core zones, slowing the spread of EAB. BotaniGard® may also be useful for the treatment of healthy trees surrounding outlier infestations to facilitate containment and possibly eradication. The ultimate role of this fungal pathogen in the overall EAB management in North America, however, will depend on further research on optimizing application methods, rates, frequency, environmental and nontarget effects, as well as management decisions made by program managers in the field.

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## IMPACT OF *BEAUVERIA BASSIANA* AND IMIDACLOPRID, ALONE AND IN COMBINATION, FOR CONTROL OF EMERALD ASH BORER IN A NEWLY INFESTED ASH NURSERY

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### ABSTRACT

We are investigating the potential of the fungus *Beauveria bassiana* (strain GHA), alone and in combination with imidacloprid, for use against the emerald ash borer (EAB), *Agrilus planipennis*. We treated approximately 400 *Fraxinus pennsylvanica* and *F. americana* (height ca. 5-6 m) at a commercial tree nursery with fungus alone, imidacloprid alone at two rates, fungus plus the lower rate of imidacloprid, or a formulation blank as control. Imidacloprid (Bayer) was applied as an early season drench in late May, and the fungus (BotaniGard® ES, Laverlam) and formulation blank were applied biweekly three times between mid-June and mid-July. Initial EAB infestation was low. However, in 2007, more than half the trees had beetle exit holes. We monitored spore deposition and estimated spore persistence on leaves and bark. At least four genotypes of *B. bassiana* were present in soil before any sprays, and none of them was identified as the GHA strain. After sprays, we readily reisolated strain GHA from leaves (up to 6 weeks post-spray), bark, and soil. A drop in fungal persistence with time was less pronounced on bark than on leaves. We have developed a real-time polymerase chain reaction (PCR) method to quantify fungal DNA from environmental samples. We found that efficacy of 1-, 4-, and 24-hour adult exposures to freshly GHA-treated leaves and bark to be high. Beetles exposed to leaves and bark sampled one and two weeks after fungal application showed much lower mortality. Mortality of beetles exposed to leaves treated with both imidacloprid and the fungus was equal to, or greater than, that of beetles exposed to leaves treated with fungus alone. We have completed two seasons of a multi-year study.

## THE ANTICIPATED HOST SWITCH: A NEW BRACONID PARASITOID IN MICHIGAN

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### ABSTRACT

Since the discovery of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, in the United States, researchers have hoped to identify native natural enemies that might mitigate the impact of EAB. Formal studies conducted in Michigan demonstrated the presence of a suite of pathogens and parasitoids associated with EAB. Unfortunately, none of these were abundant; fungi accounted for 2% and parasitoids counted for less than 0.5% EAB mortality (Bauer et al. 2005). Woodpecker predation can have a greater impact, causing over 50% mortality in some sites but varying considerably within and among sites (Cappaert et al. 2005, Lindell et al. 2007). However, there is no evidence that these predators have the capacity to regulate EAB populations.

Over time, as EAB populations build and expand, predators or parasitoids native to North America could potentially develop into significant natural enemies of EAB. Exotic pest species are frequently attacked by native natural enemies (Cornell and Hawkins 1993). For example, a native ichneumonid, *Lathrolestes luteolater*, has become an effective biocontrol of *Profenusa thomsoni*, the introduced birch leafmining sawfly (Digweed et al. 2003), and pine shoot beetle, an exotic scolytid, is attacked by several native predators and parasitoids that are adapted to locate prey in bark and phloem (Kennedy and McCullough 2002).

A natural enemy could shift or expand its host range under several scenarios. For example, a parasitoid of a phloem or wood-boring beetle on a non-ash host could become abundant and then encounter EAB in stands where infested ash co-occur with the non-ash host. Alternately, populations of EAB may expand into an area where there is overlap with the range of a previously allopatric buprestid parasitoid that is highly compatible with EAB. In this paper, we document the discovery of an established parasitoid of EAB. This currently unknown species is either a heretofore unobserved EAB parasitoid or has recently expanded its host range to include EAB.

**The site.** Seven Lakes State Park is 3 miles west of Fenton, Michigan. The park encompasses 600 ha of old field, mature oak-hickory forest, and early successional hardwoods. Since 2004, scientists from Michigan State University have conducted several EAB studies at the park, owing to the exceptional abundance of green and white ash. Because the ash are in numerous discrete stands, the degree of EAB infestation is patchy; the majority of trees are dead or heavily infested, but uninfested trees also occur.

**The discovery.** While dissecting trees at Seven Lakes in September 2007, we began to encounter the cocoons of parasitoid wasps in EAB galleries. Wasp larvae were also present (Figure 1), feeding externally on fourth instar larvae.



Figure 1. Larva (L) and cocoons (R) of *Atanycolus* sp. in galleries of emerald ash borer.

We followed this initial observation with a broader survey throughout the park to determine the distribution and prevalence of the parasitoid. A minimum of three ash trees were dissected within ash stands at each of 10 sites. Each tree was evaluated by removing the bark from four sections on the main stem, each 40 cm long. All live EAB larvae were tallied by life

stage and the number of parasitoid cocoons and larvae recorded. The proportion of parasitism was calculated as the number of parasitized EAB larvae divided by the total number of EAB larvae encountered; all four sample sections were weighted equally in determining the average for a tree.

**Distribution at Seven Lakes.** The locations of sites, which represent most of the significant ash stands in the park, are indicated in Figure 2. Rates of parasitism for each site are given in Table 1. Overall, EAB larval density in dissected trees averaged 60 larvae per m<sup>2</sup> (range 0-280 per m<sup>2</sup>). The parasitoid was found at 9 of 10 sites, indicating it is broadly distributed within the park. Parasitism within individual trees reached 83% (excluding a tree at site 2, which had a single cocoon and no live EAB; i.e., 100% parasitism). The highest parasitism rates were clustered at sites 1-3, an area of densely stocked, nearly pure ash forest.

**Further observations.** We observed parasitoid feeding externally and cocoons that were located in the terminus of the

Figure 2. Parasitoid survey sites at Seven Lakes State Park. Circles define 100m zones. Number labels for circles are keyed to data presented in Table 1.

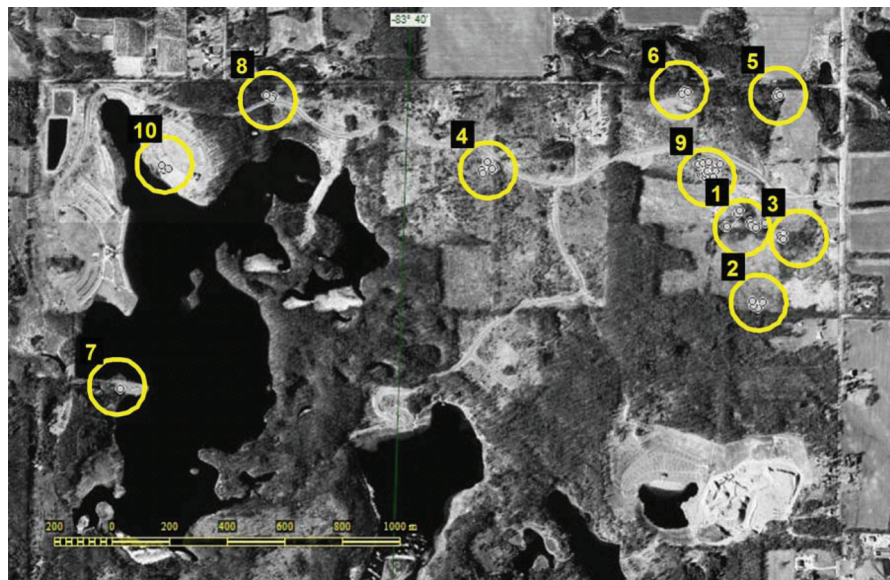




Table 1. Parasitism rates of EAB larvae at survey sites in Seven Lakes Park.

Site	No. trees	% positive <sup>1</sup>	Percent of larvae parasitized <sup>2</sup>		
1	12	75	50	83	21
2	4	75	73	100	42
3	4	75	42	84	19
4	7	71	25	54	3
5	3	100	17	40	2
6	3	67	18	35	2
7	1	100	3	---	---
8	2	100	2	3	2
9	12	8	2	---	---
10	3	0	0	---	---

<sup>1</sup>Refers to percent of trees surveyed where *Atanycolus* sp. was present.

<sup>2</sup>Data from positive trees where *Atanycolus* sp. was present.

host gallery. In every case, the hosts were fourth instar larvae and parasitoids were solitary. By October 18, 95% of parasitoids had formed cocoons. Less than 10% of cocoons observed had been vacated: i.e., we found little evidence of the parasitoid generation that had produced the larvae and cocoons present during the fall.

We made a cursory exploration of stressed or dead elm, oak, and quaking aspen trees to evaluate other phloem or wood-boring larvae that might serve as hosts of the parasitoid. We observed several cerambycid larvae in elm and *Agrilus liragus* in aspen, but there was no clear evidence of parasitism among those species.

**Identity and origin of the parasitoid.** A few live adults of the parasitoid were collected from beneath the bark of dissected trees in September. These were sent to Paul M. Marsh (U.S. National Museum, ret.), a braconid specialist. He identified the genus as *Atanycolus*, which includes species that attack buprestids native to Michigan (e.g., *Atanycolus charus* on *A. anxius* [Loerch and Cameron, 1983] and *Atanycolus* sp. on *Melanophila fulvoguttata* [Graham, 1943]). However, our specimens did not appear to be a known North American species and may be nonindigenous. Further taxonomic work is ongoing.

We propose three explanations for the origin of this unknown species at Seven Lakes:

1. *An Asian parasitoid of EAB was introduced to the United States, either simultaneously with the introduction of EAB or later.* This hypothesis appears unlikely, given the absence of evidence of this *Atanycolus* species in five years of intensive research by many groups. Furthermore, Seven Lakes State Park is a considerable distance from international ports of entry, industrial areas, warehouses, or other likely locations for introduction, where its presence has not been noted.
2. *A rare (and previously undescribed) native or nonindigenous Atanycolus species became abundant during a population surge of a non-EAB primary host. The parasitism of EAB that we observed was incidental.* This idea is implausible given the abundance and dominance of ash and EAB at Seven Lakes Park. It is likely that EAB outnumbers other similar hosts by several orders of magnitude; thus the

prevalence of the parasitoid can only be accounted for if EAB is now the primary host.

3. *A rare (and thus undescribed) native or nonindigenous Atanycolus species has overcome an ecological or genetic barrier and adopted EAB as a host.* This explanation seems most likely, and we consider it most likely that the *Atanycolus* species is now a defacto parasitoid of EAB. It is not a known parasitoid of other common cerambycid or buprestid hosts. It has established among tens of thousands of trees distributed over several kilometers. It is present in numbers that suggest EAB has been a host for multiple generations.

**Future directions.** Our preliminary observations suggest that the Seven Lakes *Atanycolus* parasitoid has the potential to contribute to control of EAB. We are planning a program of research to include these topics:

1. Identification and origin. We are rearing additional specimens to aid systematists with identification. If the *Atanycolus* is not identified as an Asian EAB specialist, we will investigate whether there are alternate hosts that might have brought the species into contact with EAB. Given the size of the wasps and the known host range and searching behavior of *Atanycolus*, cerambycids and buprestids are the likeliest candidates (Kenis and Hilszczanski 2004). Studies will involve surveying stressed and dead trees at Seven Lakes and introducing the *Atanycolus* to borer-infested material in the lab and field. We will also begin to survey other areas near Seven Lakes Park to establish the population level of the parasitoid in the region.
2. Basic biology. Assuming that the *Atancolus* is a new or little-studied species, we will need to describe its basic biology: life cycle and phenology, reproductive capacity (M:F ratio, fecundity), environmental requirements, and other factors that affect its potential for affecting EAB populations.
3. Biocontrol potential. A better understanding of the parasitoid biology and empirical data related to parasitism rates are needed to assess its potential as a biocontrol agent. Further surveys at Seven Lakes (and other sites, if discovered) will suggest how rates of parasitism vary with habitat features, EAB density and other factors. Options such as controlled release of the parasitoid at other sites may be useful to determine dynamics of the parasitoid populations and interactions between the parasitoid and EAB at varying densities.

**Summary.** A braconid parasitoid of EAB, an *Atanycolus* sp. heretofore unknown in North America is well established within a 600-ha site near Fenton, Michigan. Average rates of parasitism at nine locations varied between 2% and 73%. Given the ubiquity and prevalence of this parasitoid at the site, we advocate further research to assess the biological control potential of this species.

**Acknowledgements.** Thanks to Houping Liu, USDA Forest Service, and Paul M. Marsh for assistance with species identification.

