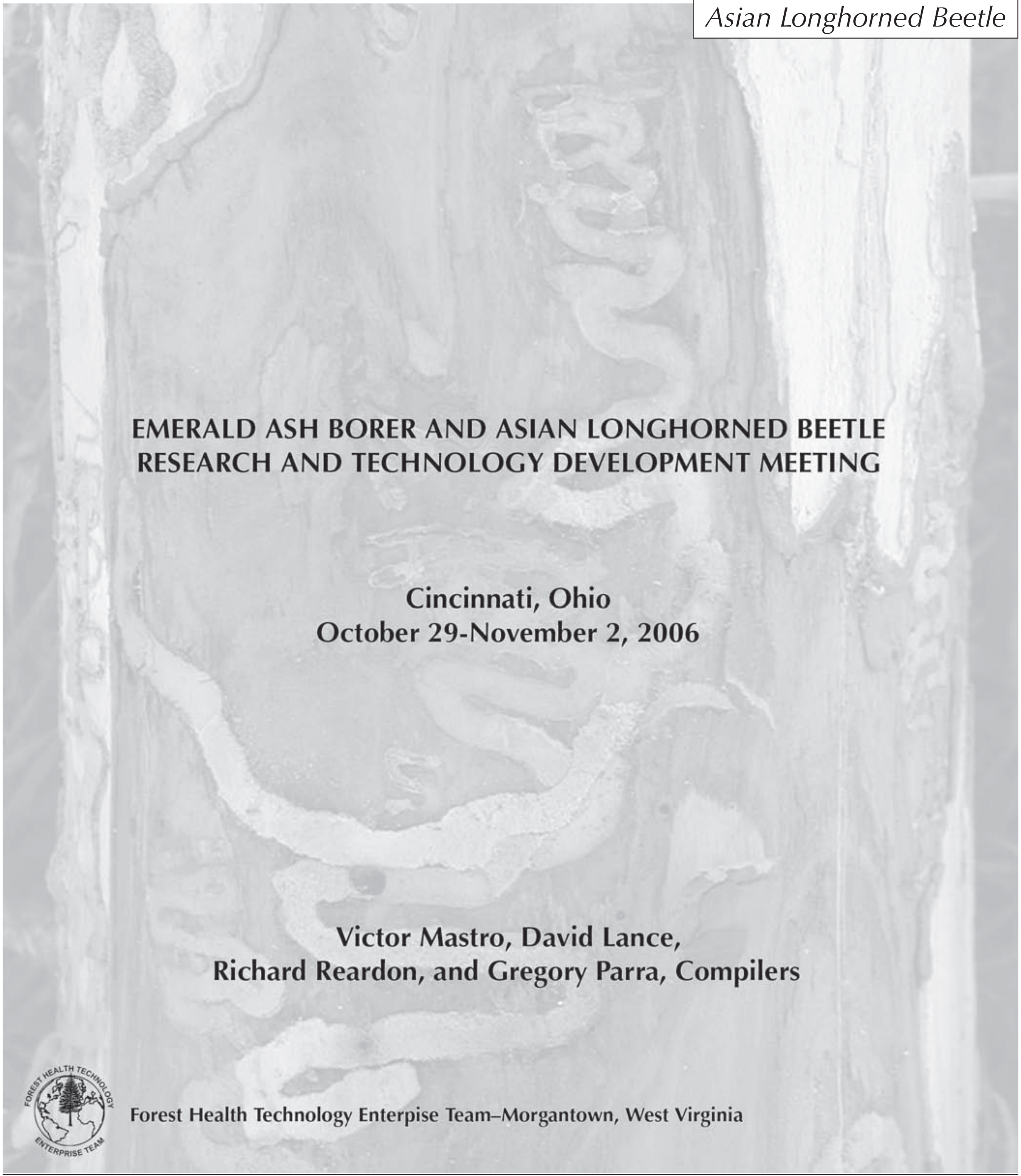
Forest Health Technology Enterprise Team

TECHNOLOGY
TRANSFER

Emerald Ash Borer
Asian Longhorned Beetle



EMERALD ASH BORER AND ASIAN LONGHORNED BEETLE RESEARCH AND TECHNOLOGY DEVELOPMENT MEETING

Cincinnati, Ohio
October 29-November 2, 2006

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

Forest Health Technology Enterpise Team–Morgantown, West Virginia

U.S. Department of Agriculture
Forest Service
Animal and Plant Health Inspection Service

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EMERALD ASH BORER AND ASIAN LONGHORHED BEETLE RESEARCH AND DEVELOPMENT REVIEW MEETING

October 29–November 2, 2006
Millenium Hotel
Cincinnati, Ohio

Compiled by:

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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 five states—Michigan, Ohio, Indiana, Illinois, and Maryland—have known infestations. The infestation also extends into five counties in southwestern Ontario, Canada. 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 and areas of forest settings are estimated to be in the billions of dollars. The Forest Service currently is doing a more in-depth economic assessment (N. Schneeberger, pers. comm.).

When the EAB was first discovered in North America, there was little information on this insect. Within its native range, EAB behaves similar to many of the North American buprestid species, attacking only weakened or dying trees. Its natural range is not completely known, and there is 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 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. Active management has attempted to eradicate the 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. Control options have also been limited; to date, only ash tree removal has been applied as a control tool. This approach is expensive, and its success is compromised by the current limitations of 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 projects. Included in the current report, we can see the first fruits of this expanded research program as well as some of the preliminary products of ongoing research. These include a 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. More products of these combined efforts will follow in the years to come.

The first discovery of a breeding population of Asian longhorned beetle (ALB), *Anoplophora glabripennis*, in North America occurred in New York City in 1996. This discovery was probably at least 15 years after the insect had been introduced. At that time, large-diameter maples in parks and along streets had already been killed by ALB activity. Subsequently, satellite infestations were found in Suffolk and Nassau Counties, New York, and Hudson County (Jersey City), New Jersey. Additional infestations, arising from at least two separate introductions, were also found in and around Chicago, Illinois, and in Linden, Carteret, and Rahway (in Union and Middlesex counties), New Jersey. An

ALB infestation was also discovered in Toronto (Ontario) Canada. In response, the infestations in Illinois and Jersey City appear to have been eradicated, and substantial progress has been made to eradicate the beetle at the other sites.

When ALB was first discovered, it was similar to the emerald ash borer in that few scientific papers describing its biology, behavior, or management were available. Since then, through USDA (through Animal and Plant Health Inspection Service, Agricultural Research Service, and Forest Service offices) and university research efforts, we all have a better understanding of its adult behavior, host range, and dispersal capabilities. Survey methods, control strategies, and effective regulatory treatments have been developed and put to use in dealing with this pest.

Despite the past and pending successes of ALB eradication efforts, available survey and control options are still less than optimal and expensive to employ. The pace of research has slowed as the programs have matured, but continued products should be delivered in the near future to help the programs achieve their goals.

Emerald Ash Borer and Asian Longhorned Beetle Research and Technology Review Meeting

October 29–November 2, 2006

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U.S. National Prospective	C. Kellogg
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Femmes fatales: pathogen transmission during mating and reduction in reproduction of Asian longhorned beetle females infected with *Metarhizium anisopliae*	Ann Hajek
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EMERALD ASH BORER PROGRAM REPORTS

EMERALD ASH BORER STATE UPDATE: OHIO

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ABSTRACT

The Ohio Department of Agriculture (ODA) followed the Science Advisory Panel's recommendation to survey the entire state for emerald ash borer (EAB) at a goal density of 18 trees per township. Visual survey, detection trees, and destructive sampling were all methods used to identify new EAB populations. Between January and June 2006, 9,673 detection trees were set across Ohio. Both public and private lands were used with permission from all landowners. At this time, 43 positives trees were found, ten in new areas previously unquarantined. Regardless of apparent severity of infestation, quarantines are now assigned to entire counties instead of townships or portions of counties.

Ohio has no eradications planned at this time. Only infestations found that are small, have a known source, are far beyond the leading edge and are likely to be eradicated will be considered for removal measures. Widespread eradications have been suspended because of their expense, the forecasted lack of federal funds, increasing pushback from the public, and the speculation that large, multi-year infestations may not be eliminated by tree removal.

Compliance Agreement issuance has been steadily increasing. The process has been streamlined by determining whether movement of regulated articles to or from the facility is intrastate or interstate and by dividing responsibility for monitoring between USDA and ODA employees. Currently, 121 active agreements have been signed with facilities that consent to be inspected regularly. Firewood Blitzes have played a major role in the regulatory program: at road blocks, campground entrances, and ferry docks, over 12,413 vehicles were encountered by ODA staff and over 8,000 pieces of potentially infested firewood intercepted.

In an effort to reach the campers who are potentially spreading EAB through firewood, 448 "Don't Move Firewood" campground signs were distributed across the state to both public and private parks. To help motorists be aware of the quarantine lines, 40 signs were posted at state and interstate routes exiting from quarantined areas. 14 press releases were distributed to media outlets statewide detailing quarantine updates in Ohio and in surrounding states. The general public has been very receptive and is responsible for alerting program officials to many possible outbreaks. Over 3,264 hotline calls were answered in the office and 385 webmail inquiries sent through the website. Ninty-two public events were held, most in conjunction with other EAB Task Force agencies to explain the regulations concerning EAB infestation. Included in that number are numerous presentations and booths at trade shows, industry fairs, and outdoor festivals designed to educate Ohio citizens about EAB.

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 valuable ash resource, with heavy mortality being observed throughout Essex County, Ontario, and adjacent areas in 2006. With the recent confirmation of EAB in the City of London, Middlesex County will become the fifth south-western Ontario county to be regulated by the Canadian Food Inspection Agency (CFIA) for EAB.

Canada's strategy is to slow the spread of EAB through effective quarantine, regulations and communications strategies. While past control actions, such as the establishment of an Ash Free Zone in 2004, are believed to have had a major impact in support of this objective, tree removal and the application of registered pest control products are not considered cost-effective options at the present time. The Canadian Food Inspection Agency (CFIA) considers the primary obstacle to control to be the relative paucity of effective surveillance and early detection tools, and until such time as EAB can be detected at low population levels around outliers and the leading edge accurately determined, large scale control actions cannot be considered a viable strategy.

Throughout 2006, detection surveys were conducted at high-risk sites such as campgrounds, nurseries, parks, and sawmills across Ontario and in other areas of Canada for EAB presence and as partial fulfillment of phytosanitary certification requirements for export markets. In areas abutting counties known to be infested, the survey strategy was to detect outlier populations. This was accomplished through a combination of detection and delimitation surveys. Canada does not employ the use of "trap" trees in its surveillance programmes, but rather, relies on visual inspection of trees in risk-based grid systems.

Quarantine of infested and high-risk areas is seen as the most important regulatory tool at the present time. To this end, Canada imposes quarantines 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 of Quarantine to all property owners within a 5-km radius of a known detection site. In Lambton and Elgin Counties, both methods of quarantine are in place concurrently, creating a quarantine zone within a regulated area. This is considered a valuable tool in slowing the intra and inter-county spread of EAB in areas which are not considered to be generally infested.

The CFIA is committed to continued collaboration with its Canadian and U.S. federal and state partners on the development of effective surveillance, regulation, and control strategies for EAB and the prioritization and funding of research initiatives.

Emerald Ash Borer:
Biology, Behavior, and Ecology

EXPANDED EXPLORATIONS FOR EMERALD ASH BORER IN ASIA AND IMPLICATIONS FOR GENETIC ANALYSIS

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ABSTRACT

Emerald ash borer (EAB) is considered native to northeast China, Korea, Japan, Taiwan, Mongolia, and eastern Russia. We are using genetic analyses to determine the origin of North America's EAB infestations; however, acquiring samples from countries other than China has proven difficult. To increase the diversity of EAB populations sampled in Asia, an expanded survey was conducted in areas of South Korea, Japan, and China from June-August 2006.

Live ash trees (*Fraxinus* spp.) were visually observed for EAB exit holes and/or crown dieback. If symptoms were observed, bark was peeled at breast height to inspect the trees for larvae. Leaves and branches were sampled using an aerial net to sweep for adult beetles. In South Korea, six new field sites were evaluated for EAB at the end of June to mid-July. *Fraxinus rhynchophylla* was the primary tree species at our sites. Larvae were collected at five of the six field sites (Mt. Juri, Mt. Muju, Mt. Sangju, Mt. Wolak, and Jurisan National Park), while two adults were collected at the Suwon site. A single adult was also collected at Mt. Sangju. The majority of larvae collected in South Korea were early instars. Six field sites were evaluated in two provinces in China (Jilin and Tianjin) from mid-July to mid-August with Roger Fuester (USDA-ARS). Live larvae were collected at three of the six field sites

(Dagong in Tianjin province and JingYueTan Park and Jiang Nan Forest in Jilin province). All live larvae were placed on artificial media developed by Dr. Leah Bauer (USDA-FS), shipped to her containment room at Michigan State University to rear any parasitoids, and healthy raised EAB will be used for genetic evaluation. Finally, eight field sites were evaluated in Japan throughout the remainder of August 2006. No evidence of EAB was detected at seven of the eight sites (Otsuki, Morioka, Iwaki-san, Aomori, Odate, and the University of Tokyo Tanashi and Chiba stations), while two larvae were collected from *F. lanuginosa* at Mt. Zao near Shirioshi City. These larvae, however, cannot be distinguished as EAB or *A. koyoi* , another *Agrilus* specie attacking *F. lanuginosa* in this area of Japan. Therefore, these larvae were placed on artificial media to rear to the adult stage.

Healthy EAB specimens will be used for genetic analysis to determine 1) the geographic origin of North American EAB populations; 2) the number of EAB introductions; 3) invasion history; 4) possible changes in EAB biology; and 5) sites in Asia for discovery of potential EAB biological control agents. Specimens will be evaluated by a variety of genetic techniques, including mitochondrial cytochrome oxidase I (COI) gene sequencing, amplified fragment length polymorphisms (AFLP), nuclear gene sequencing (wingless (*Wg*), phosphoenolpyruvate carboxykinase (*Pep*CK), cytochrome c (*Cytc*), elongation factor-1 (EF-1)), and microsatellite analysis. Preliminary data from samples collected before the summer of 2006 observed COI haplotype diversity in South Korea, while the common haplotypes shared by all Chinese and North American EAB individuals exists in each of the Korean populations sampled, and a single Japanese specimen had a 3.7% divergence from the common haplotype (Bray et al. 2006).

We expect that genetic analyses of our expanded data set will improve resolution of the EAB populations, allow us to determine which populations are most closely related to each other, and see if the North American infestations resulted from single or multiple introductions of this pest. Knowledge of EAB genetics will be useful in understanding the invasion dynamics of the beetle and to help identify geographic localities of potential biocontrol agents.

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In Japan: Minemitsu Kaneko (Japan Wildlife Research Center) and Takaharu Hattori (private collector).

HOST SELECTION BY EMERALD ASH BORER: CHEMICAL ECOLOGY AND BEHAVIORAL STUDIES

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ABSTRACT

We compared host selection and feeding behavior of the emerald ash borer, *Agrilus planipennis* Fairmaire, on its native host (Manchurian ash) and on North American ash species exotic to the beetle (green, black, white, blue, and European ash). We examined whether beetles could locate hosts by olfaction and investigated chemical differences in volatile profiles of native and exotic hosts. In a multiple-choice assay, beetles preferred to land and feed on three (green, black, and white ash) of the five exotic host species tested. While beetles consumed every ash species offered to them, the native Manchurian ash and the exotic blue ash were least preferred in feeding bioassays. The six ash species in our study differed significantly in relative amounts of chemical compounds that elicited electrophysiological activity by beetle antennae, and beetles landed less frequently on visual silhouettes of artificial trees, suggesting that olfactory cues are used to locate and discriminate among host species in nature. North American ashes exotic to the beetle are more palatable and probably less resistant than Manchurian ash, which coevolved with the beetle in its native range. Physical and/or chemical characteristics of blue ash might render it less preferred than other North American ashes. Attractive volatiles from North American ashes can be used as blends to trap beetles in flight. Similarly, less attractive odours, potential antifeedants, and genes for resistance in blue and Manchurian ash can be explored for methods to control *A. planipennis* populations.