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## PROGRESS ON BIOLOGICAL CONTROL OF EMERALD ASH BORER

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### ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis*, a buprestid native to northeastern Asia, was determined as the cause of ash tree (*Fraxinus* spp.) mortality in areas of southern Michigan and Ontario, Canada, in 2002. Infestations have been found since in Ohio, Indiana, Maryland, Virginia, Illinois, Pennsylvania, and West Virginia. Regulatory agencies have shifted from a tactic of eradication to one of management for this pest in North America. This change resulted in part from increasingly large infestations and limited capability to detect and control EAB. Classical biological control, the introduction and establishment of exotic natural enemies for sustained control of an invasive species, is being considered for management of EAB.

Considerable progress has been made since 2002 in developing an EAB biological control program. Natural enemy surveys of EAB in Michigan during 2003-2004 revealed less than 1% of immature EAB were parasitized and EAB eggs contained no parasitoids (Bauer and Liu, unpublished data). This level of parasitism was much lower than parasitism of EAB observed in China (Liu et al. 2007) and levels reported in the literature for a native species of *Agrilus* (Loerch and Cameron 1983). The lack of native natural enemies attacking EAB in the United States supports the need for biological control of EAB in North America.

In 2003, we began studying EAB and its natural enemies in ash stands in China. We found three hymenopteran parasitoids for possible use as EAB biocontrol agents in North America: a gregarious larval braconid ectoparasitoid, *Spathius agrili* (Liu and Liu 2002; Liu et al. 2003; Yang et al. 2005), a gregarious larval eulophid endoparasitoid, *Tetrastichus planipennisi* (Liu et al. 2003; Yang et al. 2006), and a solitary, parthenogenic encyrtid egg parasitoid, *Oobius agrili* (Zhang et al. 2005). Studies on the population biology of *O. agrili* and *T. planipennisi* parasitizing EAB attacking *F. pennsylvanica* planted in Jilin Province reveal the importance of natural enemies in maintaining EAB population densities below a tolerance threshold for this ash species, which is a common tree throughout eastern North America (Liu et al. 2007).

*Oobius agrili* was discovered in 2004 in Jilin Province, China (Zhang et al. 2005). In China, *O. agrili* is a solitary and parthenogenic egg parasitoid with at least two generations per year. This minute encyrtid wasp spends the winter and spring as a mature larva in EAB eggs, and adult emergence is synchronized with the EAB oviposition period during July and

August in the field. We developed laboratory rearing methods and recorded the life cycle of *O. agrili* parasitizing EAB eggs at 25° C (Bauer and Liu 2007). Using these methods, we performed no-choice assays with eggs of six *Agrilus* spp., two cerambycid beetles, and four lepidopterans. Overlap in physiological host range was found for three native *Agrilus* spp. with eggs of similar size to EAB. For these three species, paired choice assays revealed *O. agrili* strongly preferred to oviposit in EAB eggs laid on ash than in eggs of other *Agrilus* spp. on their respective host plants (Bauer and Liu 2007).

*Tetrastichus planipennisi* was discovered in 2003 in Jilin and Liaoning Provinces of China (Liu et al. 2003) and later in Heilongjiang Province (Yang et al. 2006). *Tetrastichus planipennisi* oviposits into the haemocoel of actively feeding third- and fourth-instar EAB larvae. In China, this tiny eulophid wasp completes at least four generations per year and overwinters as mature larvae inside the host gallery. After chewing a small emergence hole in the tree bark, adults emerge the following spring, with an average of 35 (range 5 to 122) adults emerging from a single host larva. We developed laboratory rearing methods and recorded the life cycle of *T. planipennisi* parasitizing EAB larvae at 25°C (Liu and Bauer 2007). Using no-choice assays, groups of female and male *T. planipennisi* were exposed to actively-feeding larvae of eight buprestid species, five cerambycid species, and a wood-boring sawfly, all implanted in small branches of their host plant. We also assayed larvae of a tenebrionid beetle and two lepidopteran species by implantation in small ash branches and sphingid larvae by exposure on host leaves. *Tetrastichus planipennisi* rejected all species except actively-feeding EAB larvae implanted in ash branches (Liu and Bauer 2007).

*Spathius agrili* was first reported in Tianjin, China (Liu and Liu 2002), where it is a prevalent parasitoid of EAB in stands of *F. velutina*, an ash species native to southwestern United States and parts of Mexico. In Tianjin, the emergence of *S. agrili* adults is well synchronized with the availability of third- and fourth-instar EAB larvae, its preferred host stages, and completes three generations per year (Yang et al. 2005). Females oviposit through the tree bark, paralyzing the larva and laying a clutch of eggs on the integument. At maturity, larvae of *S. agrili* spin a cocoon and pupate within the host gallery. No-choice laboratory assays of larval wood-boring insects from China and North America showed some overlap in the physiological host range of *S. agrili*, although successful parasitism was significantly lower in non-hosts than in EAB; no borers in genera other than *Agrilus* were attacked. Therefore, we evaluated the ecological host range of *S. agrili* using an olfactometer to determine the attractiveness of certain host plants. We found *S. agrili* was attracted to *F. pennsylvanica*, *F. velutina*, and a willow (*Salix babylonica*) only in Y-tube olfactometer tests. In nature, if parasitoids are not attracted to the host tree, they are unlikely to encounter and parasitize non-target larvae. In China, no *S. agrili* or *T. planipennisi* were reared from six species of field-collected *Agrilus* larvae ( $n = 2,074$ ). Considering the combination of evidence from no-choice and olfactometer tests, the lack of *S. agrili* reared from other *Agrilus* spp. in China, and that native *Spathius* spp. were rarely reared from EAB in North America, we predict only incidental non-target parasitism (Gould et al. 2007).

Given the known risk of EAB to North American ash resources, the high potential benefit of these parasitoids in suppressing EAB populations, and the relatively low potential risk to native *Agrilus* spp., we submitted permit requests in January 2007 to USDA APHIS for release of each species in Michigan. After extensive review by federal and state scientists,

land managers, and university faculty members during a 60-day public comment period, it was agreed that the potential benefits outweighed the potential risks, and APHIS issued a finding of no significant impact (FONSI). Release permits were issued at the end of July, and field releases began in central and southeastern Michigan. In 2007, adult *O. agrili* (female n = 1406) were released in July and August at two sites in Ingham Co., Michigan; adult *T. planipennisi* (female n = 1360) were released from July through October at two sites in Ingham Co., Michigan; adult *S. agrili* (female n = 311) were released in August and September at one site each in Gratiot, Shiawasee, and Oakland counties, Michigan. We will evaluate the sites for establishment and dispersal of the parasitoids over the next five years or more. Additional research sites will be determined this winter for 2008 parasitoid releases.

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## POPULATION BIOLOGY OF EMERALD ASH BORER AND ITS NATURAL ENEMIES IN CHINA

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### ABSTRACT

*Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), also known as emerald ash borer (EAB), was first discovered in Michigan and Ontario, Canada, in 2002 following investigations of declining and dying ash trees (*Fraxinus* spp.). *Agrilus planipennis* has also spread to Ohio, Indiana, Maryland, Virginia, Illinois, Pennsylvania, and West Virginia by natural dispersal and transport of infested ash materials. As of 2007, over 25 million ash trees have been killed by this pest in Michigan alone. The adverse effects of *A. planipennis* on forest biodiversity, ash resources, and urban areas in North America are high as ash trees are widely distributed and planted throughout North America.

In its native country of China, *A. planipennis* was considered only a minor and periodic pest of ash trees—presumably due the presence of natural enemies and host resistance. The introduction of North America ash species in recent decades, however, elevated *A. planipennis* to pest status in some areas and increased its distribution to additional locations in northern China.

In 2003, we initiated a classical biological control project for *A. planipennis* by studying its population dynamics and natural enemy complex in China. During our initial exploratory surveys for ash trees and *A. planipennis* in northeastern China, including Jilin and Liaoning Provinces, we found two parasitoid species attacking third- and fourth-instar larvae on Manchurian ash (*F. mandshurica*) (Liu et al. 2003). One of these parasitoids was a previously unknown gregarious larval endoparasitoid, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), which we found in both provinces during the course of our study (Liu et al. 2003). The other parasitoid was a gregarious larval ectoparasitoid, *Spathius agrili* Yang (Hymenoptera: Braconidae). This finding expanded the known range for *S. agrili*, which was previously known only from the more southerly Tianjin City, where it attacks *A. planipennis* larvae in stands of Arizona ash (*F. velutina* Torr.). In 2004, we discovered *Oobius agrili* Zhang and Huang (Hymenoptera: Eulophidae), a previously unknown solitary and parthenogenic parasitoid that attacks the eggs of *A. planipennis* (Zhang et al. 2005), in Jilin Province.

For this study, we surveyed field populations of *A. planipennis* in Jilin Province, China, during 2004 and 2005 and studied: 1) the seasonal dynamics of *A. planipennis*; 2) seasonal abundance of its egg parasitoid, *O. agrili*; 3) seasonal abundance of its larval endoparasitoid, *T. planipennisi*; and 4) impact of these two parasitoids on host populations in the field. Results showed that in our field site in Jilin Province, *A. planipennis* had an asynchronous, one-year life cycle in green ash trees (*F. pennsylvanica*), with larvae overwintering in all four instars. At least two generations of *O. agrili* were observed on *A. planipennis* during the egg period in 2005, with parasitism reaching 56.3% in July and 61.5% in August. A portion of the *O. agrili* population diapaused within host eggs in the fall and winter months and emerged the following spring and summer, resulting in post-season parasitism of 28.6% in June of 2004, 12.0% in May of 2005, and 43.8% in November of 2005. Up to four generations of *T. planipennisi* emerged from host larvae, with an average larval parasitism of 22.4% within a range of 0 to 40.4%. *Tetrastichus planipennisi* overwinter as larvae within the host or host galleries and emerge the following spring.

These two parasitoids were important in the population dynamics of *A. planipennis* on green ash, with an estimated 73.6% reduction of EAB population densities during 2005 (Liu et al. 2007). The characteristics of these parasitoids—such as high parasitism rates, short generation time, high reproduction rate, parthenogenesis in *O. agrili*, and life-cycle synchrony with host—suggest these parasitoids may prove useful in the management of *A. planipennis* in North America as biocontrol agents.

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## FIELD HOST RANGE TESTING OF *SPATHIUS AGRILI*, A PARASITOID OF EMERALD ASH BORER: EVALUATING NONTARGET IMPACTS

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### ABSTRACT

The introduction of the emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), into the Midwest from Asia has had a devastating affect on ash (*Fraxinus* spp.). As the emerald ash borer's ability to spread became better understood and its distribution in the Midwest increased, biocontrol became an increasingly important option. Prior to the identification of emerald ash borer in Michigan, *Spathius agrili* Yang (Hymenoptera: Braconidae) was discovered attacking EAB in its native range in China. Subsequent laboratory host specificity testing with North American woodborers and olfactory testing of various tree volatiles, including ash, indicate *S. agrili* is not predicted to have a significant impact on native North American fauna.

Field releases of *S. agrili* at three sites in Michigan were initiated in the late summer of 2007 to determine ability to establish and to monitor the predicted minimal nontarget impacts. Three woodborers native to North America are being monitored: the twolined chestnut borer (*Agrilus bilineatus* [Weber]), the bronze birch borer (*A. anxius* Gory), and the redheaded ash borer (*Neoclytus acuminatus* [Say]).

Host trees (diameter at breast height ~15 cm) for the nontarget *Agrilus* species were moved to the release sites: pin oak (*Quercus palustris*) for the twolined chestnut borer and European paper birch (*Betula pendula*) for the bronze birch borer. In the laboratory, bolts of host trees infested with these species were placed in rearing tubes and adults collected upon emergence. Adults were held for 10 days, fed with foliage and honey:water, and then released into containment cages around the trunks of the host trees, where it was anticipated that they would lay eggs. The cages also included a sapling of the host tree with foliage and small twigs of foliage with their stems in a moist bag. After two weeks, the cages were removed.

Larvae of the redheaded ash borer were brought to the release sites as larvae feeding in ash logs of 1m long. Three logs were brought to each release site as well as three ash logs infested with emerald ash borer larvae. Pairs of redheaded ash borer-infested and emerald ash borer-infested logs were strapped 1 m high to the trunks of emerald ash borer-infested trees in the immediate area of the planned *S. agrili* releases.

After a sufficient cold period, the transplanted oaks and birches will be cut into sections. These sections and the ash logs infested with EAB and red-headed ash borers will be placed in individual rearing tubes to monitor *Spathius* emergence. A subset of the wood will be debarked to confirm the presence of nontargets and possible parasitism.

# ACCURACY ASSESSMENT OF REMOTE SENSING IMAGERY FOR MAPPING HARDWOOD TREES AND STRESSED ASH TREES

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## ABSTRACT

The emerald ash borer, *Agrilus planipennis* Fairmaire, was first identified near the Detroit, Michigan, area in July 2002. Through natural dispersal and human-assisted spread, the lower peninsula of Michigan is now considered generally infested, along with portions of Illinois, Indiana, Ohio, Pennsylvania, Maryland, West Virginia, and Ontario, Canada. Currently, detection methods rely heavily on visual surveys and labor-intensive trap-tree surveys. The ability to identify ash trees and stressed ash trees from airborne imagery would greatly assist regulatory efforts aimed at stopping the spread of this pest. Remote sensing can be a useful tool for mapping vegetation, and advances in sensor technology and image analysis continually improve the information content of the imagery. This study investigates the use of hyperspectral imagery, LIDAR (LIght Detection And Ranging) data, and high-resolution panchromatic imagery in conjunction with ground-based spectral data to: 1) differentiate ash trees from other northern hardwood species, 2) differentiate stressed ash trees from healthy ash trees, and 3) establish what time during the growing season is optimal for identifying ash trees and their health status.

In 2006, we were able to collect hyperspectral imagery over 150 square kilometers at locations in northern Michigan (Lower Peninsula), southern Michigan, and northern Ohio in both early June and late August. We also collected LIDAR data and high-resolution panchromatic imagery during early June. Analysis of the 2006 airborne data set is in progress, and we have four different groups examining the data with diverse objectives. Remote Measurement Services, LLC (Houston, Texas), is looking at the data with a proprietary analysis technique that isolates geologic and biologic component data through a fusion of the hyperspectral imagery with the LIDAR and panchromatic datasets. Clark Labs at Clark University (Worcester, Massachusetts) is using traditional hyperspectral analysis techniques for vegetation classification and segmentation technology to identify individual tree crowns. The USDA Forest Service (Durham, New Hampshire) is analyzing data to examine chemical ecology. Finally, ITT Space

Sciences Division (Rochester, New York) is developing sub-band engineering approaches to vegetation classification. Our objective in comparing the different analytical methodologies is to identify a system that can process hyperspectral data in a timely fashion in an effort to make remote sensing technology operationally useful in program management.

Preliminary analysis results by Remote Measurement Services guided an assessment of their classification process. The process uses GBC-Health™ and GBC-Class™ transformations to differentiate between levels of vegetation stress and identify species differences. Panchromatic imagery and LIDAR data were used to segment the tree canopy into individual tree crowns. Classification was then performed on individual trees. An average overall accuracy of 77% for all flight areas was achieved for differentiating ash trees from all other tree species. However, there are several issues regarding over- and under-segmenting the imagery that can lead to confusion in classification of some tree canopy types. Further analysis is being done to address segmentation issues.

Classifying ash into five separate health categories based on canopy was done on a pixel level using only the hyperspectral imagery for both June and August. Health classes were defined as: H1 = 100% canopy, H2 = 76-95% canopy, H3 = 51-75% canopy, H4 = 26-50% canopy, and H5 = 5-25% canopy. Tables 1 and 2 list the average correctly classified ash trees at both a pixel level and at a complete tree level for both the June and August data. Our ground reference data set was divided into two sets: one set was made available to the analysts for training purposes and a second, verification set was retained for accuracy assessment.

An interesting result of analysis indicates that higher classification accuracies might be achieved using late August data rather than early June data. Accuracy of 60-70% in several of the flight areas and in ash health classes show the promise of this type of survey methodology. With further refinement of analysis methods, including definition of the extent of ash variability, accuracy of 70-80% is quite possible. Remote Measuring Systems will continue their analysis, and a complete classification accuracy assessment will be conducted for their results.

Table 1. Average correct ash health score classification using GBC-Class™ analysis for the June 2006 hyperspectral data.

Training Data	# Trees	Pixels Correct	Min	Max	Trees Correct	Min	Max
Green Ash H=1	88	60.80%	33.50%	82.10%	63.00%	38.50%	75.00%
White Ash H=1	28	58.28%	0.00%	97.10%	47.56%	0.00%	100.00%
Black Ash H=1	1	78.60%	78.60%	78.60%	100.00%	100.00%	100.00%
Ash H=2	60	57.44%	13.80%	94.90%	52.11%	0.00%	100.00%
Ash H=3	21	58.26%	0.00%	78.10%	58.34%	0.00%	100.00%
Ash H=4	15	74.40%	61.50%	93.80%	87.77%	80.00%	100.00%
Ash H=5	7	85.23%	75.80%	100.00%	100.00%	100.00%	100.00%
<b>Verification Data</b>							
Green Ash H=1	84	36.59%	7.70%	61.30%	38.81%	0.00%	70.00%
White Ash H=1	18	66.30%	46.70%	100.00%	69.03%	50.00%	100.00%
Black Ash H=1	0						
Ash H=2	38	39.05%	0.00%	67.70%	33.46%	0.00%	70.00%
Ash H=3	29	42.80%	9.70%	76.20%	50.63%	0.00%	100.00%
Ash H=4	15	30.03%	18.00%	48.50%	8.33%	0.00%	25.00%
Ash H=5	8	49.93%	0.00%	87.50%	66.67%	0.00%	100.00%

Table 2. Average correct ash health score classification using GBC-Class™ analysis for the August 2006 hyperspectral data.

Training Data	# Trees	Pixels Correct	Min	Max	Trees Correct	Min	Max
Green Ash H=1	88	61.26%	27.70%	76.60%	66.49%	30.80%	100.00%
White Ash H=1	25	62.66%	10.80%	93.80%	63.98%	0.00%	100.00%
Black Ash H=1	1	85.70%	85.70%	85.70%	100.00%	100.00%	100.00%
Ash H=2	59	66.90%	44.40%	88.90%	67.89%	33.30%	100.00%
Ash H=3	16	83.06%	75.20%	100.00%	78.34%	50.00%	100.00%
Ash H=4	11	71.27%	53.80%	100.00%	77.77%	50.00%	100.00%
Ash H=5	5	87.90%	64.70%	100.00%	100.00%	100.00%	100.00%

Verification Data							
Green Ash H=1	84	48.30%	0.00%	87.80%	52.91%	0.00%	100.00%
White Ash H=1	15	71.33%	54.10%	85.70%	72.23%	50.00%	100.00%
Black Ash H=1	0						
Ash H=2	51	63.87%	26.90%	96.30%	78.10%	33.30%	100.00%
Ash H=3	27	49.66%	5.40%	86.40%	50.14%	0.00%	100.00%
Ash H=4	10	23.73%	8.00%	37.60%	8.33%	0.00%	25.00%
Ash H=5	6	69.60%	41.70%	93.80%	100.00%	66.70%	100.00%

## HYPERSPECTRAL REMOTE SENSING FOR EARLY DETECTION OF INVASIVE PESTS

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### ABSTRACT

Use of hyperspectral technologies to assess vegetation stress has been well-documented over the past several decades. However, taking these technologies from research to management applications has proven challenging. A multi-agency effort was conducted in 2006 to examine the capability of a commercially available sensor (SpecTIR VNIR) to map ash decline due to the exotic emerald ash borer (EAB) in Michigan and Ohio. Previously successful calibration techniques involved relating detailed decline measurements on the ground to known stress-sensitive indices and wavelengths from airborne hyperspectral imagery. Following these methods, a six-term linear regression model based on chlorophyll and stress-sensitive indices predicted a 0-10 continuous decline rating with an  $R^2 = 0.71$  and an average jackknifed residual of 0.61. Translation of this continuous rating to a five-class variable (commonly used in forest health assessment) resulted in 97% accuracy. The ability of this instrument to assess early decline is based upon calibration with field measurements of photosynthetic activity, a drop in which is typically the first symptom of forest stress. Use of this measurement enables early identification of infestations and could be used to improve the efficacy of control and monitoring efforts. While this decline prediction is not stress- or species-specific, it will enable land managers to target field efforts and monitor forest health across larger geographic scales.

# THE PRACTICAL UTILITY OF HYPERSPECTRAL REMOTE SENSING FOR EARLY DETECTION OF EMERALD ASH BORER

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## ABSTRACT

Hyperspectral remote sensing technology has been used in forest ecology research for the last decade to examine landscape scale patterns of foliar chemistry (nitrogen, cellulose, and lignin) (Martin and Aber 1997), stand productivity (Smith et al. 2002), and soil nitrogen dynamics (Ollinger et al. 2002). More recently, techniques have been developed to map the location of eastern hemlock stands and tree stress along the hemlock woolly adelgid infestation front (Pontius et al. 2005). To date, all of these efforts have relied on a NASA-operated sensor that is dedicated to support research projects.

Commercially available hyperspectral remote sensing imagery has become operationally viable. A successful demonstration project (jointly sponsored by APHIS and USFS) was conducted in 2006 over EAB infested areas of Michigan and Ohio (see the previous abstract, this publication, by Pontius et al.). It is possible to create detailed maps showing the location of *Fraxinus* spp. and to map pre-visual stress for forest tree species, including *Fraxinus* spp.

Early detection of EAB infestation is an important aspect of any management or eradication strategy. Hyperspectral remote sensing data in existing detection and management strategies to enhance our efforts in dealing with this pest. Complications include the expense of imagery, complex image processing techniques that require a high level of analyst expertise, and the time required to produce final maps.

In order to examine whether this technology is ready for integration into existing survey and detection efforts, we outlined a framework for a hypothetical project to be accomplished during the summer of 2008, with resulting species and stress maps available by January of 2009. The hypothetical project would map 2 million acres at 4-meter spatial resolution along the infestation front. In order for a project of this scope to be successful, it would require input from field personnel familiar with the targeted areas (Figure 1). The other variable to be considered when evaluating operational viability is cost. Currently, the imagery is commercially available, but the process for analyzing it is still in development and consequently not available on a commercial basis. The cost of this hypothetical project is approximately \$350,000, or about 18 cents per acre.

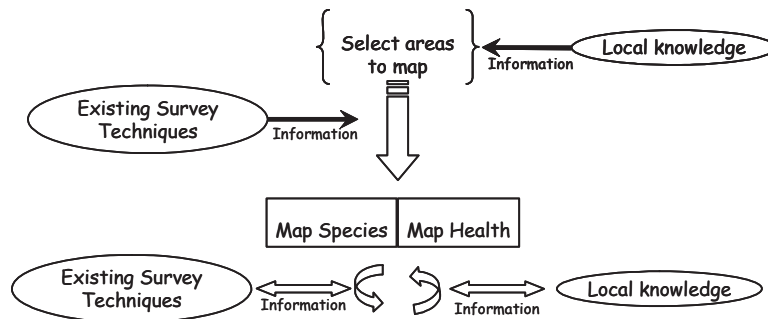


Figure 1. A conceptual model for integrating local knowledge and existing survey techniques into the selection of areas to map. Existing survey techniques and local knowledge are also integrated into species and stress maps in order maximize the usefulness of the data products.

We have documented the conceptual framework along with all the relevant variables in order for a land management agency to evaluate the practicality of using hyperspectral remote sensing imagery to facilitate early detection of EAB infestation.

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## COMPARING EMERALD ASH BORER-DETECTION TOOLS

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### ABSTRACT

Since the discovery of emerald ash borer (EAB) in southeastern Michigan in 2002, this exotic wood-boring pest has spread to neighboring states. Low adult beetle density has limited the ability to detect EAB at the leading edge of its spread. If detected early, different response strategies may allow resources managers to reduce the spread rate of EAB. The objective of this study was to identify the effectiveness of different trapping techniques for detection of EAB at low adult density.

Trapping techniques for EAB were tested in 2007 at 57 sites in Michigan (38), Indiana (10), and Ohio (9). Eight detection tools were selected and a single replicate of each trap type was utilized at each site. Traps included an ash tree girdled within the lower 1.5 m of the stem with a clear sticky band wrapped above the wound, an ash trap tree girdled as above during the previous (2006) field season with a clear sticky band above the wound, an ash trap tree girdled as above with a purple sticky band wrapped above the wound, a large ash tree with a DBH of approximately 30 cm girdled as above with a clear sticky band wrapped above the wound, un-girdled ash trap trees wrapped with a clear sticky band at breast height, an ash trap tree girdled 3 m above the ground and wrapped with a clear sticky band at breast height, a purple prism trap hung 3 m above the ground from the base of an ash tree canopy, and an un-girdled non-ash trap tree wrapped with a clear sticky band at breast height. Of the sites located in each state, twelve sites with trap trees girdled in 2006 were also used for the 2007 study in Michigan, six in Indiana, and seven in Ohio, though six of these sites did not have trees of suitable size for a girdled large ash trap tree.

After establishment in April to May, 2007, traps were monitored bi-weekly, and all EAB adults were collected and sexed during the summer flight season. During mid-flight season, trap tree species were identified, and basal area was measured at each site and calculated for each tree species. For analysis, sites that captured fewer than 200 adult EAB were considered low-density sites, and sites that captured 200 or more adult EAB were considered

high-density sites. Mean capture rate per day was calculated for each trap, and differences in the ranks of each trap type within the two density categories were analyzed with an ANOVA. A Chi-squared test was used to test for the independence of the species of ash selected for the trap type and the detection of EAB at low density. A logistic regression was used to identify the relationship between the detection of EAB at low density and live ash basal area for each trap type.