VISUALLY MEDIATED PARATROOPER COPULATIONS IN THE MATING BEHAVIOR OF *AGRILUS PLANNIPENNI*S (COLEOPTERA: BUPRESTIDAE)

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ABSTRACT

Emerald ash borer (EAB) were examined in the laboratory and in the field for a spectrum of pre-mating behavior, including the use of chemical, visual, and acoustic cues. In captivity, mating is initiated by beetles at least ten days old and appears to be based simply on random contact with a member of the opposite sex. In both lab and field, females are far more sedentary than males and spend a large portion of time remaining still and feeding. Feral females move to the trunk and large branches of ash trees when temperatures are higher to search for oviposition sites. In the field, male EAB search the tree during flight and will attempt to copulate with dead beetles of both sexes pinned to leaves. All evidence suggests that males find potential mates in short-range space using visual cues. Equal numbers of feral males approach male and female dead, pinned models; however, considerably more time is spent attempting copulation with dead females rather than males, suggesting a contact chemical cue. Chemically washed beetles of either sex elicit the same number of approaches, but comparatively less time is spent in investigation of these beetles than unwashed females by feral males. Unwashed males elicit very short periods of investigation. Sticky traps prepared from dead, pinned female beetles attract only feral male *A. planipennis* at a rate similar to that at which purple sticky traps of similar size capture both sexes. We suggest the use of visual cues in enhancement of EAB monitoring in the field.

EMERALD ASH BORER FLIGHT ESTIMATES REVISED

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ABSTRACT

Using computer-monitored flight mills, we found that tethered emerald ash borer (*Agrilus planipennis*) adults can fly up to the equivalent of 2.8 km/day at speeds greater than 1.5 m/sec (3.5 mph), with mated females flying twice as far as unmated females ($P < 0.0001$), suggesting that gravid females are programmed to make dispersal flights. This is supported by the absence of a correlation ($R^2 = 0.0005$) between distance flown and female size (Figure 1). Females "flown" for 8 hours per day and then allowed to rest, feed, and drink for 16 hours continued to make long flights for up to five days: the maximum distance flown was 9.84 km in four days, with 50 percent of beetles flying over 4 km per day and 10 percent flying over 7 km per day.

It is difficult and can be misleading to draw conclusions about free flight from flight mill data. There are at least four potential sources of error in the assumption that *flight mill speed = true flight speed of the insect in free flight:*

- Inertial drag of the armature (work expended turning the armature)
- Torsional drag of the magnet (dynamo effect)
- Inconvenience of being attached to the armature (glued at the pronotum)
- Lift provided by the armature (all flight effort results in forward motion).

Thus, it is not clear whether true flight speed is greater or less than the speed recorded on a flight mill. Flight mill data are best interpreted relatively, as we did when comparing flight by males, unmated females, and mated females. In order to be able to draw conclusions about flight in the wild, flight mills must be calibrated: there are very few instances in which this has been possible.

Free flight speed estimates were obtained using high speed photography of beetles flying in a space between two mirrors as part of an experiment to determine the maximum feasible load for a beetle with a weight glued to the pronotum. The position in three dimensions of a flying beetle was determined by analysis of the position of the beetle and its two images in each film frame. An average speed was calculated from the change in position of each beetle from frame to frame. Figure 2 shows the relationship between flight speed and load as a percentage of beetle weight. The regression equation was used to remove the effect of weight from the flight speed to obtain a distribution of free flight speed. Figure 3 shows the mean flight speed in free flight to be three times that of the mean flight mill speed; the standard deviations are not significantly different ($t = 0.70$, $p > 0.48$) and neither distribution is significantly different from normal. Thus, we conclude that the impact of the flight mill is to reduce the measured flight speed by a factor of three. Assuming the duration of EAB flight on the flight mill is not greater than the same individual would achieve in the wild, we now revise the distribution of flight distance up by a factor of three (Figure 4; note the logarithmic abscissa), which predicts that 10 percent of gravid females are capable of flying over20 km per day.

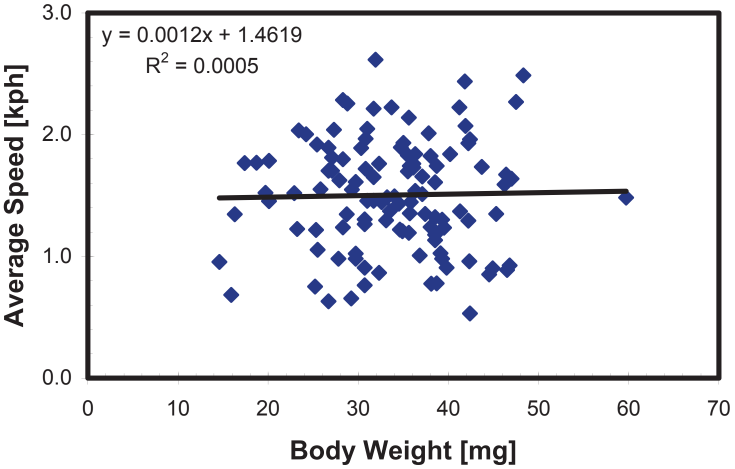

Figure 1: Effect of beetle size on female flight speed.

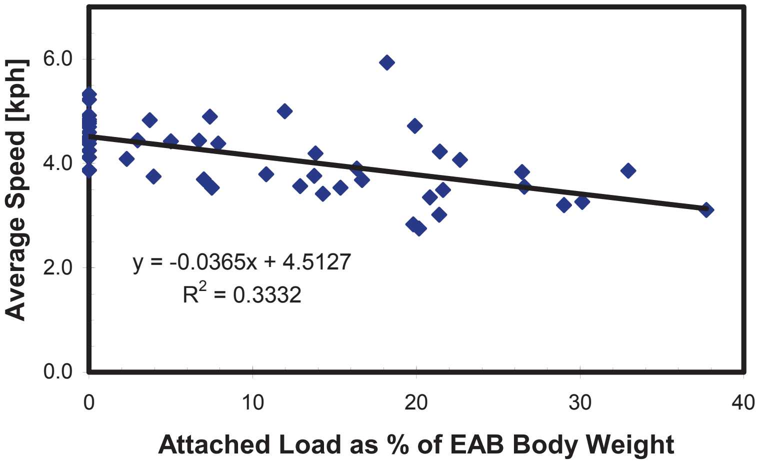

Figure 2: Effect of payload on female flight speed.

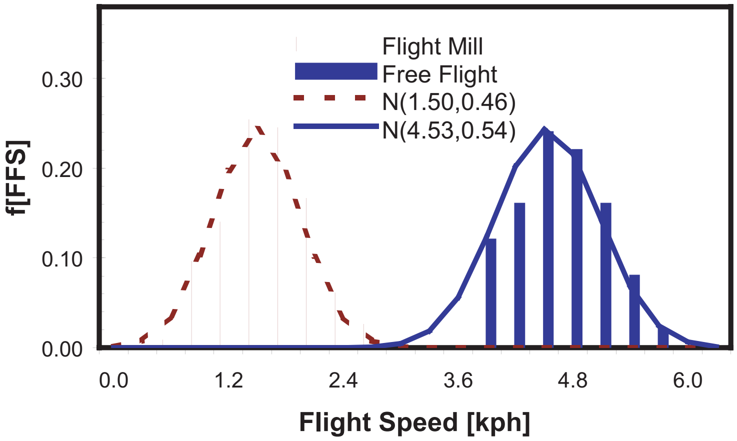

Figure 3: Distributions of female flight speed.

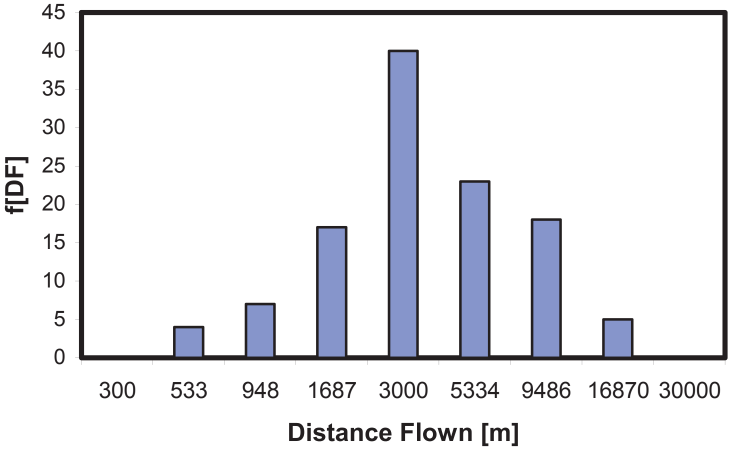

Figure 4: Predicted flight range of gravid female EAB.

DISPERSAL BEHAVIOR OF *AGRILUS PLANNIPENNIS* (FAIRMAIRE) (COLEOPTERA: BUPRESTIDAE): RELEASE-RECAPTURE STUDIES

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ABSTRACT

In this and in a previous year's study, we investigated the emerald ash borer's (*Agrilus planipennis* Fairmaire) adult dispersal behavior from a central release in an area with varying physiographic and vegetative features. Host tree (*Fraxinus*) species and density varied in the site selected for study in 2006.

Traps were arrayed in roughly concentric circles up to 300 meters out from the central release point, with a total of 118 trap trees set. There were three different trap types:

1. Eight mesh panels (1 meter by 0.5 meters) strung together by 0.5-meter lengths of rope and hung vertically 10 meters parallel to an ash tree's trunk using a pulley system (total of 56 trees).
2. A high and low purple prism trap associated with an ash tree. The high trap was strung 10 meters from the ground and the low one was attached to rebar directly below the high trap. Each of the prism traps was baited with 4 ml of manuka oil (total of 49 trees and 98 traps).
3. A high and low purple prism trap associated with a non-ash tree, set up in the same manner as the those associated with ash trees (total of 14 trees and 28 traps).

Beetles used in this study were collected in the laboratory by placing infested ash wood in emergence barrels. The adult emerald ash borer emerging from these barrels were collected daily and marked with day-glow powder. They were then placed in jars and released the morning following their collection. The color of day-glow used was changed weekly in order to track the released beetles travel over time.

There were two separate series of releases preformed. The first release began on May 20 and totaled 11,500 beetles over a four-week period. The overall recapture rate for this release was 4.5 percent. The second release began on June 28, and a total of 7,300 beetles were released over a three-week period. The recapture rate for this release was 2.2 percent. A total

of 670 beetles were recaptured through the study, an overall recapture rate of 3.6 percent. One beetle was recaptured at the furthest trap, 305 meters from the release point. This beetle was collected less than two weeks after its release. Male and female recapture patterns are similar. Also resident feral beetles were captured in patterns similar to marked released beetles. We also analyzed feral beetle capture rates by trap height for all three trap types. A Chi-square analysis showed that, for panel traps and purple prism traps on ash, significantly more beetles were caught on high traps (P = 0.001). Chi-square analysis of puple prism trap height on non-ash trees showed no significant difference between beetles caught on high and low traps.

MODELING POTENTIAL MOVEMENTS OF THE EMERALD ASH BORER IN OHIO

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ABSTRACT

The emerald ash borer (EAB, *Agrilus planipennis*), is threatening to wipe out native ashes (*Fraxinus* sp.) from North America and so far is accomplishing this across large sections of Michigan, Ohio, Indiana, and Ontario—with infestations in Illinois and Maryland as well. We are attempting to model its future movement by adapting a model developed for the potential movement of tree species over a century of climate change. We have two model variants: an 'insect-flight' model and an 'insect-ride' model to assess potential movement.

Both models require spatial estimates of basal area of ash available to the insect and the relative abundance of the insect. We used classified Landsat data, calibrated with Forest Inventory and Analysis (FIA) data and other plot-level data, to estimate ash quantities per 270x270 m cell. For initial conditions of EAB abundance, we estimated zones of infestation for each year from 1998-2005 using known EAB location information and other data.

With the 'flight' model, probability of movement depends on EAB abundance in the source cells (270 m cells), the quantity of ash in the target cells, and the distance between them. To estimate abundance, we assume an 11-year cycle along a normal curve, with maximum abundance at year 6 and minimum abundance at the initial colonization time as well as after the ash trees have died within the cell. With the 'insect-ride' model, we utilized GIS data to weight five factors related to potential human-assisted movements of EAB-infested ash wood or just hitchhiking insects. The five factors are roads, urban areas, tree nurseries, various wood products industries, and especially campgrounds. For campgrounds, we are developing a gravity model that considers traffic volumes and routes between areas of EAB infestation and distances to campgrounds. Each layer has buffer weights that, when combined, result in a map of zones of enhanced probability of EAB colonization.

The 'flight' and 'ride' models were then combined to yield a map of colonization potential in the central part of Ohio being modeled. When actual EAB finds were overlaid on this probabilistic map, 62 percent of finds fell within our highest class of probability, and 83 percent of finds fell within a zone of high probability of colonization.

In Ohio, the movement of EAB so far has been greatly hastened by human movements, and 67 percent of the outlier EAB finds in Ohio occurs within 2 km of major roads. This seems to implicate 'hitchhiking', in which the insect catches a ride on a vehicle (radiator or defroster or some other place on the vehicle). However, firewood, nursery stock, and wood products also are implicated for some outliers.

DEFINING THE "EDGE" OF ISOLATED EMERALD ASH BORER INFESTATIONS: SIMULATION RESULTS AND IMPLICATIONS FOR SURVEY AND HOST REMOVAL

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ABSTRACT

In August 2006, two ash trees harboring emerald ash borer (EAB) larvae, *Agrilus planipennis* (Coleoptera: Buprestidae), were discovered in Prince George's County, Maryland. One tree, with three galleries, was a sentinel tree planted in April 2006 within the 800-m (½-mile) ash-free buffer surrounding the nursery (38° 43.71' N, 76° 53.36' W) that had received infested ash trees from Michigan in 2003. The other, a native tree girdled in November 2005 to serve as a trap tree, contained numerous larvae. This tree was about 134 m (440 feet) outside of the buffer and 610 m (2,000 feet) from the infested sentinel tree. These discoveries and additional infested trees found later in a broader survey of the area showed that the removal of all ashes within 800 m of known infested trees in 2003 was an inadequate response, leaving undetected galleries intact outside of the removal zone. The '800-m rule', based on field observations to date, assumptions about EAB dispersal capacity and previous simulation studies, is clearly in need of revision.

In this study I revised the simulation model I developed in 2004, which was based largely on data gathered in 2003 by Deb McCullough (Department of Entomology, Michigan State University) at a field site near Tipton, Michigan (McCullough et al. 2003, Sawyer 2004, 2005). The model assumes that larva galleries are distributed around a parental point source at distances described by an exponential probability function. The original model did not track individuals or include stochastic (random) processes. I modified the model by adding stochastic elements representing dispersal direction, mating success and the disposition of "fractional" galleries, essentially making it operate in an individual-based mode at low population densities.

The objectives of this new work were to (1) investigate whether the concept of an "edge" is meaningful in reference to a population's spatial distribution; (2) examine how an infestation's outermost limit depends on population size and other variables; (3) study the influence of random events on the location of this edge; and (4) consider the implications of these findings for EAB surveys, host removal as a control method and regulatory actions.

Simulation results showed that, except for very small initial populations, the distribution of most EAB galleries around a point source is little affected by the inclusion of stochastic

elements in the model. For example, with a distribution model based on McCullough's data, 95% of first-year galleries consistently fell within 300 m of the point source if there were 20 or more founders (based on 50 runs of the model). Ninety-nine percent (99%) of galleries fell within 450 m of the origin for initial populations of 200 or more emerging EAB adults. The location of these percentiles did not vary for populations larger than these. In contrast, the location of the most-distant gallery (the "edge" of the infestation) depended very much on the size of the original population. Also, due to stochastic elements in the model, results varied from run to run regardless of population size. The position of the outermost first-year gallery ranged from 300 to 900 m from the epicenter for a small infestation initiated by 20 beetles, moving outward to 850-1,250 m for a large infestation initiated by 2,500 beetles (based on 50 runs of the model in each case). The absolute number of galleries established more than 800 m from the epicenter also depended on the initial population size, ranging from a single gallery in one run of the model (out of 50) when there were just 10 founders, to a range of one to five galleries (avg. 2.5) in each of 50 runs for 2,500 founders.