At low adult EAB density, the capture rate per day for girdled large trap trees was significantly higher than the other trap types. At high adult EAB density, girdled large ash trap trees had the highest capture rate per day, but was not significantly higher than the purple prism trap. For sites categorized as high adult EAB density, the mean diameter at breast height (DBH) for the ash trees associated with the hanging purple prism trap was not significantly different than the DBH for the girdled large ash trap tree. However, at low density, the mean DBH was significantly different between the two trap types. This suggests that at both low and high adult EAB density, the size of the trap tree is important, with trees approximately 30 cm DBH capturing more adult EAB per day.

Emerald ash borer detection was independent of the species of ash selected for the trap tree at low EAB adult densities. Trap tree selection for detection surveys may not need to focus on a single species as EAB at low population densities may not seek out a single species of ash. The odds of detecting EAB at low adult densities were reduced by 5.3 percent with each increase of 1 m<sup>2</sup>/ha of live ash basal area. Current-year-girdled ash trap trees, high-girdled ash trap trees, and un-girdled ash trap trees had the greatest reduction in the odds of detecting EAB as the live ash basal area increased. For the current-year-girdled trap trees, the mean DBH was approximately half of the girdled large ash trees, and they were a much smaller proportion of the ash resource available to EAB as live ash basal area increased. However, at low densities of EAB adults, the odds of detecting EAB with a girdled large ash trap tree increased by 5.4 percent with each increase of live ash basal area by 1 m<sup>2</sup>/ha. Also, as total forest basal area increased and the ratio of live ash basal area to total forest basal area increased, only the large trap trees increased in the odds of detecting EAB. These girdled large ash trap trees capture more EAB adults per day, and the odds of detection increase because they are still a large component of the available ash resource for EAB even as the live ash basal area and the total forest basal area increases. In addition, for trap trees above 25 cm DBH, increases of 1 cm/DBH resulted in an increase of nearly 9 percent in the odds of detecting EAB.

Capture rates of EAB adults are variable and detection of adults is still limited; however, utilizing large ash trees with diameters approximately 30 cm may provide a more effective detection tool for EAB adults. Adult EAB may be more attracted to these large trees, which may be experiencing size-related declines in growth rates and a reduced ability to respond to attack.

## THE '06 TRAP TREES IN '07

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### ABSTRACT

To date, use of girdled trap trees remain the most effective method employed by regulatory and resource management agencies for detecting low-density populations of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire. Locating suitable trees can be difficult, and felling and debarking trap trees is expensive. Alternative options for EAB detection could include enhancing the attraction of adult EAB to ash trees without requiring destruction of the trees. Preliminary studies with ash seedlings showed that the stress-eliciting compound methyl jasmonate (MeJa) caused changes in foliar volatiles similar to those induced by insect feeding and physical stress, which attracts beetles. In addition, blends of volatile compounds associated with ash leaves or bark elicit a positive response by adult EAB. Manuka oil contains many of the same volatile compounds present in ash bark and has been found to be attractive to EAB in trapping experiments.

In 2006, we initiated a study to compare adult EAB capture rates and larval densities on trees that were a) girdled, b) exposed to MeJa, c) had Manuka oil dispensers attached to the trunk, or d) left as untreated controls. We used a randomized incomplete block design and implemented the study at five sites with EAB densities that varied from relatively high (more than 35 EAB captured per tree) on control trees to very low (fewer than three EAB captured per tree). All treatments were included at four sites (n=40 trees per treatment). At the fifth site, three of the four treatments (girdle, MeJa, and control) were included (n=30 trees per treatment). Blocks of trees were selected to represent a range of sun exposure. Exposure of each individual tree was ranked as a) fully exposed, b) super dominant tree/canopy mostly exposed, c) 2-3 sides open, d) 1 side open/edge, or e) closed canopy/shaded. Sticky bands, 30 cm wide, were placed 1 m aboveground on the trunk of each tree.

Adult EAB were removed from sticky bands weekly throughout the summer to monitor adult beetle activity. Results showed that beetle activity peaked in early July, consistent with results from previous years' studies. Girdled trees captured significantly more EAB than control trees at all sites, regardless of EAB density. The number of EAB captured on the trees treated with MeJa or Manuka oil tended to be slightly higher than the number on the control trees; however, the differences were not significant.

Half of the blocks of trees at each site were felled and peeled to assess larval densities. Girdled trees at all sites had significantly more EAB larvae per m<sup>2</sup> than the other trees; trees treated with MeJa or Manuka oil did not differ significantly from the controls.

The amount of exposure to sun, as predicted, significantly affected adult beetle capture and larval density. Trees that were fully exposed to sun, had two or three sides exposed to sunlight, or were dominant above the canopy captured significantly more adult EAB ( $34.0 \pm 7.9$ ,  $13.1 \pm 2.7$ , and  $9.4 \pm 3.0$ , respectively) than trees that were only open on one side or were in a closed canopy ( $3.2 \pm 1.1$  and  $2.1 \pm 0.8$ , respectively). Open and partly-open grown trees also had significantly higher larval densities than trees grown in closed or partly-closed canopies.

We compiled data from 237 trees used in trap tree studies we conducted over the past four years (2003-2006) and analyzed them to determine if adult capture on sticky bands could be used as a predictor of larval density in trees. Larval density increased linearly as adult capture rates increased ( $y=1.8x + 24.0$ ;  $R^2=0.45$ ).

We continued to evaluate the remaining trees in 2007 at four of the sites used in 2006; at the fifth site, new trees were selected. Trees used as controls in 2006 remained as control trees in 2007, trees girdled in 2006 now represented a two-year girdle treatment, and trees previously treated with Manuka oil were girdled in the spring of 2007 (one-year girdle). Trees treated with MeJa in 2006 were wounded by removing a vertical strip of bark of the same area of bark as a standard girdle, leaving most of the phloem around the circumference of the tree intact.

Results show that beetle activity peaked in mid-June in 2007, slightly earlier than previous years. Beetle densities increased three- to 10-fold between 2006 and 2007 at all five sites. Seven of the 20 trees girdled in 2006 died by early summer in 2007. High numbers of adult EAB were captured on all trees remaining from 2006. We plan to fell and dissect the remaining trees this winter to quantify the increase in larval density from 2006 to 2007 among treatments.

## DOUBLE-DECKERS AND TOWERS: EMERALD ASH BORER TRAPS IN 2007

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### ABSTRACT

Effective and efficient methods to detect and monitor emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, have been a high priority for scientists since this invasive pest was identified in 2002. In 2006, our objectives included development of a practical trap design suitable for operational programs and evaluation of lures. In 2007, we continued this work and assessed additional trap designs. Here we briefly review results from 2006 and present data from our 2007 research.

In 2006, we designed a multi-component “double-decker” trap that incorporates both visual factors and olfaction. The double-decker trap consists of two of the purple plastic panel traps used by USDA APHIS. Panels are attached at 6 feet and 10 feet to a 10-foot-tall purple PVC pipe 4 inches in diameter. The pipe slides over the top of a t-post pounded into the ground (4-5 feet tall). No additional support is required. The three-sides of each panel trap are coated with clear Pestick and checked weekly from late May through August to collect EAB adults.

Visual factors integrated in the trap include a tall, vertical silhouette similar to that of a tree and the color purple, which has consistently been shown in numerous studies to attract EAB. In addition, the traps are set in the open, at least 10 m from the edge of a wooded area. This placement ensures that traps are highly visible to beetles and that the traps are exposed to full sun for all or nearly all of the day. Our observations of beetle activity plus data from previous studies have consistently shown that adult EAB are more active in sunny conditions than in the shade. For example, in a previous trap tree study, an average of 31 beetles per tree were captured on sticky bands on ash trees fully exposed to sun compared to less than three beetles per tree on ash trees in the same stand but growing under shade. Moreover, placing traps in the open away from nearby trees reduces potential competition between our lures and volatiles emitted by adjacent ash trees with varying but unknown levels of stress.

Like other buprestids, EAB are not known to use long-range pheromones. Lures for EAB are comprised of compounds associated with foliage or bark/wood from stressed ash trees. Placing traps in full sun and away from the edge of wooded areas provides a highly vis-

ible physical target for EAB beetles that is associated with a relatively unique volatile plume unlikely to be overwhelmed by those of nearby ash trees.

We initially tested the double-decker trap design in 2006 at six sites where EAB density ranged from moderate to low. Lures evaluated in 2006 included a four-component leaf blend lure (*cis*-3-hexanol, *trans*-2-hexanol, *trans*-2-hexanal, hexanal) (Poland et al. 2006) and Manuka oil, a commercially available product chemically similar to ash bark/wood volatiles (Poland and McCullough 2007, Crook et al. 2007). We also applied rough texture to some of the panels to simulate rough bark. Previous studies have shown EAB density is generally higher on ash trees with rough bark than on adjacent smooth-barked trees (Anulewicz et al. 2007a, 2007b). Five to ten randomized blocks were established at each site and checked weekly. We calculated the average number of EAB captured per trap on traps with all three baits (leaf blend lure, Manuka oil, and texture), with the leaf blend + texture, Manuka oil + texture, and with the leaf blend + Manuka oil. More than 4,060 EAB were collected from the 40 blocks of traps in 2006. Results showed that the leaf blend lure and the leaf blend lure plus Manuka oil caught significantly more EAB than traps with Manuka oil alone. Texture did not affect trap catch (see Poland and McCullough 2007).

In 2007, we again used the double-decker traps, leaf blend lure, and Manuka oil; however, we also evaluated a tower trap design, a single-panel trap design, and crude extracts of ash foliage and of bark/wood as lures. The tower trap consisted of two purple panels, 16 and 20-foot-high, on a 20-foot-tall purple PVC pipe braced against a t-post and supported by guy wires. Single panel traps were suspended from rebar poles 5 to 6 feet high. On June 7, we baited some traps with a crude extract made from foliage collected from ash trees girdled in May 2006. This extract was replaced on July 2 with an extract made from foliage of trees girdled in May 2007. A crude extract of ash wood/bark removed from the trees girdled in May 2007 was also added to traps in early July.

Using a randomized block design, we monitored EAB attraction to five different trap/lure combinations, including 1) an unbaited double-decker trap; 2) a double-decker with leaf blend and Manuka lures; 3) a double-decker with leaf blend, Manuka oil and crude extracts; 4) a tower trap with leaf blend, Manuka oil, and crude extracts; and 5) a single-panel trap suspended from a 4-foot-tall rebar with leaf blend, Manuka oil and crude extracts. A total of 31 blocks were established at eight sites; EAB densities were moderate at one site and low or very low at six sites. At one site, in Michigan State University's W.K. Kellogg Forest, EAB was not known to be present.

We collected a total of 4,172 EAB from the 155 traps used in 2007. Activity of EAB peaked in mid- to late June, roughly 1 to 2 weeks earlier than in previous years. We captured 80% of the beetles between June 15 and June 28, which corresponded to roughly 780 to 1,040 accumulated degree-days (base 50° F).

The double-decker traps baited with the leaf blend and Manuka oil lures captured significantly more EAB than any of the other trap/lure combinations. On average, double-decker traps that included the leaf blend lure and Manuka oil captured 39 to 46 EAB per trap. In comparison, the tower traps, unbaited double-decker traps, and baited single panels captured  $\leq 20$  EAB per trap on average. The crude extracts did not significantly increase EAB capture. There was little difference in EAB capture between the upper and lower panels of any of the

traps. Average EAB capture was similar among the single panel traps, the lower panels of unbaited double-decker traps, and individual panels on the tower traps. Individual panels on the baited double-decker traps, however, captured significantly more EAB per panel than individual panels on any of the other traps.

At the Kellogg Forest site, where EAB was not known to be established, a baited double-decker trap captured four EAB. This represents the first recorded “detection event” of EAB with traps and lures at this site. Two girdled trap trees roughly 150 m away from this trap were felled, debarked, and found to be uninfested.

In addition, at another site, one block of traps were installed on a slight rise 300 m away from the nearest ash tree. Despite this distance, we collected a total of 67 EAB on the two baited double-decker traps (one with and one without crude extracts), 25 EAB on the tower trap, 15 EAB on the unbaited double-decker trap, and three EAB on the single panel trap. These results show that the traps effectively attracted dispersing EAB from at least 300 m away.

In summary, our 2007 results demonstrate that trap catch is enhanced by both visual and olfactory elements. The double-decker traps (positioned in full sun) baited with the four-component leaf blend lure and Manuka oil detected EAB in very-low-density settings and attracted EAB from roughly 300 m away. Some operational issues should be considered if traps are to be used programmatically for EAB detection or monitoring. Pestick was re-applied to several traps following heavy rains and occasionally when an accumulation of flies or other insects obscured a panel and had to be scraped off. We noticed that EAB occasionally fell off the panels, especially after rains. Checking traps at two-week intervals may be desirable during peak EAB activity periods if resources permit.