These basic results show that, while percentiles of a population's distribution are well defined and stable, its outmost "edge" is not. Random events may place galleries beyond the 800-m host removal zone. However, with default parameter values (based on McCullough's field data), so few galleries are expected at that distance, spread over such a large area, that it seems unlikely a viable population would remain after cutting. In fact, to avoid extinction of the remnant population, some parameters of the model, such as the rate of increase or the mean dispersal distance, had to be changed. The discovery in 2006 of multiple galleries per tree at the Maryland site calls into question some assumptions of the model, especially the ideas of random dispersal and continuous deposition of eggs, according to a probability model, as females disperse. Processes not represented in the current model, such as attraction to host trees, attraction to mates, and laying eggs in a clustered distribution, would increase the likelihood that an infestation will re-establish from a small number of surviving galleries. It is apparently for such reasons that the 800-m host-removal tactic is an insufficient response.

Simulations (with default parameter values) showed that if host removal is delayed, it becomes more and more likely as time passes that EAB galleries will become established beyond 800 m. After three years of simulated population increase and spread (in the absence of control measures), the 99th percentile of gallery distribution advanced 150 m, from 450 m to 600 m from the original epicenter. More importantly, the "edge" of the infestation was also found farther from the source point. In 50 runs of the model, the outer-most galleries were found 700-1,200 m from the epicenter in the first year, 900-1,400 m from the source in the second year, and 1,150-1,900 m (more than a mile in some cases) from the origin in the third year, emphasizing the importance of quick action in containing the infestation at reasonable cost.

Survey and detection at the "edge" of an infestation are problematic. The low population density makes detection difficult and uncertain. The large area to be covered leads to high costs for both survey and host removal. Regulatory actions become increasingly costly and affect more people. Nevertheless, simulation results suggest that host trees should be removed out to 1,600 m (one mile) from the point source in the first year of an EAB infestation, and to a minimum of 2,400 m (1.5 mi.) for a three-year old infestation. Intensive survey and monitoring (using trap trees) should be conducted in the surrounding area for several years.

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RESURRECTED FROM THE ASHES: A HISTORICAL RECONSTRUCTION OF EMERALD ASH BORER DYNAMICS THROUGH DENDROCHRONOLOGICAL ANALYSIS

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ABSTRACT

The invasive emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), was identified in southeastern lower Michigan in July 2002 and was found to be responsible for the death or decline of several million ash (*Fraxinus* spp.) trees. We intensively surveyed southeastern lower Michigan to determine where emerald ash borer-induced decline of ash trees appeared to be most severe. Over an 1,800 square-kilometer geographic area encompassing what was commonly believed to be the epicenter of the known core infestation in Detroit, Michigan, we used tree ring analyses to examine the historical dispersal patterns and spread of emerald ash borer. Two to 4 increment cores and/or cross-sectional samples from emerald ash borer-killed green ash trees were preferentially collected over declining or non-stressed ash trees on at least a 2.4 × 2.4-kilometer sampling grid 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 crossdate samples to a known master chronology compiled from ash trees surrounding the sample area.

Preliminary crossdating 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 as early as 1996-1997. Analyses indicate that ash mortality radiated farther out from the reconstructed epicenter each year. In related dendrochronological research conducted at several emerald ash borer outlier sites in Michigan, we have found that an area is typically infested for 3 to 4 years before tree mortality occurs. In turn, this would suggest that emerald ash borer was introduced and became established in southeastern lower Michigan in the early to mid-1990s.

Additional analyses are currently in progress to verify the accuracy of the preliminary crossdating analyses. We are also in the process of crossdating samples collected over a 15,000 square-kilometer geographic area encompassing the original seven counties quarantined for emerald ash borer in 2002. The greater dendrochronological reconstruction of emerald ash borer dynamics will reveal: 1) whether or not emerald ash borer initially became established somewhere other than the Westland-Garden City area, 2) temporal and spatial dynamics of early outlier sites within the core infestation, and 3) how emerald ash borer spread historically throughout southeastern lower Michigan.

TWO YEARS UNDER THE BARK: TOWARDS UNDERSTANDING MULTIPLE-YEAR DEVELOPMENT OF EMERALD ASH BORER LARVAE

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ABSTRACT

Multiple-year development of emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), larvae has significant implications pertaining to continued development of effective management strategies for improved control of this invasive pest. When emerald ash borer was identified in 2002, little was known about it's biology, and a single-year generation was assumed. Our reconstructions of emerald ash borer population dynamics at several outlier sites in Michigan have suggested that 2-year development of emerald ash borer larvae occurs and may be typical at sites with low emerald ash borer densities. Additionally, we have used dendrochronological analyses to verify 2-year development of emerald ash borer larvae on green, white, black, and blue ash at several sites throughout Michigan. We have also found on several occasions that emerald ash borer larvae exhibit an increased rate of development on stressed trees (e.g., trees with damaged canopies and girdled trees).

In 2006, the effect of tree stress on emerald ash borer larva development was examined using a randomized complete block design on 90 healthy green ash in a plantation. Treatments included girdling, applications of methyl jasmonate (a stress elicitor), and untreated controls. Pairs of 2-week-old emerald ash borer adults were caged on trees in June and allowed to infest the trees. Galleries under the cages were excavated in late August/early September, and rates of emerald ash borer development wereassessed. Preliminary results indicate that a significantly greater proportion of emerald ash borer larvae developed to late instars on girdled trees compared to control trees and trees with the methyl jasmonate applications.

Our research to-date suggests that: 1) 2-year development of emerald ash borer larvae is typical at outlier sites; 2) increased rates of larva development occur on stressed trees; and 3) multiple-year development is likely associated with low levels of tree resistance and low densities of larvae. Implications of this research were discussed in relation to developing effective management strategies, including the effect of 2-year larva development on rates of spread, considerations associated with eradicating high-priority outlier populations, and detection and survey of isolated emerald ash borer populations.

EMERALD ASH BORER–HOST RELATIONSHIPS

CONSTITUTIVE AND WOUND-INDUCIBLE DEFENSE RESPONSES OF ASH PHLOEM

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ABSTRACT

We conducted a time course study on the production of defense proteins and soluble phenolics in response to mechanical wounding and emerald ash borer (EAB), Agrilus planipennis Fairmaire, homogenates in the phloem of mature white ash (*Fraxinus americana)* and green ash (*F. pennsylvanica)* grown in a common garden in Ohio. On each tree, wounds were created with a boring tool and treated with EAB homogenate or water. Samples of phloem around the original wounds were taken over a three-day time interval. These samples were compared to an unwounded control site taken at the same times on the same tree. Activities of trypsin inhibitor (TI), polyphenoloxidase (PPO), peroxidase (POD), chitinase, β-glucanase, and total soluble protein content were compared among treatments. For both white and green ash, both TI and chitinase activities varied through time and responded significantly to wounding. Total protein content of the phloem of green ash also varied through time and increased in response to wounding. Soluble phenolics were detected by HPLC in phloem extracts, but were similar between species and largely unaffected by wounding.

We also compared constitutive protein and phenolic levels in young Manchurian ash (*F. mandshurica)*, white ash, and green ash to study the enhanced field resistance displayed by

Manchurian ash to EAB. Phloem extracts of Manchurian ash showed a significantly higher browning rate and TI and PPO activity, but a lower POD activity at the constitutive level than the native ashes. Manchurian ash also had a distinct HPLC profile of soluble phenolics relative to the native ashes, including both quantitative and qualitative differences.

Although several proteins and phenolics failed to respond to wounding, we found some evidence for induction of defense proteins in wounded phloem of native ashes, but responses varied little in the presence and absence of EAB homogenates. This modest response to wounding may explain why native North American ash trees are quite susceptible to EAB attack. In turn, the faster browning rate, higher TI and PPO activity, and distinctive phenolic profile of Manchurian ash could explain why they are both more resistant to and more likely to survive an EAB attack than native white and green ashes.

EVALUATION OF RURAL ASH RESOURCES IN UPPER AND LOWER MICHIGAN THREATENED BY THE EXOTIC EMERALD ASH BORER

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ABSTRACT

The Rural Ash Monitoring Plot project has developed a permanent, statewide monitoring plot network in forests containing ash (*Fraxinus* spp.) in order to monitor the status and health of ash in Michigan's rural forests. The project was initiated in 2004 and is a collaboration between Michigan Technological University and the University of Michigan. The goals of this project are 1) to characterize the condition of ash resources in rural forests in Michigan, 2) to characterize forest changes in response to exotic invasive species such as emerald ash borer (*Agrilus planipennis*), 3) to provide a database for use in monitoring and management of ash resources, and 4) to provide a network of research plots with data that are available for use in other research projects.

Plots are distributed across Upper and Lower Michigan, and measurements are adapted from the USDA Forest Service Forest Health Monitoring protocols. Data collected include stand-level information, plot-level information, and tree-level information. In the Upper Peninsula, 73 permanent monitoring plots were installed between 2004 and 2005 and revisited in 2006. Emerald ash borer was not present in any plots, with the exception of the plot at Brimley State Park, which was eliminated as part of an emerald ash borer eradication cut in 2005.

The majority of ash trees in the Upper Peninsula were characterized as healthy, mature, codominant trees. Very few of the trees were damaged, and damage types were not consistent across species or sites. Most damage was recorded as loss of apical dominance. Continued monitoring of these plots will enable us to characterize the impacts of emerald ash borer on rural forests in Michigan. These plots are available for use in association with other research projects.

LIVING WITH EMERALD ASH BORER: DEVELOPMENT AND IMPLEMENTATION OF AN ASH REDUCTION MODEL TO REDUCE THE POPULATION POTENTIAL OF EMERALD ASH BORER

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ABSTRACT

The exotic emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), is established in a number of states, including Michigan, Indiana, Ohio, and Ontario. At high population densities, all green, black, and white ash trees are apparently susceptible to attack and can be expected to die. Emerald ash borer larvae develop in the phloem of ash trees in stems and branches above approximately 2.5 cm in diameter. Removal of ash from high priority areas such as those stands in close proximity to outlier populations will reduce the population density of this insect.

The surface area of over 500 ash trees was measured using standing trees as well as cut trees throughout Lower and Upper Michigan. White, green, and black ash trees in open grown and forested settings were all represented. There are strong quadratic relationships between diameter at breast height and calculated surface area of the tree, but these quadratic relationships differ significantly between open grown and forest grown trees when the dif-

ferent ash species are considered separately. Multiple models have been developed for use in management prescriptions to reduce the amount of ash available to emerald ash borer. These models are based on ash species and crown light exposure. Information on ash species and the light exposure for most of the trees in a stand (i.e., forested or open grown trees) may allow managers to use a more specific model to fit their stand.

Other relationships between diameter, surface area, and volume of phloem are being determined. These relationships, in addition to others involving tree vigor, form, and growing conditions, have been integrated into models characterizing the amount of ash phloem in a forest stand. Using these models with trees-per-acre information from a stand and stock table, it is possible to determine diameter limits for cutting to meet prescribed ash phloem reduction targets. By reducing emerald ash borer populations through phloem reduction and decreasing the removal of the smaller trees in a stand, this model will enable the genetic diversity of ash to be optimized during ash reduction efforts. Similar models are available for use when the management goal is to retain large trees within a stand. Applied models help land managers to make scientifically quantifiable decisions relating to ash reduction in forests. Forest resource managers are able to access the models online at *www.ashmodel.org* and determine the diameter limit for removal of ash to achieve the phloem reduction target within the context of other forest management goals.

PREDICTING EMERALD ASH BORER-INDUCED CHANGES IN FOREST TREE SPECIES COMPOSITION

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ABSTRACT

The death of ash trees causes changes in community composition, structure, and successional trajectories of forested areas and may facilitate the invasion of exotic plant species.

To study and predict these changes, we are monitoring plots in forested areas across Ohio and developing modeling methods to predict the successional trajectories of these areas. In addition to recording the identities and sizes of tree species in the plots, we are also monitoring light levels and invasive species as conditions change due to ash mortality. In areas where ash is a dominant species, we will also do seed bank studies. We are tracking the condition of individual ash trees to assess rates and patterns of decline as well as the factors (stand size, ash density, species composition) that may affect decline. Preliminary data from natural areas near Toledo shows the potential for rapid decline in ash condition in infested sites.

We will model the succession of these stands, as well as other stands with data provided by collaborators, using the Forest Vegetation Simulator (FVS). FVS is a non-spatially-explicit model of tree growth and survival that is used by the US Forest Service and National Parks. We will use ordination and cluster analysis to identify different groups of stands that are predicted to behave similarly. We will parameterize the model to explore emerald ash borer effects on invasive shrub species, which are abundant in Ohio. The information generated by our studies will allow land managers to know what their forests will look like during and after emerald ash borer infestation and enable them to develop management strategies.

PATTERNS OF EMERALD ASH BORER-INDUCED ASH DECLINE AND MORTALITY IN THE FORESTS OF SOUTHEASTERN MICHIGAN

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis*, invasion of the forests of the Huron River Watershed in southeastern Michigan may result in drastic changes in forest structure and composition. The objectives of our study were to (1) quantify landscape-level spatial patterns of ash decline and mortality, (2) assess whether patterns of colonization and decline vary among black ash (*Fraxinus nigra*), green ash (*F. pennsylvanica*), and white ash (*F. americana*), and (3) determine whether observed spatial patterns of ash decline and mortality changes over time.

During 2004 and 2005, 31 permanent vegetation plots were established in forest stands within eight state and metro parks within the Upper Huron River Watershed. In 2004 and 2005, surveys were conducted in 11 and 20 stands, respectively. In 2006, all 31 stands were reassessed to quantify progression of ash dieback and mortality. Forest stands were selected to represent a moisture gradient with black, green, and white ash as the major ash component on hydric, mesic, and xeric sites, respectively. Forest stands were also chosen to represent an

ash mortality gradient that decreased with distance from the presumed epicenter of EAB infestation in the township of Canton, Michigan. Within each forest stand, three 0.1 ha circular plots were placed along a single transect. Within each plot, all understory and overstory plants were identified to species, and their diameter at breast height (DBH) and density (stems/ha) were recorded, and species diversity indices were calculated. On each ash tree, EAB colonization was quantified by documenting the density of adult emergence holes and woodpecker attacks on the main bole at 1-2 m from the ground. Each ash tree within a plot was assigned a dieback rating that ranged from 1 to 5, with '1' designated as a healthy tree with full crown and no sign or symptoms of EAB attack and '5' designated as a dead tree.

In 2004 and 2005, there was a significant curvilinear relationship between mean tree dieback rating and mean EAB attack density/m^2 of bark surface area on the main bole (P-value < 0.001; $R^2 = 0.72$), suggesting that EAB was the major source of ash decline and mortality.

There was no relationship between percent ash mortality and any stand-level variables including ash density, ash basal area, total basal area, total tree density, and species diversity. Thus, all ash stands are susceptible to colonization by EAB irrespective of stand diversity or density, suggesting that silvicultural practices may have little potential for preventing EAB colonization. The only significant relationship detected was a negative correlation between percent ash mortality and distance from the epicenter of infestation ($P = 0.001$; $R^2 = 0.35$).

Black ash experienced greater EAB-induced decline and mortality than white or green ash species (ANOVA, $P = 0.001$), although all three species were severely impacted. There were little differences among size classes, with ash trees ranging in DBH from 2.5 to 22.5 cm experiencing approximately equal degrees of decline and mortality. A comparison of ash mortality between plots that were sampled in 2004 and 2006, and in 2005 and 2006 indicated that ash mortality increased by 19-23% over 1-2 years. The slope of the line describing the negative relationship between ash mortality and distance from the epicenter remained unchanged between 2004-2005 and 2006 (2%/km). However the y-intercept in 2006 increased by 22 percent, suggesting that ash mortality is increasing rapidly and will reach 100 percent in all plots within the next few years.

Where ash was present in the overstory, it was the most common species in the understory and seedling layer. Common understory associates included maple (*Acer* spp.), basswood (*Tilia* spp.), and cherry (*Prunus* spp.). This suggests that as ash mortality progresses, community composition will shift in favor of these three genera. These results indicate that as EAB continues to spread it has the potential to substantially change the structure and composition of North America's central hardwood forests.

ASH DIEBACK SURVEY IN MICHIGAN

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ABSTRACT

A visual survey of ash dieback in southeast Michigan was initiated in 2003. Because the core infestation of emerald ash borer in Michigan appears to be centered about 20 miles west of downtown Detroit, in Canton, we used the freeway system to sample ash dieback. We drove all of the expressways coming out of Detroit, stopping at each exit to estimate dieback. The first ten ash trees (> 4" dbh) found were compared with a standard set of ash dieback photographs to visually estimate the level of canopy thinning or dieback for each tree. If ten ash trees were not found near the exit ramp, we turned away from the expressway and drove until ten trees were found. Ash trees in all ownerships were surveyed in the survey, including freeway property, ditch banks, commercial property, private yards, public parks, and woodlots. All ash species (*Fraxinus*) were included in the survey. Dead ash trees were included, but not stumps. The survey continued outwards from Detroit in all directions until at least three consecutive sites were found where trees averaged less than 25% dieback. The survey was repeated in 2004, 2005, and 2006. In 2005 and 2006, emerald ash borer exit holes were also counted using high-quality binoculars at all sample sites in the western portion of the survey (from Jackson to Ann Arbor to Flint to Lansing). Reliability of binocular counts of exit holes was verified by cutting down 15 trees (10-20" dbh) and counting exits again.

In 2003, the most severely impacted area was along I-275 between Novi and I-94. Along this stretch of about 20 miles, most of the ash trees were dead or dying, and canopy dieback averaged greater than 80 percent (Figure 1). In 2004, dead ash trees were found west to Ann Arbor and up to 10 miles south of I-94. In 2005, dying ash trees were found all the way to Flint to the north and nearly to Monroe to the south. In 2006, dead ash trees were observed south to the Ohio border, west to Jackson and Lansing, and north to Flint and Saginaw. For the first time in 2006, outlier infestations that originated from the movement of infested wood or trees became apparent in our survey.

Binocular counts of exit holes on one side of the trunk (going up as high as possible) of an ash tree correlated well with the actual number of exit holes found on all sides of the main trunk after trees were felled ($r^2 = 0.85$). In 2005, the number of emerald ash borer exit holes per 10 trees at each of 33 sample sites correlated well with the average level of canopy dieback at the same sites ($r^2 = 0.59$). No exit holes were found at any site that averaged less than 25% dieback, and at least one exit hole was found on all sites that averaged greater than 40% dieback. Canopy dieback of ash trees in our survey was then plotted against the distance away from the center of the emerald ash borer infestation near Canton, Michigan. Using the regression line for this relationship, the average distance away from the center of the EAB

infestation where trees averaged 40% canopy dieback in 2003 was 12 miles compared with 40 miles in 2006 (Figure 2).

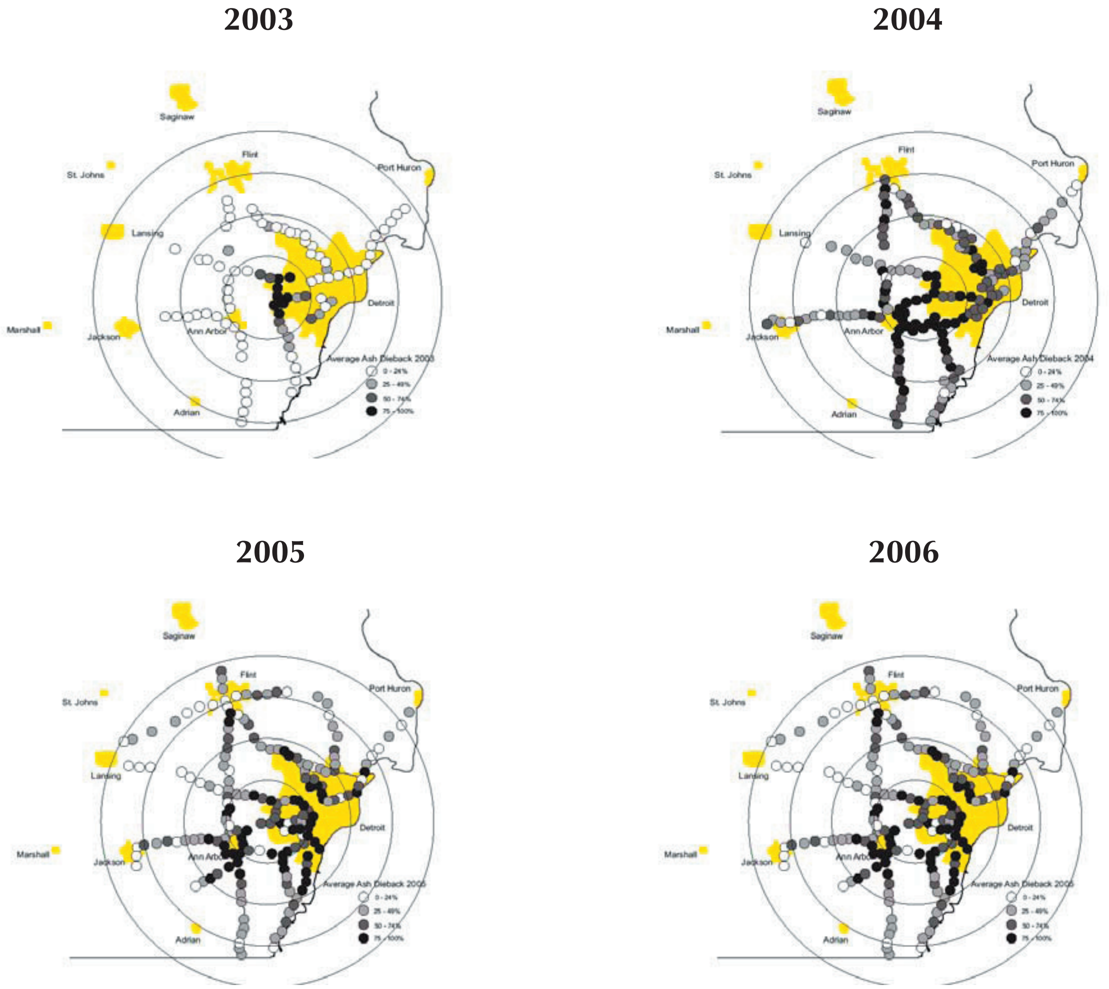

Figure 1. Ash canopy dieback in Michigan from 2003-2006. Circles are 15 miles apart.

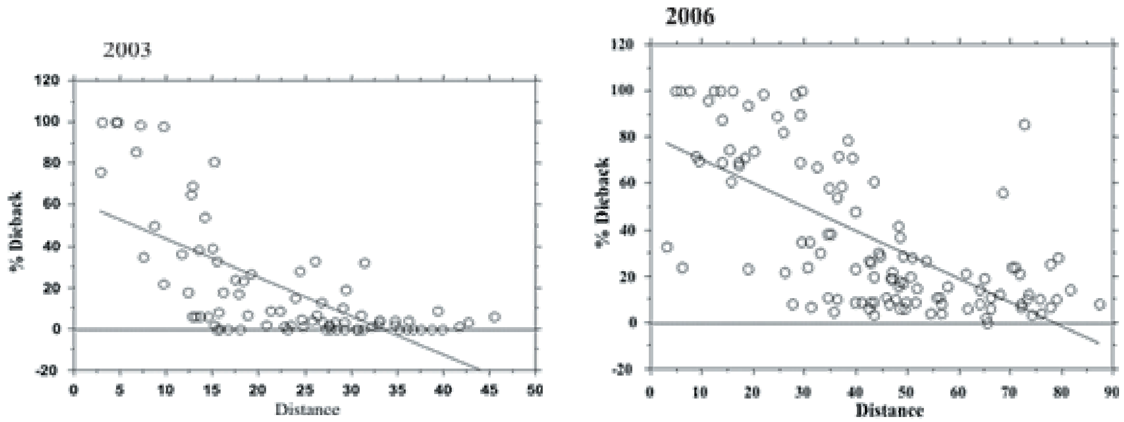

Figure 2. Relationship of ash canopy dieback to distance from center of infestation in 2003 and 2006.

THE EMERALD ASH BORER ERADICATION PROTOCOL: ENVIRONMENTAL IMPACTS AND NATIVE PLANT COMMUNITY RESPONSES

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ABSTRACT

This project investigates the environmental impacts of the emerald ash borer eradication program and further identifies how the plant community is responding to those changes. Specifically, the goal is to examine how changes in the light environment and soil compaction alter dominance and distribution of the plant community. This project is being conducted at Pearson Metropark, located in Lucas County, in North West Ohio. The Ohio Department of Agriculture quarantined Lucas County in 2004 and called for the removal of every ash tree within a one-half mile radius of a positively infested tree. In the spring of 2005, the eradication protocol began at Pearson Metropark and was supposed to have a minimal impact possible to the habitat. However, habitat conditions become altered dramatically with the abrupt changes to the environment caused by the removal of so many trees.

Changes to the environment include an increase in the number and size of gap formations that occur within a short period of time because multiple trees are being removed from area all at once. In addition, the effect of heavy vehicles used to remove trees may lead to an increase in soil compaction. These factors are likely to influence the composition of the existing plant community; however, of greater concern is whether or not this level of disturbance further facilitates a secondary spread of invasive plant species. Often invasive plant species have the ability to dominate and outcompete native species in disturbed habitats. This project is designed to describe the successional stages of plant colonization after the eradication protocol and further determine the degree of invasion potential between the cut and uncut areas.

The sampling design consists of fourteen 20x25m plots (eight uncut plots and six cut plots). The light environment was assessed using six 180° fisheye hemispherical photographs taken from each plot. These fisheye images are imported into a software program called Hemiview to determine changes in solar radiation regimes (direct and diffuse radiation). Results include increases in duration and intensity of light reaching the forest floor. There are greater global site factor (GSF) measurements found in cut plots, where ash trees were removed, compared to uncut plots (Figure 1).

Soil compaction measurements were also taken in all fourteen plots. Using a soil penetrometer, six readings were taken at five different depths (3, 6, 9, 12, and 15 inches) in each plot to measure the pressure in pounds per square inch (PSI) needed to penetrate through the soil profile. The degree of soil compaction was found to be greater in the cut versus uncut plots, with those results detectable at each depth of the soil profile (Figure 2).

A baseline of the plant community structure was assessed before cutting began and then again after cutting was completed. Measurements for overstory composition and structure were collected, with similar information gathered for the shrub layer and the herbaceous understory. Preliminary results indicate that the plant community in cut areas is beginning to show an increase level of sensitivity to invasive plant species. While these results represent a response time of only 2-3 months after the initial eradication cutting in 2005, it appears as though the 2006 patterns will be similar. The changes to the environment imposed by the eradication protocol observed thus far appear to create ideal opportunities for the spread of invasive plant species.

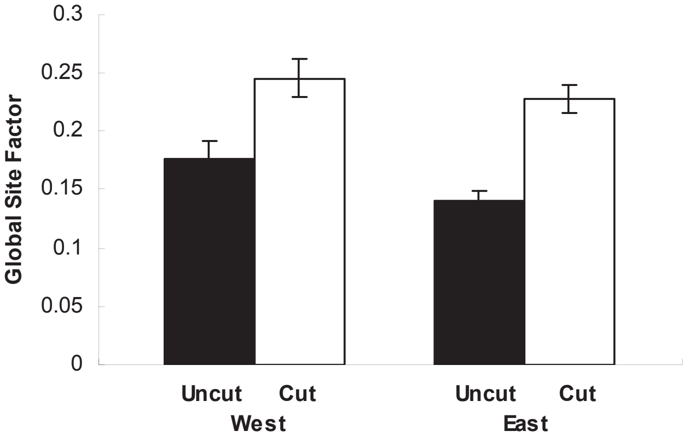
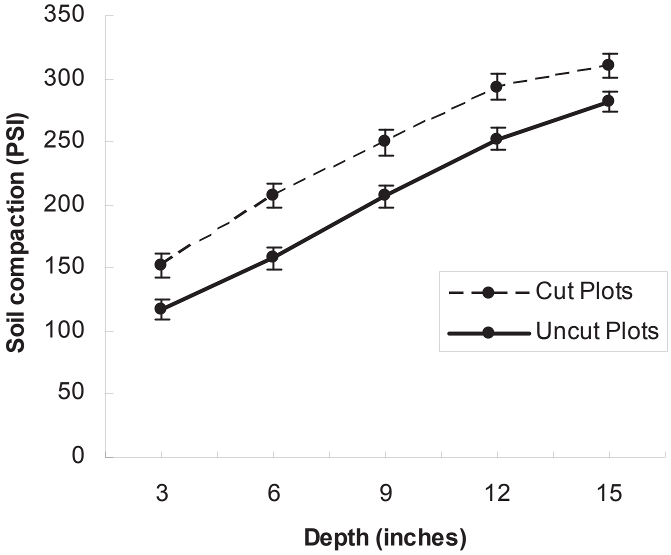


Figure 1. Variation in the proportion of radiation (Global Site Factor—GSF) that occurs under the forest canopy in cut and uncut plot compared to that of open sky. Significantly greater GSF occurred in the cut plots compared to the uncut plots (P<0.0001).

Figure 2. Soil compaction in cut plots and uncut plots at five different depths in the soil profile. Analysis of variance revealed significant differences in soil compaction between cut plots and uncut plots (P<0.0001). Bars indicate standard errors.



CHEMICAL CONTROL OF EMERALD ASH BORER

DISTRIBUTION OF TRUNK-INJECTED ^{14}C IMIDACLOPRID IN *FRAXINUS* TREES: A TEST OF THE SECTORED–FLOW HYPOTHESIS

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ABSTRACT

In a previous study at Michigan State University, researchers used ^{14}C labeled imidacloprid to track trunk-injected pesticide movement in ash trees (Cregg et al. 2005). The study demonstrated that imidacloprid moves slowly but steadily through the tree over time and accumulates in the leaves. A large proportion of imidacloprid metabolites are removed from the tree system as a result of leaf litterfall, and there is a low concentration reservoir of imidacloprid equivalents found near the injection point.

The sampling protocol of the earlier study, however, did not permit a thorough examination of spatial variability of imidacloprid distribution in the ash trees. Orians et al. (2004) demonstrated that sap flow in trees may be sectored or integrated depending upon tree species. Highly sectored flow could result in an uneven distribution and variable efficacy of trunk injected pesticide. The objective of the current study is to determine the extent to which movement of imidacloprid is sectored within the trunk of ash trees.

Understanding the flow of the pesticide through trees will enable us to improve the efficacy of trunk injection treatments and better understand the persistence and potential for extended emerald ash borer (EAB), *Agrilus planipennis*, control.

METHODS

On June 27, 2006, we injected 32 trees (16 *Fraxinus americana*, 16 *F. pennsylvanica*) with 25 µCi ^{14}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 using a 5/16" drill bit. Stem injection tubes were inserted into the holes and imidacloprid was applied at 30 PSI using a bicycle pump. Stem injection tubes were removed after all fluid was taken up by the tree. 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.

Fine roots, trunk cores, and leaves were sampled at 0, 2, 7, 21, 60, and 98 days after injection (DAT). Tree stems were sampled only once at 60 DAT because the trees were small. Samples were oven dried, ground with a mortar and pestle, and oxidized in a biological tissue oxidizer. The resultant 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%).