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## LAB AND FIELD RESPONSES OF EMERALD ASH BORER TO SELECTED WAVELENGTH REGIONS OF THE VISIBLE SPECTRUM

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### ABSTRACT

The main aims of this research were to:

- Measure reflectance and wavelengths of ash leaves and purple coroplast as well as adult emerald ash borer (EAB), *Agrilus planipennis*, elytra and abdomen surfaces.
- Measure retinal responses of *A. planipennis* to a range of different wavelengths in the 200-900 nm spectrum using electroretinogram methods.
- Select several colors based on above measurements and study *A. planipennis* attraction to them in field tests.

An ASD FieldSpec Pro contact probe spectrophotometer set to full range scan mode was used for measuring all reflectance curves. Green ash leaves had a wavelength of between 540-560 nm with 10% reflectance. Previous research has shown that girdled and herbicide treated trees tended to have a slightly higher reflectance than healthier trees. We, therefore, wanted to examine differences in reflectance in addition to wavelength as it affects EAB attraction to visual traps. Male and female elytra were measured at 540 nm and had a 5 percent reflectance. The abdomens of males and females were both seen to have a 650 nm wavelength and 6-7% reflectance. Coroplast purple was seen to have a 430nm wavelength and a 20 percent reflectance.

Electrophysiological retinogram recordings from the eyes of male and female adults showed that both sexes were most sensitive to 340 nm (Ultraviolet), 430 nm (in the purple range), 450-460 nm (in the blue range) and 540 nm (in the green range). Females were seen to be particularly sensitive to 650 nm (red), whereas males were not. Based on these measurements, light and dark blue, purple, green, and red colors were selected for field testing alongside purple coroplast controls. In a study that had all these colors individually painted on translucent coroplast prism traps and hung at 1.5 m above ground level outside of an infested ash stand, no significant differences were seen between green, red, or purple treatments. Dark



blue had the lowest trap catch and was significantly lower than red, light green, or dark purple. When purple and green treatments were hung at 13 m above ground level, a clear difference between treatments was observed: we saw that both dark and light green traps had a mean trap catch of approximately 300 insects. Purple coroplast controls averaged approximately 70 insects. At 13 m height, there was no significant difference in catch between the two different reflectances of 540 nm green tested.

For traps hung at 1.5 m height, control purple coroplast and dark purple traps averaged between 40-50 insects per trap, and catches were significantly higher than light purple traps. No differences were seen between green and purple traps. Glue on traps was seen to increase reflectance by only 2.5 percent.

## FIELD RESPONSES OF EMERALD ASH BORER TO MULTICOMPONENT LURES

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### ABSTRACT

During 2007, the efficiency of purple prism traps baited with several different ash volatile lure treatments was investigated for emerald ash borer, *Agrilus planipennis*, with respect to the number of adult beetles captured. Two natural oil distillates (Manuka and Phoebe oil) previously shown to contain high concentrations of green ash bark volatiles that are antennally active to *A. planipennis* were compared and combined with a four-component leaf lure developed by the U.S. Forest Service.

In three separate field studies, Manuka oil-baited traps caught significantly more adult beetles than unbaited control traps. Manuka oil in the release rates of 5 mg, 50 mg, and 500 mg all caught more insects than unbaited control traps. Catch did improve with increased dosage but was not significant between the three release rates tested. On traps placed at 1.5 m height, Manuka oil lures combined with a four-component leaf lure caught significantly more beetles than leaf lure-baited traps or unbaited traps. On traps placed at 13 m height in the tree canopy, Manuka oil lures increased trap catch significantly by itself or when used in combination with the four-component leaf lure. Unbaited traps, leaf lure-baited traps, and a 50 mg Manuka oil-baited trap all caught significantly more beetles when placed at 13 m height.

In a field test comparing and combining Phoebe oil with Manuka oil, Phoebe oil-baited traps caught significantly more beetles than either Manuka oil baited traps or unbaited control traps. We hypothesize that Phoebe oil's improved attractancy to *A. planipennis* over Manuka oil is due to the additional presence of 7-epi-sesquithujene.

## FURTHER INVESTIGATIONS IN DEVELOPING A TRAP FOR EMERALD ASH BORER: HOW TRAP HEIGHT AFFECTS CAPTURE RATES

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### ABSTRACT

The key to an effective pest management program for the emerald ash borer is a survey program equipped with tools for detecting and delimiting populations. Traps placed at 13 m (in the mid-canopy) had previously been shown to catch more beetles than traps placed at lower heights. Studies in 2007 focused on the interactions between 1) trap height and placement in relation to an ash woodlot, 2) trap height and trap color, and 3) trap height and host-produced volatiles.

To study the interaction between trap height and placement in relation to an ash woodlot, we placed traps at one of five locations: 1) 13 m high, 15 m inside an ash woodlot; 2) 13 m high along the edge of the woodlot; 3) 1.5 m high, inside the woodlot (directly below 1); 4) 1.5 m high along the edge (directly below 2); and 5) 1.5 m high, 15 m outside of the woodlot in an adjacent field. Overall, traps placed at 13 m height caught more beetles than traps placed at 1.5 m height. Traps placed inside the woodlot and along the edge caught significantly more EAB than traps placed in the field, but were not significantly different from each other. This differed from studies conducted in previous years, in which traps placed in the field and along the edge caught more beetles than traps placed inside the woodlot. Woodlots this year had more open canopies with sparse foliage; the amount of light entering these stands may not have been substantially different than the amount reflecting off of traps placed along the edge or in the field. Beetles tended to accumulate later in the season on traps placed in the woodlot or along the edge. This may be an indicator of beetles dispersing from the woodlot to other locations later in the season.

Four colors were painted onto translucent prism traps and tested at two heights (13 m and 1.5 m) and compared with catches from a Coroplast stock purple control. The five colors were: 1) Coroplast purple, 2) dark green, 3) light green, 4) lavender, and 5) dark purple. Trap catch at 13 m height was significantly higher than at 1.5 m height for the two green colors but not for any of the shades of purple. Accumulation of beetles on traps was slower for all colors on traps placed at 1.5 m than at 13 m height. This was especially true of dark green traps.

Three lure treatments were compared with a blank control on purple prism traps, again placed at 13 m and 1.5 m height above ground level. The lures were 1) Manuka oil, 2) a leaf blend developed by the U.S. Forest Service, and 3) a combination of Manuka oil and the leaf blend. There was no significant difference between traps placed at 13 m or 1.5 m height when baited with the combination lure. Traps baited with the other lures and the unbaited control caught more beetles when placed at 13 m than at 1.5 m height.

Total trap catch from a trap height study conducted in 2006 (over 3,600 EAB caught) and the color height interaction study from 2007 (over 9,600 EAB caught) were fit to degree-day models (base 50°F) to help determine the best time to hang traps in order to catch the earliest flight. Based on the model, 5%, 50% and 95% of the total number of beetles caught on traps occurred at 537, 737, 1,009 and 579, 795, 1,082 growing degree-days, respectively, for 2006 and 2007.

# IDENTIFICATION AND ANTENNAL ELECTROPHYSIOLOGY OF ASH BARK VOLATILES FOR THE EMERALD ASH BORER

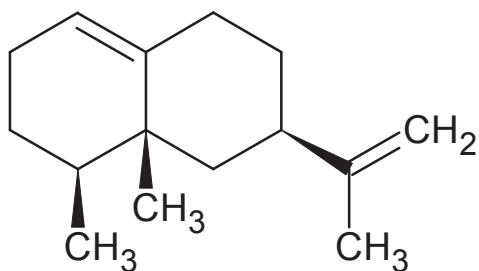
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## ABSTRACT

Biologically active bark volatiles from ash trees (*Fraxinus* spp.) might be used as tools in monitoring the presence of the invasive emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae). Two compounds identified from the volatile emissions from white ash bark were readily sensed by both male and female EAB antennae. The key isolation procedure was silver nitrate/silica HPLC. Identification was by GC-MS, NMR, polarimetry, and micro-chemical reactions, the results compared to literature data and standards obtained from natural plant sources.

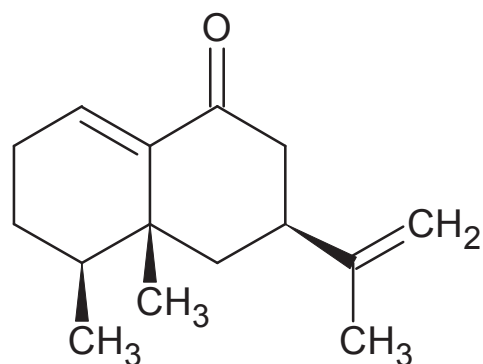


eremophilene

One of the compounds was identified as eremophilene (absolute stereochemistry at left). This sesquiterpene is present as a trace constituent in the essential oil of the Manuka tree (*Leptospermum scoparium*), and sesquiterpene-enriched Manuka oil fractions have been reported to be attractive to EAB in the field (Crook et al. 2005, 2006).

The second compound was 7-epi-sesquithujene, reported previously from the bark of green ash and obtainable from the essential oil of *Phoebe porosa*. Phoebe oil distillates are reported to be attractive to EAB in the field (Crook et al. 2005, 2006).

The current EAB lures are mixtures of sesquiterpines that are derived from natural oils. The presence of non-host compounds in these lures might negatively affect the efficacy of the current lures. However, none of the biologically active sesquiterpines are commercially available, and chemical synthesis of these compounds is not practical.



eremophilone

Eremophilene (absolute stereochemistry at left) can be obtained in relatively large amounts through the simple chemical conversion of the natural ketone, eremophilone. This ketone is the most abundant compound in the commercially available oil of Buddha wood (*Eremophila mitchelli*) and can be easily isolated in high purity.

## REFERENCES

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- Crook, D.J., A. Khrimian, J.A. Francese, I. Fraser, T.M. Poland, and V.C. Mastro. 2006. Chemical Ecology of Emerald Ash Borer. P. 79 (abstract) in: V. Mastro, D. Lance, R. Reardon and G. Parra, compilers. *Proceedings of the Emerald Ash Borer Research and Technology Development Meeting*, October 31-November 1, 2006. Cincinnati, Ohio. USDA Forest Service, FHTET Publ. 2007-04.

## CHEMICAL ECOLOGY AND BEHAVIORAL STUDIES ON THE EMERALD ASH BORER: AN UPDATE

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### ABSTRACT

In 2006, we tested host selection and feeding preference of the emerald ash borer (EAB) on four species of ash species (green, black, white, and blue ash) that are native to North America but exotic to the beetle. For comparison, we also included Manchurian ash (which is native to the beetle) and European ash (which is exotic to the beetle) in the test. Beetles were given a choice among the six species of foliage.

Leaves were placed in screen cages and 30 beetles (males and females separately) were released in the middle of the cage. We counted the number of beetles that landed on each ash species and measured the amount of foliage they consumed. Males landed in highest numbers on green ash, followed by black ash and then white ash. Blue, European, and Manchurian ash foliage attracted fewer male beetles equally. For females, there was no significant difference in landing among green, black, and white ash foliage. Female landing on blue ash results were not significantly different from green, white, European, or Manchurian ash; however, there were significantly fewer landings on European and Manchurian compared to green, black and white.

When feeding, males did not discriminate among green, black, and white ash foliage, or among black, blue, European, or Manchurian ash foliage. Females fed almost equally on green, black, white, and European ash foliage but significantly less on blue and Manchurian ash foliage.

In 2007, following up on these results, we tested beetle feeding behavior on green ash compared to Manchurian ash. Beetles consumed foliage from both species, but they consumed significantly more green ash foliage than Manchurian ash foliage. One possible explanation is that Manchurian ash foliage might have higher nutritive value than green ash foliage, and so beetles might require lower consumption to achieve similar fitness. Alternatively, Manchurian ash might contain compounds that limit beetle consumption. If the alternate hypothesis is true, and if larval feeding responsible for tree mortality follows a similar pattern as adult

feeding, then it may partly explain why Manchurian ash in the beetle's native range is not as extensively damaged by herbivory as North American ash species.

Having established that EAB prefer North American ashes to Chinese ash, the next question was what would happen if the two were hybridised. Koch et al. (this volume) crossed white ash (the pollen donor) with Chinese ash (the flower) to obtain two putative hybrids, 'chiam 1' and 'chiam 2', which are siblings. We had four tree genotypes in these hybrids, and we tested beetle landing and feeding on all four of them. We also compared the profiles of volatiles the hybrids emitted to those of their parent species.

There was no statistical difference among the number of beetles that landed on the four genotypes nor among the amounts of foliage they consumed. With respect to amounts consumed, it appears that the hybrids have taken on some characteristics of the American parent. In their volatile profiles, the two hybrids were similar to each other but different from both parents; volatile composition may be a trait that is not directly inherited.