RESULTS

Imidacloprid equivalents in leaves varied with time, orientation to the injection point, and whorl height (Figure 2). For branches in the plane of the injection point (0°), imidacloprid equivalent concentration in leaves increased throughout the growing season. Leaves of branches opposite of the injection point (180°) had lower concentrations than the 0° branches and did not increase significantly after 20 DAT. Imidacloprid equivalent concentrations in leaves of branches at right angles to the injection point (L90° and R90°) were generally intermediate to the leaves on the 0° and 180° branches. The difference in imidicloprid equivalent concentration between the 0° and 180° branches was lower in whorl 3 than whorl 1, suggesting that flow becomes more integrated with tree height.

Imidacloprid equivalent concentrations in roots, stems, and trunk cores 60 DAT were significantly lower than the concentration of imidacloprid equivalents found in leaves (Figure 3). This pattern is consistent with the previous study and suggests that the movement of imidacloprid occurs primarily in the xylem.

SUMMARY

The results of the current study are consistent with the sectored flow hypothesis. Imidacloprid equivalent concentration is higher in leaves of branches directly above the injection point (0°) as opposed to leaves of branches on the opposite side of the injection point (180°). The relatively low amount of imidacloprid in other plant organs, such as roots, suggests that imidacloprid moves through trees primarily through the xylem. Based on the steady increase in imidacloprid equivalent concentration in leaves throughout the growing season in this study and our previous work we hypothesize that imidacloprid moves through the xylem from a reservoir of insecticide near the initial injection point.

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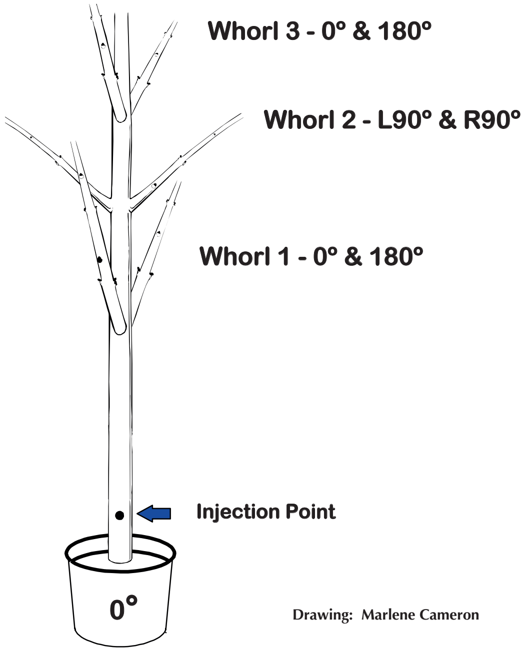

Figure 1. Tree injected at 0° to the first whorl of branches. Mean distance from injection point to the first whorl = 1.28 m. Mean distance between whorls = .18 m.

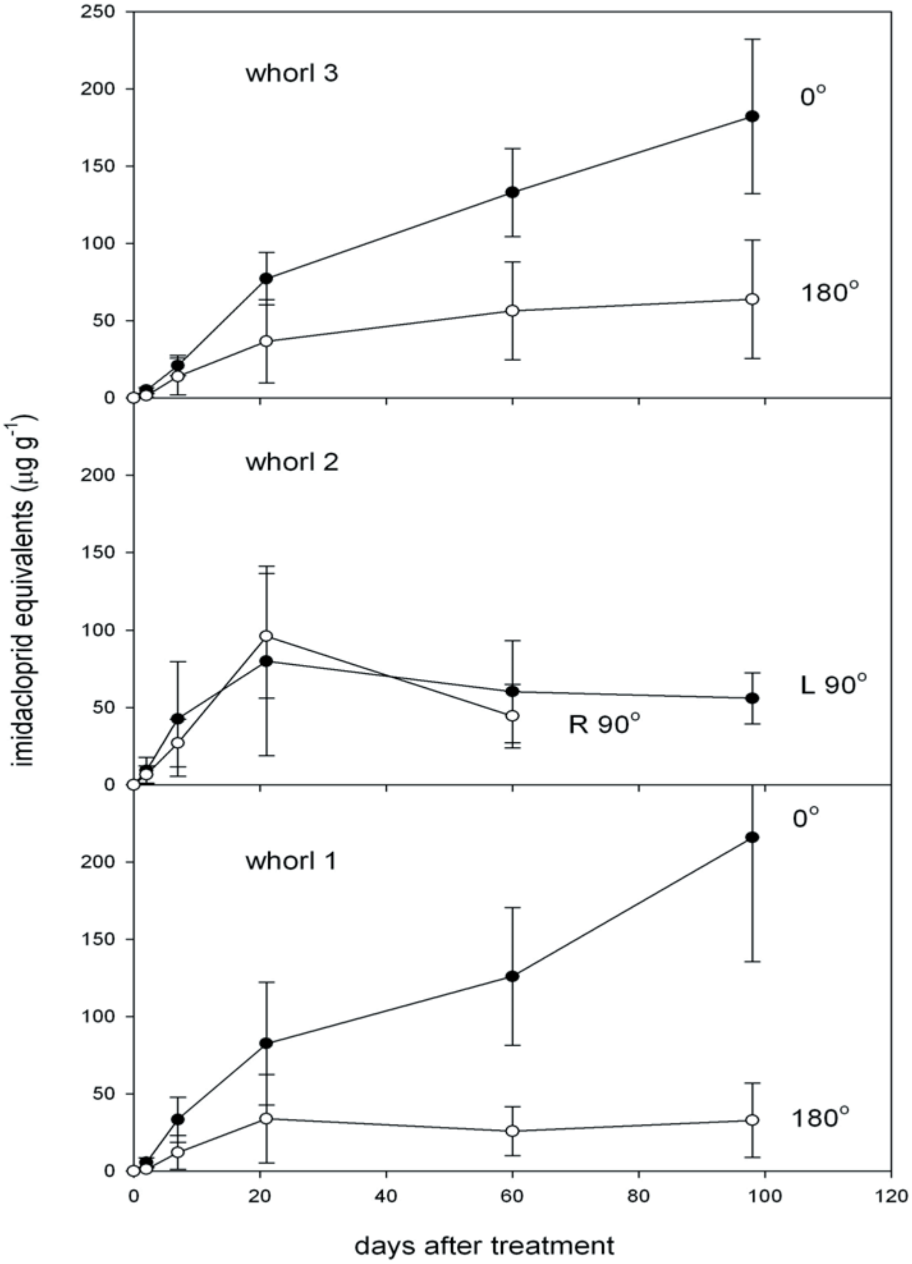

Figure 2. Concentration of Imidacloprid equivalents found in leaves of *Fraxinus americana* trees injected at 0° to the first whorl of branches.

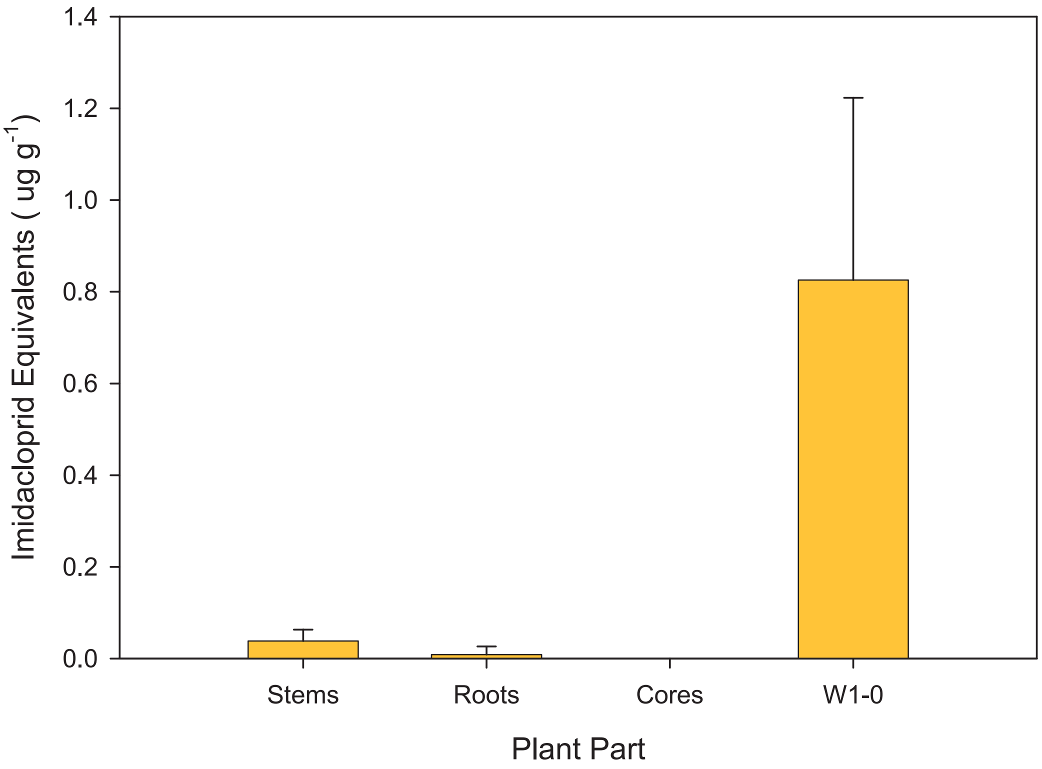

Figure 3. Imidacloprid equivalent concentration found in stems, roots, trunk cores and leaves (whorl 1, 0º) of *Fraxinus americana*, 60 days after trunk injection.

MORTALITY, FEEDING, AND BEHAVIOR OF MALE AND FEMALE EMERALD ASH BORER ADULTS IN RESPONSE TO INGESTION AND APPLICATION OF IMIDACLOPRID

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ABSTRACT

INTRODUCTION

Imidacloprid, a neonicotinoid insecticide, targets the insect nicotinic acetylcholine receptors. Trunk injection with imidacloprid is used to control the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). The effectiveness of trunk injection treatments is variable, however, suggesting that the chemical may not be completely or uniformly distributed within the tree. As a continuation of *The distribution of trunk-injected ^{14}C-imidacloprid in Fraxinus spp.: A test of the sectored-flow hypothesis* (see Tanis et al., 2006 EAB meeting abstracts), we determined the efficacy of imidacloprid through ingestion of treated leaves in male and females of EAB, estimated the feeding consumption for both sexes, and studied the behavior of intoxicated adults when they are exposed to treated leaves. In addition, we determined the toxicity of imidacloprid to male and female adults of EAB in oral bioassays and evaluated the efficacy of other neonicotinoids in adults of EAB by leaf dip bioassays.

METHODS

Trunk injection of ^{14}C-imidacloprid mixed with imidacloprid was conducted according to Tanis et al. (2006 EAB meeting abstracts). To determine the efficacy of injected imidacloprid, fresh leaves were collected 2, 10, and 57 days after treatment (DAT). Leaves were collected from the first and third whorls of each stem (see Tanis et al. 2006). Leaf stems were placed in water-filled vials. The vials were positioned inside cups containing four adult male or female EAB. Mortality, assessed as knock down and dead beetles, was recorded 24, 48, and 72 hours after treatment. Intoxicated beetle behavior was observed by exposing a single male or female adult beetle to leaves at 10 DAT. Elements of behavior including feeding, walking, stationary, grooming, vomiting, pumping abdomen, opening the wings, excretion, flight, and legs twitching were recorded using the program Observer.

For oral bioassays, imidacloprid was dissolved in dimethyl sulfoxide (DMSO), and serial dilutions were made with water. A volume of 0.5 µl of the insecticide solution was placed in the mouth of adults using a pipette. Four adults were treated per dose. At least seven doses and six replications were performed. The mortality was evaluated at intervals from 2 to 72 hours. Probit analysis was conducted on the 72 h treatment.

To evaluate the efficacy of neonicotinoids (imidacloprid, thiamethoxam, chloathianidin, dinotefuran, thiacloprid, and acetamiprid), leaf petioles were dipped in 20 ml vials containing insecticide solutions (0.1, 1, 10, and 100 ppm). The leaf-containing vials were placed in cups along with four male or female beetles. Mortality was evaluated 48 hours after treatment.

RESULTS

The position of the branch did not affect the toxicity of imidacloprid, as leaf uptake of the compound occurred quickly even in whirl 3 branch (Figure 1). Residues of imidacloprid and metabolites resulted in 80 percent mortality of adult EAB 2, 10, and 57 DAT (Figure 2). Females consumed more foliage than males in the control and the treated leaves collected 2 and 10 days after treatment (Figure 3). This higher amount of foliage consumption in females is important because they are less susceptible to imidacloprid than males (Figure 4). Therefore, compensation for higher feeding resulted in equal efficacy in males and females treated with leaves collected from the field. Toxicity of imidacloprid was greater in the females than males, as presented in Figure 4. The oral LD50 for female was 8.6 ng in comparison with 2.4 ng for males. The difference in weight males (29 mg) versus females (44 mg) may be one of the factors for reduced susceptibility of females to imidacloprid.

The sequence of behavior of females when exposed to imidacloprid treated leaves is presented in Figure 5. Symptoms of intoxication were observed at 35 minutes after exposure. This delay in toxic effects in the treated leaves may be due to slow release of imidacloprid in chewed foliage inside the insect gut. In contrast, immediate imidacloprid toxicity effects were observed in beetles when exposed to the insecticide in oral bioassays.

Efficacy of neonicotinoids by leaf bioassays showed high percent of efficacy (88 to 100%) in the concentrations 1, 10, and 100 ppm, except thiacloprid, which resulted in a mortality of 50 percent of males 48 hours after treatment.

FUTURE RESEARCH

We have preliminary toxicological data on the effect of imidacloprid metabolites in adult EAB. Topical applications of the olefine and 5-OH-imidacloprid resulted in high mortality of adult EAB. Currently, we are performing oral bioassays with the olefine, 5-OH-imidacloprid, and four other insect and plant transforming metabolites of imidacloprid to get a more complete representation of the toxicity data. In addition, we will conduct the pharmacokinetics and metabolism of 14C-imidacloprid in the adults of EAB to obtain insight on the conversion of imidacloprid to metabolites inside the insect body and the rate of excretion of parent imidacloprid.

Figure 1. Autoradiogram of ash leaves from whirl branch 3. The leaves were collected two days after ^{14}C-imidacloprid injection and exposed to a phosphor screen for 24 hours.

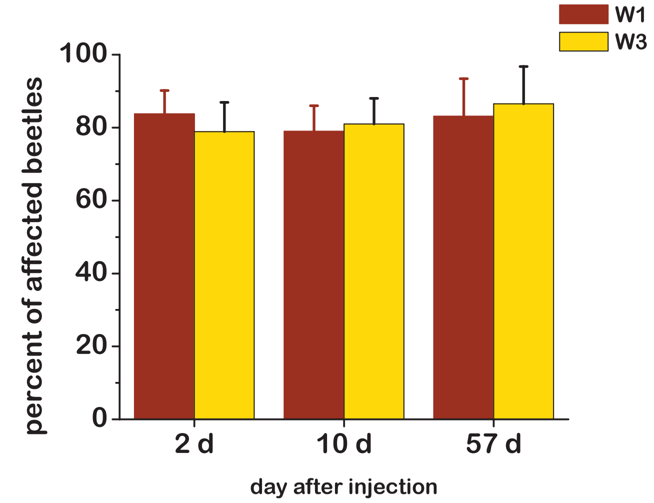

Figure 2. Efficacy of imidacloprid treated leaves from two different positions (w1= lower branch, w3=middle branch at different dates after injection.

Figure 3. Feeding of adults of EAB in untreated and treated ash leaves with ^{14}C-imidacloprid + imidacloprid.

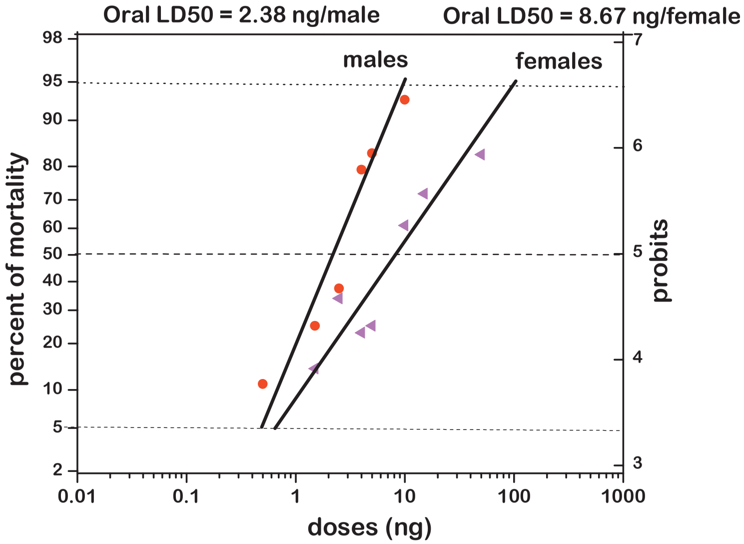

Figure 4. Log doses mortality lines of adults of EAB to oral treatments of imidacloprid.

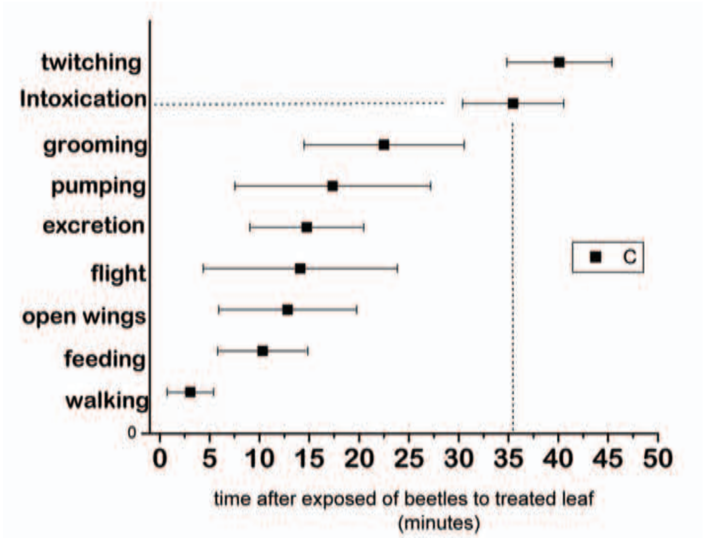

Figure 5. Sequence of elements of behavior of females of EAB after being exposed to imidacloprid-treated leaves.

UPDATE ON COMPARISON OF INSECTICIDES FOR EMERALD ASH BORER CONTROL

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ABSTRACT

From 2004 to 2006 six neo-nicotinyl insecticides and one bio-pesticide (Tables 1, 2, and 3) were tested for their efficacy against the emerald ash borer, *Agrilus planipennis* Fairmaire. All results are preliminary, and mention of a product, trade name, or company does not imply the endorsement of the authors or their affiliated governmental agencies or institutions.

The test site was located in Tianjin, China, and contained velvet ash, Fraxinus velutina Torr. The average diameter at breast height (DBH) of these trees was about 10 cm.

Parameters that were considered for comparing these insecticide treatments include (1) number of dead beetles under trees in each treatment, (2) adult mortality of caged beetles in flight season, (3) larva density and mortality of beetles counted by dissecting sample sections of tree trunk, and (4) level of insecticides in treated trees post-application. Here, we only report the number of dead beetles under treated trees for all treatment.

RESULTS

For all tests in three years, dinotefuran applied through by injection (either using the tree I.V. micro-infusion system or Mauget Generation II capsule) resulted in the highest number of dead EAB adults under treated trees (Figures 1, 3, and 5) immediately following the application. However, one year post application, the number of dead beetles collected under treated trees was much higher for trees treated with imidacloprid through soil basal injection than tress treated with other insecticides or treated with imidacloprid through trunk injection (Figures 2 and 4).

SpinTor application by cover spray did not result in high numbers of dead beetles; one of the reasons might be that we only spayed a 2-m section of tree trunk (with some twigs and leaves). The solution we obtained for acetamiprid was not suitable for trunk injection using the modified Arborjet system; therefore, after applying it to 15 trees, we had to apply it to the other 15 trees though soil drench. In most cases, the number of dead EAB adults under trees treated with acetamiprid, clothianidin, and thiacloprid did not differ from that under the untreated trees. More data are being collected and analyzed.

ACKNOWLEDGEMENTS

We thank the following people and companies for providing help or products: Mr. David Cowan (USDA APHIS Otis Laboratory), Dr. Wenxia Zhao (Chinese Academy of Forestry), Tianjin Guangang Afforestation Management Bureau; and Valent U.S.A. Co., Creative Sales Inc., J.J. Mauget Co., Arborjet Inc., Syngenta Crop Protection Inc., and Veyong Bio-chemical Co. Ltd.

Table 1. Emerald ash borer insecticide treatment in June 2004.

Insecticide	Delivery Method (abbreviations in parentheses)	Formulation
Imidacloprid	Tree I.V. micro-infusion system (Imid I.V.)	10% solution
Imidacloprid	Soil Injection: Basal Injection (Imid Soil)	Merit 2 (21% A.I.)
Imidacloprid	Trunk Injection: Mauget Gen II Capsule (Imid Mauget)	10%, 4 ml Solution
Dinotefuran	Tree I.V. micro-infusion system (Dino I.V.)	10% Solution
Dinotefuran	Soil Injection: Soil Basal Injection (Dino Soil)	Safari 20 SG%
Clothianidin	Tree I.V. micro-infusion system (C_I.V.)	10% solution
Control	Not treated (CK)	

Table 2. Emerald ash borer insecticide treatment in June 2005.

Insecticide	Delivery Method (abbreviations in parentheses)	Formulation
Imidacloprid	Soil Injection: Basal Injection (Meri75Soil)	Merit 75 WP
Imidacloprid	Trunk Injection: Mauget Gen II Capsule (Imicide)	10%, 4 ml Solution
Dinotefuran	Trunk Injection: Mauget Gen II Capsule (Dino_Mauget)	10%, 4 ml Solution
Thiacloprid	Trunk Injection: Modified Arborjet with USDA Tip (Thia_Arbo)	10% solution
Acetamiprid	Trunk Injection: Modified Arborjet with USDA Tip (Acet_Arbo)	10% solution
Acetamiprid	Soil Drench (Aceta_Soil)	10% solution
Spinosad	Cover spray (SpinTor)	SpinTor® 2 SC Naturalyte®
Control	Untreated (CK)	

Table 3. Emerald ash borer insecticide treatment in June 2006.

Insecticide	Delivery Method (abbreviations in parentheses)	Formulation
Dinotefuran	Trunk Injection: Mauget Gen II Capsule (Dino_Mauget)	X-5D25A
Dinotefuran	Soil Injection: Basal Injection (Dino_BSoil)	Safari 20 SG%
Thiamethoxam	Soil Injection: Basal Injection (Thiam_SoilBasal)	Flagship 25WG
Emamectin	Trunk injection (Ememectin_trunk)	62.4% WP by Veyong, China
Emamectin	Cover spray (Ememectin_Spray)	1% EC by Veyong, China
Control	Untreated (CK)	

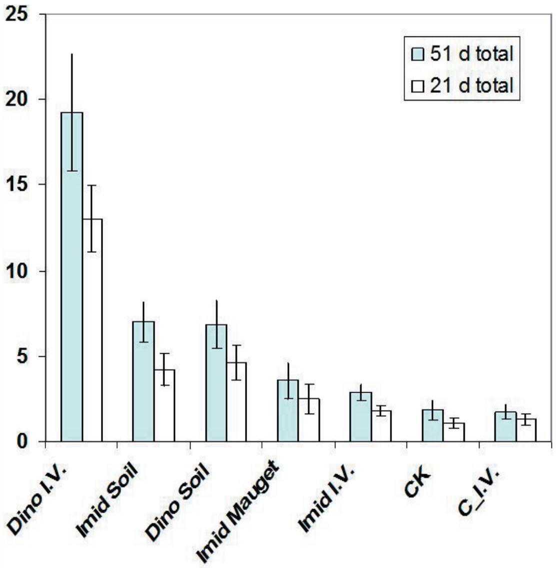

Figure 1. The number of dead emerald ash borer adults collected under trees in 2004 (collection started three days post application and for a total of 21 days and 51 days.

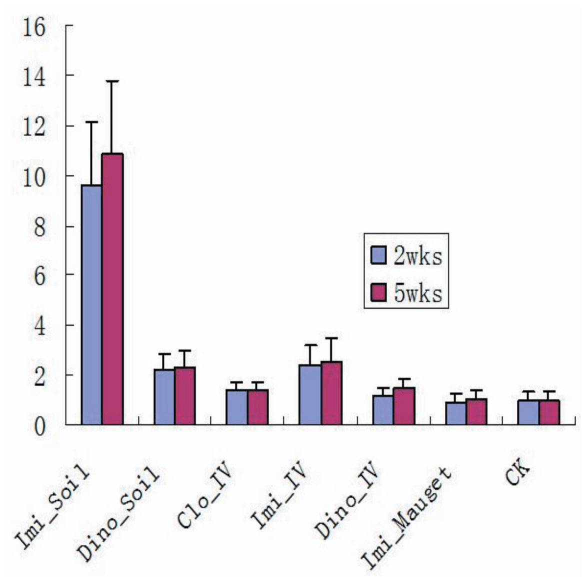

Figure 2. The number of dead emerald ash borer adults collected in May and June 2005 under trees treated in May 2004 (collection started 5/23/2005, and ended in 6/26/2005).

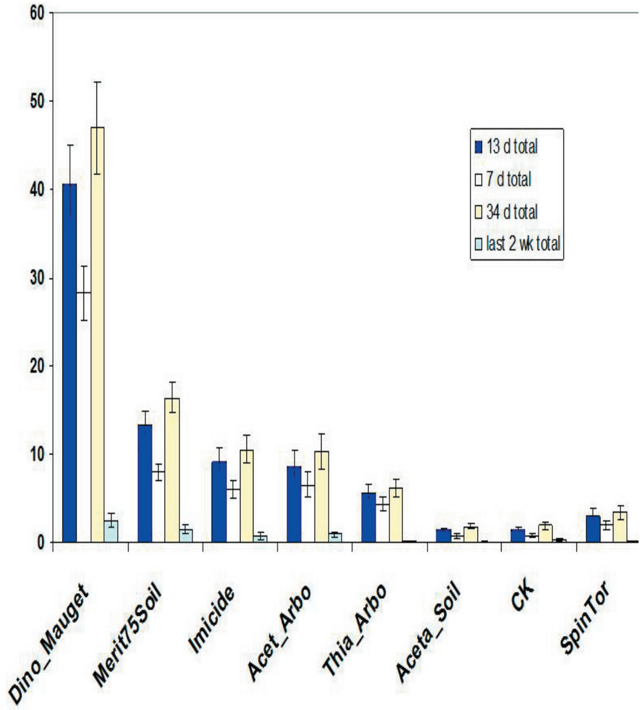

Figure 3. The number of dead emerald ash borer adults collected under trees in 2005 (collection started three days post application and for a total of 7 days, 13 days, 34 days, and June 11-June 24 (last 2-week total).

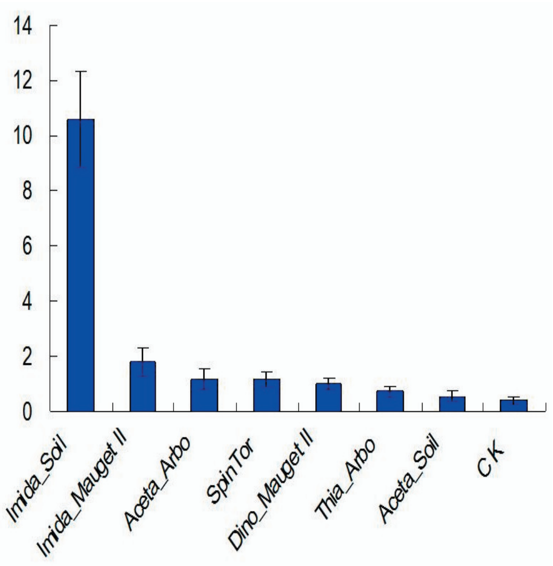

Figure 4. The number of dead emerald ash borer adults collected from 5/23/2006 to June 28, 2006 under trees treated in May 2005.

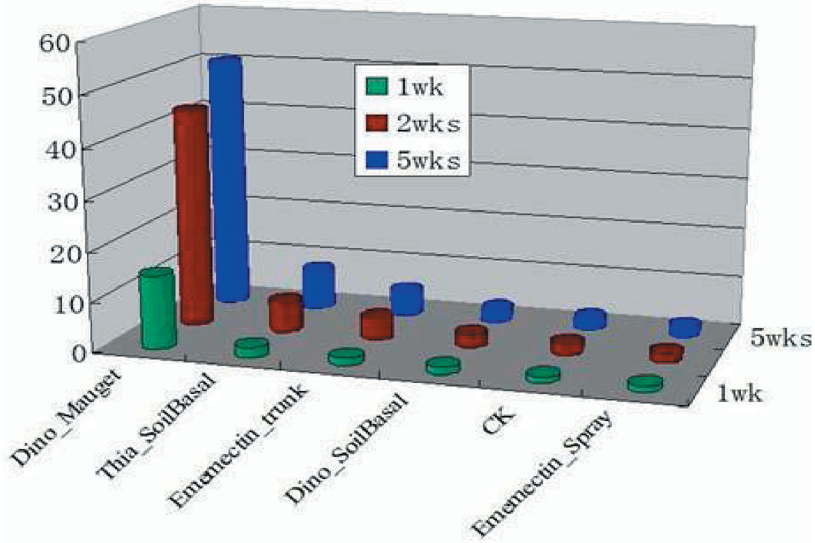

Figure 5. The number of dead emerald ash borer adults collected under trees in 2006 (collection started three days post-application and for a total of 1 week), 2 weeks (not labeled), and 5 weeks.

IMIDACLOPRID BASAL SOIL DRENCH FOR PROTECTION OF ASH TREES FROM EMERALD ASH BORER

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ABSTRACT

Ash trees at five heavily infested locations in Michigan were treated with a basal soil injection or basal soil drench of imidacloprid. A description of the ash trees and treatments at each site is in Table 1.

An imidacloprid basal soil drench applied at the label rate in May or early June each year for two years gave 95 percent control or better at the Westland and Novi sites, even in small trees (less than 6 inches DBH) heavily infested and damaged before imidacloprid was applied. All of the treated trees were healthy at the end of the test.

At sites with larger trees (8-35 inches DBH) an imidacloprid basal drench did not provide good protection the first year, but gave a high level of control in the second and third year of treatment. After 2.5 years at the Orchard Lake and Ann Arbor golf course sites, half of the treated ash trees average 60 percent dieback or less and are improving in condition compared with close to a 100 percent loss of control trees. It is possible that a higher proportion of treated trees could be saved if basal drenches are started a year or two earlier, when trees are still healthy.

The imidacloprid product available to homeowners as a basal soil drench is Bayer Tree and Shrub Insect Control, while the product used by tree care professionals as a basal soil injection is Merit 75 WP.

Table 1. Research sites for imidacloprid basal drench study, 2003-2006.