## VISUAL TRAPPING OF THE EMERALD ASH BORER AND RELATED BUPRESTIDS

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### ABSTRACT

We investigated various trapping methods to determine the visual mate-finding cues used by feral male emerald ash borer (EAB), *Agrilus planipennis*, to locate conspecifics. The results were used to detect and monitor populations of EAB under field conditions.

The most efficient method of detection of EAB by visual trapping thus far appears to be applying spray-on Tanglefoot to leaves on naturally growing ash trees (leaf-sticky-trapping, LST) and mounting an EAB (of either sex) to the leaf with a pin or fixative. Feral male EAB will approach these “dummy” beetles, land on them, and attempt to copulate with them, thus becoming entangled in the sticky surface of the trap. Feral female EAB make up a markedly small percentage of beetles captured by this method (approximately 3%), supporting the hypothesis that male EAB locate conspecifics through visual cues.

The LST method is more effective when such traps are placed high (at least 4 m above ground level) in ash trees, and still 11-36% effective (expressed as traps catching EAB versus total traps deployed at a given height), depending on height, in low-density EAB populations. At high EAB population densities, traps are 34% effective at 2 m height and 87% effective at 4 m height.

Future efforts to improve EAB LST should enable low-cost, minimal-effort monitoring of EAB even at a low population density. Further, this technique allows field personnel to detect EAB in real-time (i.e., during the current field season), as opposed to other currently available and reliable methods (such as girdled trees) that require extended periods of time to examine trees and collect EAB.

Other, but less efficient methods for capturing EAB using visual cues alone include the use of sticky colored cards, again modified by mounting EAB on the trap. Feral EAB males do not appear to discriminate between such traps based on color (yellow or blue), but preferentially approach traps with EAB mounted on them versus blank control traps.

During this study, other feral buprestids (*Agrilus subcinctus* and *A. cyanescens*, among others) were also examined in relation to any color/position preferences they may have. *Agrilus cyanescens* and an unidentified buprestid in particular were found to be preferentially trapped on blue cards bearing mounted EAB. (It is noteworthy that *A. cyanescens* is similarly colored to EAB and so may provide another ‘trap-baiting’ option.) These results lend support to the potential for a broad role of vision as the primary mate-location among the Buprestidae.

## BIOSURVEILLANCE: UTILIZING *CERCERIS FUMIPENNIS* TO DETECT INFESTATIONS OF EMERALD ASH BORER

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### ABSTRACT

Although the term “biosurveillance” is typically used to refer to disease outbreak detection, it also aptly describes the use of live organisms for the surveillance of other organisms. The use of the wasp *Cerceris fumipennis* (Hymenoptera: Crabronidae) to survey for infestations of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), represents an example of biosurveillance in this sense. *Cerceris fumipennis* is a native eastern North American wasp that stocks its underground nests with buprestid beetles. This wasp occurs throughout the current and projected North American range of EAB, where it can be found nesting in loose colonies. Recent research into foraging range and the development of mobile colonies suggest that this wasp has potential as a powerful tool for the biosurveillance of EAB.

Surveillance work involves observing female wasps as they return to their nest with prey. Covering nest entrances with clear plastic cups temporarily confuses incoming prey-laden female wasps, giving the observer time to visually identify the prey carried by each female. Once the prey has been identified the cup can be removed, allowing the wasp to enter its nest. *Cerceris fumipennis* can also be caught and relieved of their prey; a female wasp without prey will return to the forest and collect another beetle. This form of monitoring for EAB (observing the wasps at their colony) apparently does not interfere with the female wasp’s nesting behavior. Unlike the use of girdled trap trees, this form of monitoring does not have a negative impact on the surrounding forest.

*Cerceris fumipennis* is a solitary wasp (one female per nest), but the nests are clustered together in groups or ‘colonies’ of between 10 and 500 nests. Throughout the wasp’s flight season of late-June to late-August, each female will provision her nest with an average of two beetles a day. These naturally established colonies represent an efficient pre-existing tool for surveying EAB.

While investigating wasp flight range in 2007, we developed a simple equation that allows us to estimate the maximum foraging distance of a successful wasp (a female returning with prey). We calculated how quickly a female could navigate a known distance. The quickest wasp (out of 28 females tested) navigated an average of 33.4 meters per minute. This

navigation rate can be used to estimate the distance covered during a round trip foraging flight and applied to successful wasp forays. For example, on July 3 in Windsor, Ontario, wasp number 47 was away from its nest for 57 minutes and returned with an EAB. Based on the navigation rate experiment, we can now estimate that the beetle was caught within 950 m of the nest site. The foraging flight distance is a conservative overestimate because it does not take into account the time need to locate prey and the reduced flight speed of a female returning with prey.

Prior to 2007, EAB surveying with *C. fumipennis* wasps was restricted to forests adjacent to naturally established wasp colonies; however, in 2007, we experimented with mobile colonies to survey forests distant from naturally established colonies. Mobile colonies consist of a series of one meter-square steel scoops containing blocks of soil, including wasp nests, cut from a small part of a naturally established wasp colony. These scoops containing wasp colonies can be loaded onto an open trailer and moved to various locations. The female wasps spend the night in their nests so, provided that the cutting and moving takes place at night, the wasps will not be separated from their nests.

During testing of the mobile colonies in 2007, one trial involved cutting and moving active nests 350 km in a single night. No wasps were damaged in the move, and after only 24 hours at the new location, the adults had reoriented to the new surrounding and began to provision their nests with buprestids.

Results with the first experimental mobile colonies in 2007 suggest that *C. fumipennis* colonies can now be moved to areas where and when they are needed. Mobile *Cerceris* colonies can be moved to high risk sites (firewood dealers, tree nurseries, campgrounds, eradication sites, edges of quarantine zones, transects, etc.) during the night while female wasps are inactive in their nests. Once the mobile colony has been parked at the new survey site, all wasps should be observed as they come and go from their nests, with records taken for both flight times and prey items. Finally, the monitor should ensure that the colony collects at least 40 buprestid beetles (a minimum based on observation made at the Woodland Trails and Rondeau Provincial Park colonies). [Note: a quantitative minimum can be established by calculating the onset of diminishing returns from a graph that compares species diversity to the number of samples taken.]

If EAB is collected by one or more of the wasps, the flight time of the successful wasp can be used to calculate the insect's maximum foraging range. These data can then be confirmed with visual surveys for infested host material within the search areas suggested by the maximum foraging range of EAB-carrying wasps.

For further information on working with *C. fumipennis* or locating local wasp colonies, contact Philip Careless or Dr. Bruce Gill.



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# APPENDICES

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Meeting Agendas, 2007-2003

Attendees, 2007-2003



## 2007 EMERALD ASH BORER RESEARCH AND TECHNOLOGY REVIEW MEETING

October 23, and 24, 2007

### EMERALD ASH BORER PROGRAM REPORTS

U.S. National perspective	P. Bell
Michigan	G. Davis
Ohio	T. Harrison
Indiana	P. Marshall
Maryland	R.A. Bean, and C.A. Holko
Illinois	W.D. Goetsch
Pennsylvania	S.-E. Spichiger
The EAB program in Canada	K. Marchant

### RESEARCH AND DEVELOPMENT REPORTS

<b>Behavior, Biology, and Ecology of EAB</b>	<b>Moderators: Barry Lyons and Dave Lance</b>
Modeling EAB spread in Ohio and Michigan	A. Prasad, L. Iverson, and M. Peters
Dendrochronological reconstruction of the establishment, and spread of emerald ash borer	N.W. Siegert, D.G. McCullough, A.M. Liebhold, and F. Telewski
Modeling the spatial, and temporal dynamics of isolated emerald ash borer populations	N.W. Siegert, A.M. Liebhold, and D.G. McCullough
Modeling EAB dispersal using percolation theory: estimating the rate of range expansion in a fragmented landscape	R.A.J. Taylor, D.A. Herms, and L.R. Iverson
Impacts of emerald ash borer-induced gap formation on forest communities	D.A. Herms, K.J.K. Gandhi, J. Cardina, R.P. Long, K.S. Knight, A. Smith, and D.G. McCullough
Emerald ash borer genetics: an update	A. Bray, T. Poland, L. Bauer, and J. Smith
Putative pheromone for the emerald ash borer	A.A. Cossé, R.J. Bartelt, B.W. Zilkowski, and I. Fraser
Effects of cuticular chemistry on male mating behavior in the emerald ash borer	J.P. Lelito, K. Böröczky, T.H. Jones, I. Fraser, V.C. Mastro, J.H. Tumlinson, and T. C. Baker
How EAB facilitates a secondary spread of invasive plant species: impacts of EAB eradication and tree mortality	C.E. Hausman, J.F. Jaeger, and O.J. Rocha
Genetic structure of green ash: implications for the establishment of ex situ conservation protocols	M. Lang, C.E. Hausman, J.F. Jaeger, and O.J. Rocha

<b>EAB Regulatory and Outreach</b>	<b>Moderator: Dave Lance</b>
Survey of EAB distribution in China	B. Wang, Z. Xu, and V.C. Mastro
Evaluation of heat treatments for quarantine use with emerald ash borer in firewood	S.W. Myers, and V.C. Mastro
Evaluation of firewood bagging and vacuum treatment for regulatory control of the emerald ash borer	T. Poland, T. Kuhn, Z. Chen, A. Diss-Torrence, and E. Clark
<b>EAB Host-Relations</b>	<b>Moderator: Andrew Storer</b>
Emerald ash borer: a classification of tree health and degree of infestation	M.P. Peters, L.R. Iverson, and T.D. Sydnor
Three-year progression of emerald ash borer induced ash decline and mortality in south-eastern Michigan	K.J.K. Gandhi, A. Smith, R.P. Long, R.A.J. Taylor, and D.A. Herms
How fast will the trees die? A transition matrix model of ash decline in forest stands infested by emerald ash borer	K.S. Knight, R.P. Long, A. Smith, K. Gandhi, and D.A. Herms
Regenerative capacity of ash following mortality of canopy trees created by the emerald ash borer	D. Kashian, and J. Witter
Effects of host stress on EAB larval development: what makes a good home?	A.R. Tluczek, D.G. McCullough, T.M. Poland, and A.C. Anulewicz
Interspecific variation in resistance of ash to EAB	D.A. Herms, D. Smitley, E.J. Rebeck, P. Bonello, and D. Cipolinni
Development of novel ash hybrids to introgress emerald ash borer resistance into North American ash species	J. Koch, M. Mason, and D. Carey
<b>Chemical Control of EAB</b>	<b>Moderator: Ivich Fraser</b>
Potential of spinosad applications for EAB control	P.A. Lewis, D.M. Cowan, and I. Fraser
Evaluation of non-invasive trunk sprays, and trunk injection of emamectin benzoate	D.G. McCullough, D. Cappaert, T.M. Poland, P. Lewis, J. Molongoski, and A.C. Anulewicz
Imidacloprid concentration effects on adult EAB: a greenhouse study	D. Cappaert, P. Lewis, J. Molongoski, D.G. McCullough, and T. Poland
Effect of tree size and initial infestation on efficacy of annual imidacloprid applications against emerald ash borer	D. Smitley, R. Royalty, and E. Rebeck
Distribution of trunk injected <sup>14</sup> C imidacloprid in ash trees: temporal variation between spring and fall injection	S.R. Tanis, B.M. Cregg, D. Mota-Sanchez, D.G. McCullough, and T.M. Poland



Acute toxicity and feeding reduction effects of imidacloprid and its metabolites in emerald ash bore adults	D. Mota-Sanchez, B.M. Cregg, S.R. Tanis, D.G. McCullough, T.M. Poland,, and R.M. Hollingworth
<b>Biopesticides for EAB</b>	<b>Moderator: Juli Gould</b>
Emerald ash borer microbial control with the entomopathogen <i>Beauveria bassiana</i> GHA formulated as BotaniGard®	H. Liu, and L. Bauer
Impact of <i>Beauveria bassiana</i> and imidacloprid for control of emerald ash borer in an ash nursery	J.D. Vandenberg, L.A. Castrillo, H. Liu, M. Griggs,, and L.S. Bauer
<b>Biological Control of EAB</b>	<b>Moderator: Juli Gould</b>
The anticipated host switch: new evidence of a braconid parasitoid of EAB in Michigan	D. Cappaert, and D.G. McCullough
Progress on emerald ash borer biocontrol	L. Bauer, H. Liu, and J. Gould
Population biology of emerald ash borer and its natural enemies in China	H. Liu, L. Bauer, T. Zhao, and R. Gao
Field host range testing of <i>Spathius agrili</i> , a parasitoid of emerald ash borer: evaluating nontarget impacts	J.S. Strazanac, J.R. Gould, R.A. Haack, and I. Fraser
<b>EAB Survey</b>	<b>Moderators: Damon Crook and Joe Francese</b>
Accuracy assessment of remote sensing imagery for mapping of hardwood trees and stressed ash trees	D. Bartels, D. Williams, J. Ellenwood, and F. Sapio
Hyperspectral remote sensing for early detection of invasive pests	J. Pontius, R. Hallett, M. Martin, and L. Plourde
The practical utility of hyperspectral remote sensing for early detection of EAB	R. Hallett, J. Pontius, M. Martin, and L. Plourde
Comparing emerald ash borer-detection tools	J.M. Marshall, A.J. Storer, I. Fraser, J.A. Beachy, and V.C. Mastro
The '06 trap trees in '07	A.C. Anulewicz, D.G. McCullough, T.M. Poland, and D. Cappaert
Double-deckers and towers: EAB traps in 2007	D.G. McCullough, T.M. Poland, A.C. Anulewicz, and D. Cappaert
Lab and field responses of emerald ash borer to selected wavelength regions of the visible spectrum	D.J. Crook, J.A. Francese, K.E. Zylstra, D.R. Lance, I. Fraser, and V.C. Mastro
Field responses of emerald ash borer to multi-component lures	D.J. Crook, J.A. Francese, A. Khrimian, I. Fraser, and V.C. Mastro