City	Site	Number of treated trees	Number of control trees	Tree size (dbh)	Dieback at start of test
Westland	Nursery	70	70	3-5″	0%
Novi	Research farm	60	60	2-3″	20-80%
Orchard Lake	Golf course	21	23	10 - 30″	40%
Ann Arbor	Golf course	15	18	8 - 35″	20 - 80%
Troy	Street trees	10	10	12 - 20″	40%

EVALUATION OF INSECTICIDE PRODUCTS FOR CONTROL OF EMERALD ASH BORER

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ABSTRACT

‘Marshall Seedless’ green ash trees growing between the sidewalk and street in a neighborhood in Troy, Michigan, were used for this test. These trees were between 12 and 26 years old and ranged in size from 7-24 inches diameter at breast height (DBH). Trees were spaced a minimum of 50 feet apart. Lawns in the neighborhood were well maintained, but very few had an irrigation system. The trees were grouped into 10 treatment blocks with one tree receiving each treatment in each block. Each treatment was replicated 10 times, with each replicate consisting of an individual tree. The treatments were:

1. **IMA-jet trunk injection.** A solution of 5 percent imidacloprid with 5 percent ArborJet Aqueous Dilutant (AAD) made in 2004 was injected with the Arborjet Tree IV system at 35 PSI. No additional treatment was made in 2005.
2. **Onyx 13 oz., twice.** Foliage and branches were sprayed with 13 oz./100 gal. of Onyx in early and late June in 2004 and 8 oz./100 gal. in early and late June of 2005.
3. **IMA-jet trunk injection-** A solution of 5 percent imidacloprid solution was injected at a rate of 4.0 ml per inch of DBH for trees with a DBH of less than 12.0 inches, while larger trees received 8.0 ml per inch of DBH. Injections were made with an ArborJet Air Hydraulic VIPER apparatus. The injection pressure was set at 125 PSI.
4. **Onyx 32 oz., once.** Foliage and branches were sprayed with 32 oz./100 gal. of Onyx in early June in 2004 and 16 oz./100 gal. in early June of 2005.
5. **ACECAP implant.** A single cap containing 0.875 g acephate was implanted every 4 inches around the base of the tree on 4 May 2004 and 3 May 2005.
6. **BotaniGard, twice.** BotaniGard (11.3 percent *Beauveria bassiana* Strain GHA) was mixed at a concentration of 6 qt./100 gal. The entire canopy, trunk, and branches were sprayed in early and late June of 2004 and 2005. Each tree received 16-20 gal. of spray solution, in relation to its size.
7. **Untreated control.**

Test results are presented in Table 1.

Table 1. Control of emerald ash borer in Troy, Michigan, with insecticide treatments in 2004 and 2005.

2004 treatment	Larvae/m² Oct. 2004	2005 treatment	% dieback June 2005	Larvae/m² Oct. 2005	% dieback June 2006
IMA-jet injection	0.0*	None	24.3*	15.2	48.0
Onyx 13 oz, twice	3.1*	Onyx 8 oz, twice	30.0*	39.9	48.1
IMA-jet injection	1.2*	None	21.1*	24.3	59.4
Onyx 32 oz, once	1.9*	Onyx 16 oz, once	38.9	22.0	65.8
ACECAP implant	2.8*	ACECAP implant	27.5*	37.4	81.0
BotaniGard, twice	4.7	BotaniGard, twice	35.6	75.7	86.0
Untreated control	10.1	None	50.3	65.4	93.1

* Indicates treatment mean is different from control mean at P = 0.05

EFFECTS OF TRUCK INJECTION ON EMERALD ASH BORER DENSITY AND ASH SURVIVAL: A FOUR-YEAR STUDY

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ABSTRACT

When emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, was first discovered in the U.S., development of effective control methods was identified as a critical short-term research priority. We initiated studies to determine the efficacy of available insecticide products for controlling EAB larvae and adults. We developed bioassay methods to evaluate mortality of adult beetles feeding on leaves from our study trees. We also developed methods to quantify density of emerged beetles and feeding larvae. These measures yield results within one growing season, provide a means to compare treatments, and are indicative of potential product efficacy. Ultimately, a treatment is only effective, however, if it prolongs the life and preserves the aesthetic value of treated trees for a significant period of time (i.e., several years). In this study, we evaluated the survival of white and green ash treated annually for four years with trunk injections of imadacloprid products or with bidrin.

SETTING AND TREATMENTS

Our studies began in 2003, with nearly uninfested street trees in two Ann Arbor, Michigan, neighborhoods. The Forsythe neighborhood included both white ash (*F. americana*) and green ash (*F. pennsylvanica*); mean DBH was 30 and 38 cm, respectively. Ash trees in Forsythe were not abundant and were generally widely spaced; we estimated that there was ca. 1000 m^2 ash phloem per km^2. We selected 18 green ash and 18 white ash in 2003 and randomly assigned them to one of two imidacloprid treatments or to be left as untreated controls. Trees were trunk-injected in mid-May of each year from 2003 to 2006. Trees were treated with either Imicide (10% solution, one 3-ml Mauget capsule per 5 cm DBH) or Pointer (12% solution in 2003 and 5% solution in 2004-2006; 1 ml per 10.2 cm basal circum injected with a Wedgle injector) (n = 6 of each species per treatment).

In the Dartmoor neighborhood, green ash trees were abundant and larger than in the Forsythe neighborhood. We estimated that there was ca. 33,000 m^2 phloem per km^2 in this neighborhood and mean DBH was 42 cm Treatments were assigned randomly to one of four treatments or left as untreated controls (n = 6 green ash per treatment). Treatments evaluated included trunk injections of Imicide applied with Mauget capsules (rate as above), Pointer applied with a Wedgle, or bidrin (dicrotophos) applied with Mauget capsules (82% Inject-A-Cide B formulation; one 2 ml Mauget capsule per 5 cm DBH). We tested two timings for bidrin trees: 'Early' bidrin trees were treated in mid-June to target adult EAB, while 'Late' bidrin trees were treated in mid-July to target young larvae. Imidacloprid treatments were applied in mid-May of each year.

SAMPLING

We evaluated EAB density every fall from 2003 to 2006 by counting cumulative D-shaped exit holes left by emerging adult EAB and woodpecker attacks on the trunk and in the canopy of each tree. Woodpecker attacks were included because woodpeckers prey primarily on overwintering prepupae that have completed feeding and would have a high probability of emerging as adults in spring. We sampled EAB density on at least five stem or limb sections distributed from 1.5 m aboveground on the trunk to the top-most 8 cm-diameter limbs. Canopy condition was evaluated as percent dieback in July or August of each year. Our intention was to track survival and monitor EAB density and canopy for all trees in each treatment. However, in winter of 2006, the city of Ann Arbor required the removal of conspicuously damaged trees; thus, our final mortality tally includes trees that either died or exceeded 40 percent dieback in the fall of 2005.

EAB DENSITY

For green ash at Forsythe (Figure 1), EAB density in trees injected with either Pointer (via the Wedgle) or Imicide (via Mauget capsules) was significantly lower than in untreated control trees in 2004 and 2005. By 2006, all of the untreated control trees, and all but one of the trees injected with Pointer had been removed by the city due to advanced canopy decline. In 2006, larva density in the four remaining trees treated with Imicide had stabilized at a level that was less than 20 percent of the maximum density in untreated control trees.

In the white ash at Forsythe (Figure 2), larva density was generally lower in the Imicide/Mauget trees than in controls or Pointer/Wedgle trees in 2006, even though treatments did not significantly differ in any year.

At the Dartmoor site, EAB density in trees treated with insecticides did not differ from that of control trees in any year. However, when all treated trees were considered together, EAB density was less than half that in untreated control trees in 2005 (Figure 3).

CANOPY DIEBACK AND SURVIVAL

For green ash at Forsythe, mean canopy dieback remained below 25% through 2006 for the trees injected with Imicide/Mauget and 80% of those trees that survived to summer 2006. Dieback for trees injected with Pointer/Wedgle was lower than for untreated control trees through 2005. By 2006, however, all Pointer-treated and control trees had less than 40 percent dieback Table 1).

For the white ash at Forsythe, canopy dieback in trees injected with Imicide/Mauget did not differ significantly from Control trees, but 60 percent of the Imicide/Mauget trees were alive in 2006 compared to 33 percent survival of control trees. None of the Pointer-treated trees survived to 2006.

In the Dartmoor site, none of the trees injected with Pointer/Wedgle or the untreated controls survived to 2006. For all trees injected with Imicide/Mauget or Bidrin/Mauget, mean canopy dieback in 2006 was slightly less than 50 percent. Rate of tree survival was 50 percent for Imicide/Mauget, 33% for the Early injection of Bidrin/Mauget and 50 percent for the Late injection of Bidrin/Mauget.

SUMMARY

Our results, spanning four years, represent the longest evaluation of the efficacy of trunk injections for EAB control and illustrate how rapidly the density of EAB can build. The trunk injection treatments did reduce EAB density relative to untreated controls in the green ash at Forsythe and Dartmoor.

In terms of long-term tree survival and condition, however, our results are somewhat disappointing. None of the trees injected with the Pointer/Wedgle at the Forsythe or Dartmoor sites survived to 2006.

The green ash trees injected with Imicide/Mauget at Forsythe exhibited relatively low dieback, and 80 percent of these trees survived to 2006. Average dieback for the other trees injected with Imicide/Mauget, Bidrin/Mauget–Early or Bidrin/Mauget-Late ranged from 45 to 50 percent in 2006, and 33 to 50 percent of those trees survived to 2006.

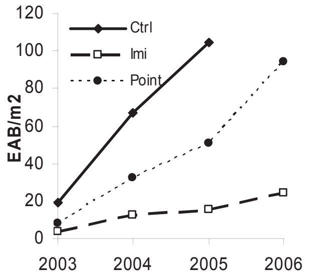

Figure 1. Emerald ash borer density (cumulative number of exit holes and woodpecker attacks per m^2) in green ash trees injected with Imicide/Mauget (Imi) or Pointer/Wedgle (Point) or untreated controls (Ctrl) Forsythe site, 2003-2006.

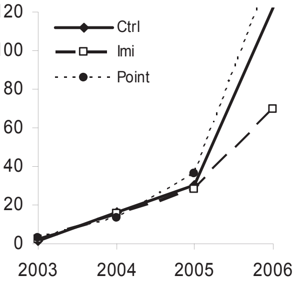

Figure 2. Emerald ash borer density (cumulative number of exit holes and woodpecker attacks per m^2) in white ash trees injected with Imicide/Mauget (Imi) or Pointer/Wedgle (Point) or untreated controls (Ctrl) Forsythe site, 2003-2006.

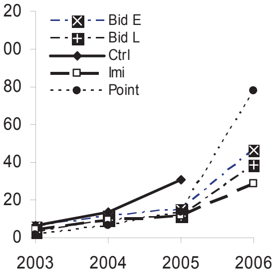

Figure 3. Emerald ash borer density (cumulative number of exit holes and woodpecker attacks per m2), in white ash trees injected with Bidrin Early (Bid E), Bidrin Late (Bid L), Imicide/Mauget (Imi) or Pointer/Wedgle (Point), or untreated controls (Ctrl), Dartmoor site, 2003-2006.

Table 1. Percent canopy dieback and survival (defined as percent of trees with less than 40 percent dieback) for green or white ash trees treated with bidrin/Mauget-Early (Bid E), bidrin/Mauget-Late (Bid L), Imicide/Mauget (Imi/Mauget) or Pointer/Wedgle (Pointer), or left as untreated controls at the Forsythe and Dartmoor neighborhoods in Ann Arbor, Michigan.

		Mean % dieback		**% Survival**	
Dataset	**Treatment**	**2005**	**2006**	**2005**	**2006**
Forsythe-green ash	Control	82	--	0	0
	Imi/Mauget	22	14	83	80
	Pointer/Wedgle	44	86	43	0
Forsythe-white ash	Control	9	52	100	33
	Imi/Mauget	19	50	86	60
	Pointer/Wedgle	18	80	100	0
Dartmoor-green ash	Control	51	--	17	0
	Imi/Mauget	14	45	83	50
	Pointer/Wedgle	25	82	50	0
	Bidrin/Mauget Early	28	49	67	33
	Bidrin/Mauget Late	20	45	100	50

EVALUATION OF NEO-NICOTINOID INSECTICIDES APPLIED AS NON-INVASIVE TRUNK SPRAYS

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ABSTRACT

Neo-nicotinoid insecticides such as imidacloprid and dinotefuron are widely used in southeastern Michigan to protect ash (*Fraxinus* sp.) shade trees from emerald ash borer (EAB), *Agrilus planipennis* Fairmaire. These products are relatively safe for humans and have few non-target effects. Trees are generally treated annually by injecting the insecticide into the soil or into the trunk. The product is translocated to the canopy, where it affects adult beetles during the summer. Questions and 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 movement of soil-injected products into soil or ground water at some sites.

We evaluated a non-invasive, efficient, and simple method of applying imidacloprid or dinotefuron to the trunk of ash shade trees. This application method involves mixing the insecticide with Pentra-Bark®, a non-toxic, bark-penetrating surfactant (Agrichem, Medina, Ohio). The formulated solution is applied directly to the bark on the lower trunk of a tree with a common garden sprayer. Pentra-Bark®, originally used as an agricultural surfactant, has recently been shown to carry fungicide products through the bark and into the xylem tissue of fruit trees, where the product is then translocated to the canopy. In our study, we sprayed the bark on the trunks of the trees until they were wet from 20 cm to 1.6 m aboveground.

The randomized complete block design was replicated at four sites to ensure that we evaluated a range of tree size and bark thickness. Mean diameter at breast height (DBH) of trees used in the study ranged from 5.0 inches at the site with the smallest trees to 15.5 inches at the site with the largest trees. There were six to 12 trees per treatment at each of the four sites. Each block consisted of trees treated with a non-invasive trunk spray of (1) imidacloprid + Pentra-Bark®, (2) dinotefuron + Pentra-Bark®, (3) a soil application of imidacloprid applied at the base of the tree with a Davey wand, (4) a trunk injection of imidacloprid applied with Mauget capsules (e.g., a positive control), or (5) left as an untreated control (Table 1). Dinotefuron treatment was not included at one site. Data were analyzed with analysis-of-

variance or the nonparametric equivalents and multiple comparison tests (when ANOVA results were significant) to assess effects of treatment and site ($P<0.05$).

To evaluate the effectiveness of the insecticide treatments, we measured residues in leaves collected from each tree in mid-June, early July, late July, and mid-August using HPLC (for dinotefuron) or ELISA (for imidacloprid). Dinotefuron levels in foliage peaked in mid-June at all three sites. Average concentrations exceeded 2.0 ppm at two sites and levels exceeded 5.0 ppm at the site with the smallest trees. Dinotefuron residues dropped relatively quickly to roughly 1.0 ppm at two sites and 2.0 ppm at the site with the small trees by early July. Imidacloprid residues have been determined for mid-June and early July only; other samples are still being processed. Trees treated with Mauget capsules generally had the highest imidacloprid residues; on average, residues averaged at least 5 ppm compared to average residues of 1.0 to roughly 3.0 ppm in trees treated with soil application or the trunk spray. Residue levels in Mauget trees were similar in mid-June and early July, while residue levels in the other treated trees increased from mid-June to early July. Residue levels were substantially higher at the site with the smallest trees compared to residues in larger trees at the other sites.

Bioassays were conducted in mid-June, early July and late July to assess survival of EAB adults 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 mid-June, EAB survival on leaves from Mauget-treated trees was significantly lower than EAB survival on other trees on Day 1 of the bioassay. By Day 4, EAB survival on leaves from all treated trees was significantly lower than survival on the untreated control trees. In early July, survival was monitored daily. Survival of EAB on trees treated with dinotefuron + Pentra-Bark® was significantly lower than on the untreated control trees on Day 1, and survival on all treated trees was lower than survival on untreated controls on Days 2-4. In the late July bioassay, EAB survival on trees treated with dinotefuron + Pentra-Bark® was significantly lower than survival on all other trees on Day 1. By Day 4, survival on all treated trees except the Mauget-treated trees was significantly lower than the untreated controls. We noted that 13 of the trees treated with the dinotefuron + Pentra-Bark® spray had leaf residues that exceeded 2.0 ppm; on 12 of those 13 trees, no EAB survived to Day 4.