Further investigations in developing a trap for emerald ash borer: how trap height affects capture	J.A. Francese, D.J. Crook, I. Fraser, D.R. Lance, and V.C. Mastro
Identification and antennal electrophysiology of ash bark volatiles for the emerald ash borer	A.A. Cossé, R.J. Bartelt, B.W. Zilkowski, and I. Fraser
Chemical ecology and behavioral studies on the emerald ash borer: an update	D.S. Pureswaran, and T. Poland
Visual trapping of the emerald ash borer and related buprestids	J.P. Lelito, I. Fraser, V.C. Mastro, J.H. Tumlinson, and T.C. Baker
Biosurveillance: utilizing <i>Cerceris fumipennis</i> to detect infestations of emerald ash borer	P. Careless, S.A. Marshall, B.D. Gill, and G. Otis

## 2007 EMERALD ASH BORER MEETING ATTENDEES

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## 2006 EMERALD ASH BORER AND ASIAN LONGHORNED BEETLE RESEARCH AND TECHNOLOGY REVIEW MEETING

October 29–November 2, 2006

### EMERALD ASH BORER PROGRAM REPORTS

U.S. National Prospective	C. Kellogg
Michigan	K. Rauscher
Ohio	L. Hunt
Indiana	R. Waltz
Maryland	R. Bean
Illinois	S. Knight
The EAB Program in Canada	K. Marchant

### RESEARCH AND TECHNOLOGY DEVELOPMENT REPORTS

<b>EAB Biology, Behavior, and Ecology</b>	<b>Moderator: B. Lyons</b>
Expanded explorations for emerald ash borer in Asia and implication for genetic analysis	Alicia Bray, Leah Bauer, Roger Fuester, Ho Yul Choo, Dong Woon Lee, Naoto Kamata, and James Smith
Host selection by the emerald ash borer: chemical ecology and behavioral studies	Deepa Pureswaran, Therese Poland, and Gary Grant
Visually mediated paratrooper copulations in the mating behavior of <i>Agrilus planipennis</i>	Jonathan Lelito, James Tumlinson, and Thomas Baker
Emerald ash borer flight estimates revised	Robin Taylor, Therese Poland, Leah Bauer, Keith Windell, and James Kautz
Dispersal behavior of <i>Agrilus planipennis</i> (Fairmaire): release-recapture studies	Ivich Fraser, Victor Mastro, David Lance, and Douglas Bopp
Modeling potential movements of the emerald ash borer in Ohio	Louis Iverson, Anantha Prasad, Jon Bossenbroek, Davis Syndor, and Mark Schwartz
Defining the “edge” of isolated EAB infestations: simulation results and implications for survey and host removal	Alan Sawyer
Resurrected from the ashes: a historical reconstruction of emerald ash borer dynamics through dendrochronological analyses	Nathan Siegert, Deborah McCullough, Andrew Liebhold, and Frank Telewski
Two years under the bark: towards understanding multiple-year development of emerald ash borer larvae	Nathan Siegert, Deborah McCullough, and Andrew Tluczek



<b>EAB Host Relationships</b>	<b>Moderator: D. Herms</b>
Constitutive and wound-inducible defense responses of ash phloem	Don Cipollini, Eusondia Barto, Alieta Eyles, Pierluigi Bonello, and Daniel Herms
Evaluation of rural ash resources in Upper Michigan and Lower Michigan threatened by the exotic emerald ash borer	Sarah Brodeur-Campbell, Jessica Metzger, Andrew Storer, and John Witter
Living with emerald ash borer: development and implementation of an ash reduction model to reduce population potential of emerald ash borer	Tara Eberhart, Andrew Storer, and Linda Nagel
Predicting emerald ash borer-induced changes in forest tree species composition	Kathleen Knight, Robert Long, and Joanne Rebbeck
Patterns of emerald ash borer induced ash decline and mortality in the forests of southeastern Michigan	Kamal Gandhi, Annemarie Smith, Robert Long, and Daniel Herms
Ash dieback survey in Michigan	David Smitley, Terrance Davis, and Eric Rebek
The EAB eradication protocol: environmental impacts and native plant community responses	Constance Hausman, Oscar Rocha, and John Jaeger
<b>Chemical Control of EAB</b>	<b>Moderator: D. McCullough</b>
Distribution of trunk-injected <sup>14</sup> C imidacloprid in <i>Fraxinus trees</i> : a test of the sectored-flow hypothesis	Sara Tanis, Bert Cregg, David Mota-Sanchez, Deborah McCullough, Therese Poland, and Robert Hollingworth
Mortality, feeding, and behavior of male and female emerald ash borer adults in response to ingestion of imidacloprid and application of imidacloprid	David Mota-Sanchez, Bert Cregg, Deborah McCullough, Therese Poland, Sara Tanis, Rufus Isaacs, and Robert Hollingworth
Update on comparison of insecticides for emerald ash borer control	Baode Wang, Ruitong Gao, Victor Mastro, Guijan Liu, Enshan Liu, Phillip Lewis, Tonghai Zhao, and Hongyan Wang
Imidacloprid basal soil drench for protection of ash trees from emerald ash borer	David Smitley, Eric Rebek, and Daniel Herms
Evaluation of insecticide products for control of emerald ash borer	David Smitley, Terrance Davis, Kevin Newhouse, and Eric Rebek
Effects of trunk injection on emerald ash borer density and ash survival: a four-year study	David Cappaert, Deborah McCullough, and Therese Poland
Evaluation of neo-nicotinoid insecticides applied as non-invasive trunk sprays	Deborah McCullough, David Cappaert, Therese Poland, Phillip Lewis, and John Molongoski

<b>Biopesticides for EAB</b>	<b>Moderator: R. Reardon</b>
Use of <i>Beauveria bassiana</i> and imidacloprid for control of emerald ash borer in an ash nursery	John Vandenberg, Louela Castrillo, Houping Liu, Michael Griggs, and Leah Bauer
Aerial application of spinosad for emerald ash borer control in woodlots	Phillip Lewis, David Smitley, Richard Reardon, and Victor Mastro
<b>Biological Control of EAB</b>	<b>Moderator: J. Gould</b>
Host preferences of Chinese EAB parasitoid wasp genera currently being considered for release in North America	John Strazanac
<i>Tetrastichus planipennisi</i> (Hymenoptera: Eulophidae), a gregarious larval endoparasitoid of emerald ash borer from China	Houping Liu and Leah Bauer
<i>Oobius agrili</i> (Hymenoptera: Encyrtidae), a solitary egg parasitoid of emerald ash borer from China	Leah Bauer and Houping Liu
Host specificity of <i>Spathius agrili</i> Yang, a parasitoid of the emerald ash borer	Juli Gould, Jennifer Ayer, Yang Zhong-qi, and Wang Xiao-yi
Explorations for natural enemies of emerald ash borer in China in 2006	Roger Fuester, Deyu Zou, Alicia Bray, Tonghai Zhao, Leah Bauer, Houping Liu, and Zhong-qi Yang
<b>EAB Survey</b>	<b>Moderators: T. Poland and D. Crook</b>
Three years of a risk-based emerald ash borer detection survey and firewood survey in Michigan and Wisconsin	Andrew Storer, Jessica Metzger, Robert Heyd, Steven Katovich, and Michael Hyslop
Developing survey techniques for emerald ash borer: the role of trap height and design	Joseph Francese, Ivich Fraser, David Lance, and Victor Mastro
A multistate comparison of emerald ash borer ( <i>Agrilus planipennis</i> Fairmaire) detection tools	Jessica Metzger, Ivich Fraser, Andrew Storer, Damon Crook, Joseph Francese, and Victor Mastro
Evaluation of a multicomponent trap for emerald ash borer incorporating color, silhouette, height, texture, and ash leaf and bark volatiles	Therese Poland and Deborah McCullough
Activity and microhabitat-selection patterns of emerald ash borer and their implications for the development of trapping systems	David Lance, Ivich Fraser, and Victor Mastro
Chemical ecology of the emerald ash borer	Damon Crook, Ashot Khramian, Joseph Francese, Ivich Fraser, Therese Poland, and Victor Mastro

Field attraction of emerald ash borer to antennally and behaviorally active ash volatiles	Therese Poland, Deepa Pureswaran, Gary Grant, and Peter deGroot
EAB attraction to girdled trees: effect of placement and timing on attraction	Ivich Fraser and Victor Mastro
Attraction of EAB to trap trees: can MeJa or Manuka oil compete with girdling?	Andrea Anulewicz, Deborah McCullough, Therese Poland, and David Cappaert
Application of remote sensing technology for detection and mapping of hardwood tree species and emerald ash borer-stressed ash trees	David Bartels, David Williams, Jim Ellenwood, and Frank Sapio
The biology of <i>Cerceris fumipennis</i> (Hymenoptera: Crabronidae) in southern Ontario and its potential for monitoring the distribution of <i>Agrilus planipennis</i>	Philip Careless, Stephen Marshall, Bruce Gill, and Gard Otis
<b>EAB Regulations and Outreach</b>	<b>Moderator: W. Wallner</b>
Estimating emerald ash borer density at local, landscape or regional scales	Deborah McCullough and Nathan Siegert
Sinks, bark, and Garlon: applied studies for emerald ash borer management	Deborah McCullough, Nathan Siegert, Therese Poland, and Robert McDonald
Evaluation of public awareness of issues relating to firewood movement and the exotic emerald ash borer in Michigan	Janet Frederick and Andrew Storer

#### ASIAN LONGHORNED BEETLE PROGRAM REPORTS

U.S. eradication program update	Christine Markham
Toronto ALB eradication program update	Ben Gasman and Janet McDonald
Research on Asian longhorned beetle in Canada	Jean Turgeon, Ben Gasman, Michael Smith, Peter de Groot, Blair Helson, Dean Thompson, Mamdouh Abou-Zaid, and Dave Kreutzweizer

#### RESEARCH AND TECHNOLOGY DEVELOPMENT REPORTS

<b>ALB Research Reports: Survey, Regulatory, and Control</b>	<b>Moderator: D. Lance</b>
Detection of the Asian longhorned beetle: Update on sentinel tree, attract-and-kill and artificial lure studies	Michael Smith, Jinqun Wu, Weizhi He, Xue-nong Xu, Gerhard Gries, Regine Gries, John Borden, Jean Turgeon, and Peter de Groot
Incidence of ALB infestation among treated trees in New York	Alan Sawyer

Femmes fatales: pathogen transmission during mating and reduction in reproduction of Asian longhorned beetle females infected with <i>Metarhizium anisopliae</i>	Ann Hajek
Natural enemies of native woodborers: potential as biological control agents for the Asian longhorned beetle	Michael Smith, Roger Fuester, Joseph Tropp, Ellen Aparicio, Daria Tatman, and Jeff Wildonger
Efficacy of lambda-cyhalothrin for control of the Asian longhorned beetle	Michael Smith, Jinqun Wu, Joseph Tropp, Weizhi He, Hongtian Su, Guoliang Zhang, Xuenong Xu, and Jiuning Li
Research update on the systemic insecticides for the control of the Asian longhorned beetle	Baode Wang, Ruitong Gao, Victor Mastro, Bingjie Wei, Junlei Liu, Ailing Zhao, and Zhichun Xu
Pesticide distribution, sampling, and residue analysis: employment of ELISA for imidacloprid detection	Phillip Lewis and John Molongoski
Post-treatment insecticide residue levels in trees following trunk and soil applications	Phillip Lewis
<b>ALB Research Reports: Biology, Rearing, and Program Management</b>	<b>Moderator: C. Markham</b>
Microbial community composition and wood digestion in the gut of the Asian longhorned beetle	Scott Geib, Ming Tien, and Kelli Hoover
Reproductive behaviors of Asian longhorned beetle	Melody Keena and Vicente Sánchez
Factors that influence Asian longhorned beetle pupation	Melody Keena
A controlled study of the healing response of host trees to simulated ALB damage	Alan Sawyer
Spatial and temporal dynamics of ALB infestations in Carteret and Linden, New Jersey	Alan Sawyer
Modeling the spread of Asian longhorned beetle in New York City: incorporating host species information	Jacqueline Lu and Gareth Russell
Update on the host range studies of the Asian longhorned beetle in a common-garden experiment	Baode Wang, Victor Mastro, Ruitong Gao, Yan Wang, and Yanfang Jin

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## 2005 EMERALD ASH BORER RESEARCH AND TECHNOLOGY DEVELOPMENT MEETING

September 26 and 27, 2005

### PROGRAM REPORTS

National Prospective	P. Bell
Indiana	B. Waltz
Ohio	T. Harrison
Michigan	K. Rauscher
Canada	K. Marchant

### RESEARCH AND TECHNOLOGY DEVELOPMENT REPORTS

<b>EAB Biology, Behavior, and Ecology</b>	<b>Moderators: B. Gill and B. Lyons</b>
Invasion genetics of EAB	Alicia Bray
Gut microflora of the emerald ash borer and other wood boring insects	A. Vasanthakumar, J. Handelsman, and K.F. Raffa
Emerald ash borer dispersal – a release and recapture study	Ivich Fraser, Dave Lance, and Vic Mastro
Spread and dispersal of emerald ash borer: a dendrochronological approach	Nathan Siegert, Deb McCullough, Andrew Liebhold, and Frank Telewski
Spread and dispersal of emerald ash borer: a coupled map lattice model approach	Nathan Siegert, Deb McCullough, Andrew Liebhold, and Frank Telewski
Modeling potential EAB spread through GIS/ cell-based/gravity models with data bolstered by web-based inputs	Louis Iverson, Anantha Prasad, Davis Syndor, Jonathan Bossenbroek, and Mark Schwartz
Is emerald ash borer an obligate migrant?	Robin Taylor, Leah Bauer, Therese Poland, and Robert Haack
<b>EAB–Host Relationships: Responses of EAB to Hosts</b>	<b>Moderator: D. Herms</b>
EAB host range and preference: lab and field studies	Andrea Anulewicz
Interspecific variation in ash resistance to EAB	Eric Rebek, Daniel Herms, David Smitley, Pierluigi Bonello, Alieta Eyles, and Don Cipollini

<b>EAB–Host Relationships: Forest Impacts</b>	<b>Moderator: D. Herms</b>
Living with emerald ash borer: modeling ash phloem removal from forests	Andrew Storer
Biological cost of eradication: consequences on the native plant community	Constance Hausman
Impact of EAB and other disturbances on rural and urban forests in Michigan	John Witter
The impact of emerald ash borer on forests within the Huron River watershed	AnneMarie Smith, Daniel Herms, and Robert Long
<b>Chemical Control of EAB</b>	<b>Moderator: D. McCullough</b>
Distribution and persistence of trunk-injected <sup>14</sup> C imidacloprid in ash trees	Bert Cregg, David Mota-Sanchez, Deb McCullough, Robert Hollingworth, and Therese Poland
Evaluation of several nicotinoids and spinosad for EAB control	Baode Wang
Insecticide control of EAB (title yet to be determined)	Dave Smitley
Long-term (3 years!) results of trunk injections for EAB control in landscape ash trees	Deb McCullough, David Cappaert, and Therese Poland
Noninvasive neonicotinoid treatments for ash logs and trees	David Cappaert, Deb McCullough, and Therese Poland
<b>Biopesticides for EAB</b>	<b>Moderator: D. Lance</b>
Efficacy of spinosad to adult emerald ash borer	Phillip Lewis
Potential uses for <i>Bt</i> in the management of EAB	Leah Bauer
The development of resistant ash to facilitate the eradication of emerald ash borer	Richard Meilan
Potential of <i>Beauveria bassiana</i> GHA for management of EAB	Leah Bauer, and Houping Liu
Simulated aerial application of <i>Beauveria bassiana</i>	Dave Smitley
<b>Biological Control of EAB</b>	<b>Moderators: D. Reardon and J. Gould</b>
Survival of emerald ash borer in chipped and ground ash	Ivich Fraser, Deb McCullough, Therese Poland, Dave Cappaert, and Vic Mastro
Egg and larval parasitoids of EAB from China: potential for biocontrol in North America	Leah Bauer, and Houping Liu
Progress on collecting, studying, and rearing parasitoids of the EAB from China	Juli Gould

Overview of Hymenoptera genera currently considered for EAB biocontrol release	John Strazanac
Exploration for EAB and its natural enemies in South Korea in 2005	Dave Williams
Research on parasitoids of buprestids in progress at the ARS Beneficial Insects Introduction Research Unit	Roger Fuester, and Paul Schaefer
<b>EAB Survey</b>	<b>Moderator: T. Poland</b>
<i>Cerceris fumipennis</i> – a useful adjunct to the EAB monitoring programs	Steve Marshall, S. Paiero, and M. Buck
Effects of trap design and placement on capture of emerald ash borer	Joseph Francese, Jason Oliver, Ivich Fraser, Nadeer Youssef, Dave Lance, Damon Crook, and Vic Mastro
Effects of tree wounding and banding on emerald ash borer capture	Ivich Fraser, Joe Francese, Jason Oliver, Nadeer Youssef, Dave Lance, Damon Crook, and Vic Mastro
Attraction of EAB to trap trees: effects of stress agents and trap height	Deb McCullough, Therese Poland, and David Cappaert
Chemical ecology of EAB in relation to bark volatiles	Damon Crook, Ivich Fraser, Joe Francese, and Vic Mastro
Identification of stress-induced plant volatiles and tests of attraction in the lab and field	Therese Poland, Cesar Rodriguez-Saona, Gary Grant, Linda Buchan, Peter de Groot, James Miller, and Deborah McCullough
Progress on EAB survey using remote sensing technology in 2004-2005	Dave Williams
Visual survey of EAB damage in Michigan	Dave Smitley
Living with emerald ash borer: detection of outlier populations	Andrew Storer, Jessica Metzger, and Robert Heyd

[A list of attendees for the 2005 meeting is not available.]

## 2004 EMERALD ASH BORER RESEARCH AND TECHNOLOGY DEVELOPMENT MEETING

October 5-6, 2004

### PROGRAM REPORTS

Canadian Program	K. Marchant
Canadian EAB Science and Survey Committee	H. Frazer
National EAB Management Plan	P. Bell
National EAB Survey Plan	D. McPartlan
Ash Reduction Strategy	N. Schneeberger
FHTET Assistance with Reduced Ash Zone	J. Adams and F. Sapio
Michigan Program Update	G. King and T. Flint
Michigan Statewide EAB Survey	A. Storer
Ohio Program Update	T. Harrison
Indiana Program Update	B. Waltz
Maryland program Update	D. Bean
Virginia Program Update	D. Martin

### RESEARCH AND TECHNOLOGY DEVELOPMENT REPORTS

<b>EAB Biology, Behavior, and Ecology</b>	<b>Moderator: B. Lyons</b>
Phenology	D. Brown-Rytlewski
EAB flight potential	R. Taylor
mtDNA sequences and AFL fingerprints for EAB	A. Bray
EAB life cycle: a reassessment	D. Cappaert
EAB development and dynamics in black ash in an outlier site	N. Siegert
Monitoring and evaluating the health of ash trees in Michigan's rural forests	J. Witter and A. Storer
<b>EAB Host Range</b>	<b>Moderator: D. Herms</b>
Interspecific variation in ash resistance/susceptibility to EAB	D. Herms, P. Bonello, D. Smitley, E. Rebeck, and D. Cipollini
Effects of community composition of forest susceptibility and response to EAB	A. Smith, D. Herms, and R. Long
Host range testing-laboratory choice test	R. Haack and T. Petrice
Host range for EAB in North America and elsewhere	A. Agius

Tree physiology and site factors affecting preferences and suitability	D. McCullough
Host range	A. Agius
Observations of the within-tree distribution of EAB in southwestern Ontario	S. Smith, P. de Groot, and L. Timms
Do purple traps, magenta and green objects improve EAB trapping efficacy?	G. Otis, M. Youngs, and G. Umphrey
<b>Chemical Control of EAB</b>	<b>Moderator: Deb McCullough</b>
Trunk surface treatments and 2003 systemic studies	R. Haack and T. Petrice
EAB survival in stumps with/without treatment	R. Haack and T. Petrice
Surface treatments and systemics	D. McCullough and D. Cappaert
Imidacloprid residues from soil-injected ash trees	P. Lewis
BotaniGard® evaluation	L. Bauer
Efficacy of trunk injection of imidacloprid and azadirachtin	N. McKenzie
Fate and metabolism of <sup>14</sup> C imidacloprid	B. Cregg and D. Mota-Sanchez
<b>EAB Survey</b>	<b>Moderator: David Lance</b>
Remote sensing	D. Williams
Remote sensing	D. Bartels
Effectiveness of visual survey and enhancements	D. McCullough
Trapping and trap trees	T. Poland, D. McCullough, P. de Groot, D. Cappaert, D. Grant, and L. McDonald
Cuticular hydrocarbons and contact pheromones	L. Hanks
Exploring the use of spatially stratified ash host distribution maps for improving the efficiency of emerald ash borer detectors	D. MacFarlane, B. Rubin, and S. Friedman
Ecological spatial patterns of ash in southern Michigan	S. Friedman, D. MacFarlane, and B. Rubin
Trap designs and colors	J. Francese
Effectiveness of tree bands	I. Fraser
Visual clues for beetle attraction	D. McCullough
Distribution of infested trees in an outlier site	L. Bauer, H. Liu, and D. Miller
Problematic <i>Agrilus</i> identifications in trap tree buprestic identifications	J. Zablony

<b>Biological Control of EAB</b>	<b>Moderators: Richard Reardon and Juli Gould</b>
Exploration in Korea for natural enemies	D. Williams
Woodpecker predation	D. McCullough
Insect natural enemies in SE Michigan and China	L. Bauer, H. Liu, and D. Miller
Exploration in China and rearing development	J. Gould and J. Tanner
Taxonomy of parasites	J. Strazanac
Foreign exploration for EAB natural enemies	P. Schaefer
<b>Regulatory Treatment of EAB</b>	<b>Moderator: Therese Poland</b>
EAB survival in firewood	R. Haack and T. Petrice
EAB survival in chips with different heat treatments	D. McCullough

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## 2003 EMERALD ASH BORER RESEARCH AND TECHNOLOGY DEVELOPMENT MEETING

September 30-October 1, 2003

<b>EAB Program Review</b>	<b>Facilitator: Victor Mastro</b>
U.S. Programs	Craig Kellogg, Deb Parland, Philip Bell, Ken Rauscher, Tim Flint, Gary King, and Frank Sapio
Canadian Program	Ken Marchant
Summary of Research Needs	Mike Stefan and Victor Mastro
<b>Biology and Behavior of EAB</b>	<b>Facilitator: Deb McCullough</b>
Overview	Barry Lyons
Life Cycle	David Roden
Dispersal	Deb McCullough
Population Dynamics	Leah Bauer, Bob Haack and Toby Petrice, Paul Schaefer, Gard Otis, Tanya Turk, and Nichole McKenzie
<b>Survey of EAB</b>	<b>Facilitator: Jason Oliver</b>
Characterization of Visual Techniques	Bob Haack and Toby Petrice
Semiochemicals	Therese Poland
Other Cues	Jason Oliver
Development of Traps for Survey	Dave Williams
Remote Sensing	Dave Bartells, Houping Liu, David MacFarlane, Deb McCullough, Dave Cappaert, and Joe Fancese
<b>EAB Regulatory Treatment and Mechanical Control</b>	<b>Facilitator: Bob Haack</b>
Presentations	Bob Haack and Toby Petrice Deb McCullough
<b>Chemical Control of EAB</b>	<b>Facilitator: Bill Wallner</b>
Systemics	Bob Haack and Toby Petrice
Cover Sprays	Phil Lewis
Application Techniques	Deb McCullough and Dave Cappaert

<b>Biological Control of EAB</b>	<b>Facilitator: Richard Reardon</b>
Pathogens	Leah Bauer
Parasites	Amy Roda and Juli Gould
Predators	Bob Haack
<b>Other Techniques: EAB Rearing</b>	<b>Facilitator: Juli Gould</b>
Presentation	Leah Bauer
<b>EAB Host Range Testing</b>	<b>Facilitator: Dan Merms</b>
Overview	Bob Haack
Suitability of Trees to Attack	Dan Herms
Site Factors	L.L. Timms
Stress Factors	Deb McCullough
Age/Size of Trees	Dave Cappaert

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