Larva density was quantified beginning in late September and continuing into October using bark windows (>500 cm^2, each) excavated on the trunk and four to six locations in the canopy. Overall EAB larva density was low at the site with the largest trees (<10 EAB/m^2), and there were no significant differences among treatments. At a second site where only imidacloprid treatments were applied (no dinotefuron treatment), the EAB population was moderate. Larva density was highly variable, however, and between-treatment differences were not significant. At the third site, with a low to moderate EAB population, trees treated with dinotefuron + Pentra-Bark®, a soil application of imidacloprid, or the Mauget capsules had significantly lower larva density than untreated controls. Trees treated with the imidacloprid + Pentra-Bark® spray did not differ significantly from other treated trees or the untreated controls. On average, trees treated with the imidacopride + Pentra-Bark® trunk spray, the soil application of dinotefuron + Pentra-Bark®, or the Mauget capsules had 50 percent, 71 percent, 75 percent and 81 percent fewer larvae per m^2, respectively, than untreated controls. Density of EAB at the site with the smallest trees was very low; these trees were not debarked and will likely be used in 2007 studies.

Our results indicate that insecticides penetrated the bark and moved into the vascular tissue of trees treated with trunk sprays of imidacloprid + Pentra-Bark® and dinotefuron + Pentra-Bark®. Thus, the insecticides could effectively translocate to the canopy. Foliar imidacloprid residues on trees treated with the non-invasive trunk spray were similar to residues in trees treated with a soil application or a trunk injection using Mauget capsules. Dinotefuron, which is highly soluble in water, appeared to translocate relatively rapidly into the canopy. Residue levels were substantially lower in early July than in mid-June, however, suggesting that the product may break down relatively quickly. Foliar imidacloprid residues were highest in mid-June and early July in trees treated with the trunk injection (Mauget capsules). Residue levels in other imidacloprid trees increased from mid-June to early July, suggesting that the product may have still been moving into the canopy or foliage. In bioassays, EAB mortality was significantly higher when adults were fed leaves from trees treated with either imidacloprid or dinotefuron compared to untreated control trees. Adult mortality on Day 4 of the bioassays ranged from 34-61 percent among imidacloprid-treated trees and 62-82 percent on dinotefuron trees. Larva density was highly variable within and among trees at all sites. Generally, larva density was lower on treated than untreated trees, but differences were significant at only one site. We plan to continue to evaluate the non-invasive application methods in 2007.

Table 1. Application rates and dates for imidacloprid (Imi) and dinotefuron (Dino) treatments applied to ash trees in 2006.

Treatment	Application method	Product	AI per inch DBH	Application date
Untreated controls	—	—	—	—
Trunk injection (positive control)	Mauget capsules (3 ml)	10% imicide	0.15 g	May 29
Soil application	Davey wand at base of tree	Macho 2F (21.4%)	1.42 g	May 22
Imi + Pentra-Bark® trunk spray	Ganden sprayer; 3.2 fl oz/inch DBH	Macho 2F (21.4%) + 3 oz Pentra-Bark®/gal	1.70 g	May 22
Dino + Pentra-Bark® trunk spray	Garden sprayer; 3.2 fl oz/inch DBH	Safari (20%) + 3 oz Pentra-Bark®/gal	1.70 g	May 22

BIOPESTICIDES FOR EMERALD ASH BORER

USE OF *BEAUVERIA BASSIANA* AND IMIDACLOPRID FOR CONTROL OF EMERALD ASH BORER IN AN ASH NURSERY

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ABSTRACT

We wish to determine the potential of *Beauveria bassiana* (strain GHA), alone or in combination with imidacloprid, for control and management of emerald ash borer (EAB), *Agrilus planipennis*. We have undertaken this work at a commercial tree nursery in southern Michigan within the EAB-infested area. Approximately 400 *Fraxinus pennsylvanica* and *F. americana* (height, ca. 5-6 m, diameter, ca. 8-12 cm) in three planting areas were arrayed in a randomized complete block design. Treatments consisted of fungus alone, imidacloprid alone at two rates, fungus plus the low rate of imidacloprid, and a formulation blank as control. Imidacloprid (Bayer Advanced Tree & Shrub Insect Control) was applied as an early season drench in late May, and the fungus (BotaniGard ES, Laverlam International) and formulation blank were applied with a hydraulic sprayer three times at biweekly intervals in June and July. We monitored spore deposition by washing leaves and bark samples and using dilution plate counting. We estimated spore persistence on leaves for up to 6 weeks after the final spray and we will continue monitoring survival on bark and in soil throughout the study.

We counted EAB emergence holes up to 2 m high on each tree. We observed emergence from only trees, but observed beetle activity within our plots and on ash trees nearby. We determined that up to four genotypes of *B. bassiana* were present in soil before any spray treatments and that none of them was the strain GHA. After sprays, we readily reisolated straing GHA from leaves (up to 6 weeks post-spray), bark and soil. Adult EAB trapped within the plots were infected with the GHA strain. This was the first year of a multi-year study.

AERIAL APPLICATION OF SPINOSAD FOR EMERALD ASH BORER CONTROL IN WOODLOTS

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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 in low population numbers. Spinosad is a biological insecticide produced by a soil bacterium that has been shown to be toxic to the adult life stage of 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. It is moderately to slightly toxic to most fish and aquatic invertebrates, practically non-toxic to avian and mammalian species, and practically non-toxic 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 it has very low potential for run-off or leaching as it binds strongly to soil.

A test application of spinosad (GF-976, a formulation with 4 pounds of active ingredient per gallon) was conducted on isolated woodlots surrounded by agricultural fields; woodlots were chosen that had apparent but low levels of EAB infestation. The six treatment and six control lots ranged in size from 8 to 30 acres and were located in Shiawassee County, Michigan. Two applications were made on June 13 and 27 at a rate of 7.2 ounces of product in one-half gallon of water per acre. Rotary atomizers were used and the volume mean diameter (VMD) ranged from 130 to 145 µ. A Section 18 "emergency exemption" registration was granted by the EPA for the application as the existing label did not allow for use of spinosad in treatment against EAB or for an aerial application over forest stands.

Impact of the application on target and non-target insects was assessed and compared with results from the control plots. Foliage was collected for analysis of spinosad residue at

regular intervals, post-application. Twelve ash trees from each plot were felled 3-4 months post-application to assess the impact of the application on the larva population; eight branches from the upper crown of each tree were collected and examined.

Dead adult EAB were found under treated ash trees in the woodlots up to seven days post-application, with the number of adults collected declining over time. Treated foliage was collected from four treated plots and a control plot at Day 1 and Day 7 post-application and then exposed to adult EAB for five days in the laboratory. Mortality ranged from 50 to 100 percent for the newly treated foliage and 10 to 50 percent for field-aged foliage, which verifies that the insecticide breaks down over time. Preliminary information from insects collected in Malaise traps shows no demonstrable effect on non-target species as a result of the treatment (Figure 1).

Larva populations vary widely between all the plots sampled, and preliminary data is currently available for four of the control and three of the treatment plots (Table 1). Because of the wide variance between and within the two treatment groups, comparisons using the number of new galleries per tree is not meaningful, but an overall relative increase of the populations can be calculated (existing larvae/emerged adults). For the spinosad plots, the relative increase was 184 percent, and for the control plots, the increase was 413 percent. This indicates that larva populations are increasing almost 2½-fold faster in the control plots than in the plots treated with spinosad.

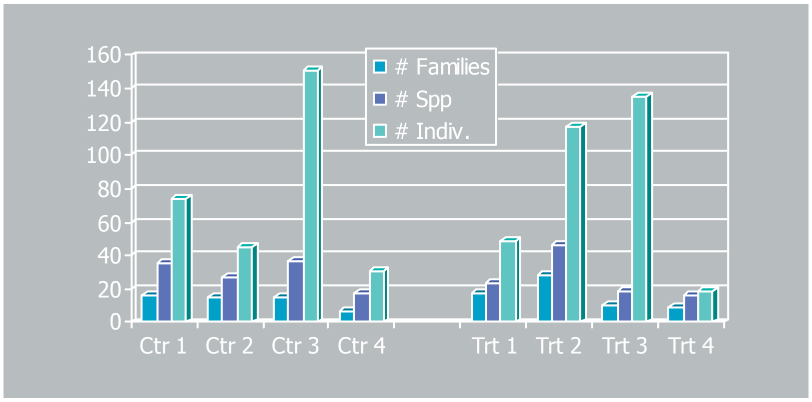

Figure1. Insects caught from 0 to 2 days after application (Lepidoptera, Hymenoptera, Coleoptera).

Table 1. Larva and adult populations in control and treatment plots.

Treatment	Exit Holes / Tree	New Galleries / Tree
Control Plots		
Plot 3	0	0.4
Plot 31	0	0.6
Plot 39	0.4	1.3
Plot 42	-	-
Plot 59	-	-
Plot 68	1.2	5.3
Treatment Plots		
Plot 1	0.2	0.5
Plot 2	-	-
Plot 8	0	0.6
Plot 61	-	-
Plot 64	-	-
Plot 74	5.3	9.6

BIOLOGICAL CONTROL OF EMERALD ASH BORER

HOST PREFERENCES OF CHINESE EMERALD ASH BORER PARASITOID WASP GENERA CURRENTLY BEING CONSIDERED FOR RELEASE IN NORTH AMERICA

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ABSTRACT

Three species of parasitic Hymenoptera reared from emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, in China are currently in quarantine and undergoing host range testing for possible release in North America. The three species parasitize different stages of EAB: *Oobius agrilus* Zhang & Huang (Encyrtidae) parasitize eggs; *Tetrastichus planipennisi* Yang (Eulophidae) parasitize early-instar larvae; and *Spathius agrili* Yang (Braconidae) parasitize late-instar larvae. All of these species were described as new to science within the last two years from recent field-collected material, with no previously collected material known. Based on literature searches for information on related species and consultation with systematists who are specialists in other members of these groups, there is limited information to estimate the host range of these three species. We feel confident, however, that they will be restricted to wood boring beetles.

Of the nine species of *Oobius* described, five were reared from eggs of Buprestidae, four of which were *Agrilus* species. One species reported as reared from robber fly eggs deposited on bark may be suspect. The subtribe Oobiina, based on *Oobius*, contains five genera, with the other genera reared only from wood boring beetle eggs. Members of the closely related genus *Avetianella* have only been reared from eggs of Cerambycidae and Buprestidae eggs. One species, *A. longoi* Siscaro, was shown to be highly specific to *Phoracantha semipunctata* F. (eucalyptus longhorned borer) when compared to *Phoracantha recurva* Newman, which feeds in the same host *Eucalytus* species. Luhring et al. (2000) studied ovipositional and developmental suitability of these congeners' eggs at different ages and maturity. Young eggs of both *Phoracantha* had similar rates of oviposition, but as eggs matured, *P. semipunctata* were significantly more preferred, and *P. semipunctata* were shown to be better hosts for the development of *A. longoi*.

Tetrastichus is a speciose genus with a highly diverse host range parasitizing a wide array of insect groups. Speaking broadly though, it is not unexpected that a *Tetrastichus* has been reared from EAB. Of the 57 chalcidoidea species that parasitize *Agrilus*, 24 are Eulophidae, and seven of these are *Tetrastichus* species, all from Eastern Europe.

Spathius is a genus composed of about 300 species, all of which we have host records for are wood boring beetle larva parasites. Nearly every species within its subfamily Doryctinae is a wood borer beetle specialist as well. The greatest species diversity of *Spathius* is in the Oriental-Australian region. In the much less specious region of North America, Matthews

(1970) showed that hosts of individual *Spathius* species are restricted to either wood borers of coniferous or deciduous trees. This might indicate olfactory cues are important within *Spathius* in initial host searching.

We are breaking new ground—thus far, successfully—with the discovery of these three EAB biocontrol candidates for release in North America. From the literature, there is scant information to develop a biocontrol program for an invasive buprestid beetle using natural enemies from its native range. The literature is useful in directing current host range and olfactometry testing, but in itself can only assist us in estimating potential nontarget effects for the EAB parasites.

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TETRASTICHUS PLANIPENNISIS (HYMENOPTERA: EULOPHIDAE), A GREGARIOUS LARVAL ENDOPARASITOID OF EMERALD ASH BORER FROM CHINA

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ABSTRACT

Agrilus planipennis Fairmaire, a buprestid native to Asia, was identified in 2002 as the causal agent of ash (*Fraxinus* spp.) decline and mortality in Michigan and Ontario. Since then, infestations have been found in Ohio, Indiana, Maryland, Virginia, and Illinois. Efforts to contain and eradicate this pest in North America are proving difficult due to the size of the infestation and lack of effective detection and control methods. As managers shift from a

policy of eradication to one of management, biological control may become an increasingly important method of controlling and slowing the spread of *A. planipennis*. With few natural enemies attacking this aggressive buprestid in North America, our research expanded to China where two new parasitoid species, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), were found attacking *A. planipennis* larvae and eggs, respectively (Liu et al. 2003; Bauer et al. 2006). In Jilin province field sites, where the distribution of these two parasitoids overlap, we estimate their combined impact resulted in ca. 74-percent reduction in local *A. planipennis* populations during 2005.

Tetrastichus planipennisi, a gregarious endoparasitoid of *A. planipennisi* third- and fourth-instar larvae, was discovered while surveying infested ash trees in Jilin and Liaoning provinces during 2003 (Liu et al. 2003) and later described (Yang et al. 2006). Field studies during 2005 in Jilin province showed parasitism rates by *T. planipennisi* increased from 16 percent in July to 40 percent in August, when the majority of host larvae were fourth instars. Each host larva produced an average of 35 parasitoids, with a range of five to 122; larger host larvae tended to produce more, but smaller, parasitoids. In the field, *T. planipennisi* completes at least four generations per year and overwinters as larvae.

We developed a standard rearing protocol for *T. planipennisi* in our containment room in Michigan using *A. planipennis* larvae dissected from infested ash logs and implanted in small ash branches. In the laboratory, *T. planipennisi* completes one generation in 20-25 days at 25ºC, and the average longevity for adults fed honey and water is 24 days for females and 14 days for males. The sex ratio for *T. planipennisi* is 3.5:1 (female:male).

We evaluated the host specificity of *T. planipennisi* using no-choice laboratory assays. In these assays, groups of female and male *T. planipennisi* were exposed to actively-feeding larvae of eight buprestids (*Agrilus anxius*, *A. bilineatus*, *A. ruficollis, A. subcinctus*, *A.* sp., *Chrysobothris femorata, C. floricola, C. sexsignata*), five cerambycids (*Neoclytus acuminatus, Megacyllene robiniae, Astylopsis sexguttata, Monochamus scutellatus, unknown sp.* in maple), or a sawfly (*Janus abbreviatus)* all implanted in small branches of their respective host plants. We also assayed larvae of a tenebrionid (*Tenebrio molitor)* and two lepidopterans (*Galleria mellonella, Manduca sexta*) by implantation in small ash branches; *Manduca sexta* larvae were also tested by exposure on tomato leaves. *T. planipennisi* rejected all species except actively-feeding *A. planipennis* larvae implanted in ash branches. These results suggest *T. planipennisi* is a good candidate for the biological control of *A. planipennis* in North America.

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OOBIUS AGRILI (HYMENTOPTERA: ENCYRTIDAE), A SOLITARY EGG PARASITOID OF EMERALD ASH BORER FROM CHINA

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ABSTRACT

Agrilus planipennis Fairmaire, a buprestid native to Asia, was identified in 2002 as the causal agent of ash (*Fraxinus* spp.) decline and mortality in Michigan and Ontario. Since then, infestations have been found in Ohio, Indiana, Maryland, Virginia, and Illinois. Efforts to contain and eradicate this pest in North America are proving difficult due to the size of the infestation and lack of effective detection and control methods. As managers shift from a policy of eradication to one of management, biological control may become an increasingly important method of controlling and slowing the spread of *A. planipennis*. With few natural enemies attacking this aggressive buprestid in North America, our research expanded to China where two new parasitoid species, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), were found attacking *A. planipennis* larvae and eggs, respectively (Liu et al. 2003; Bauer et al. 2006). In Jilin province field sites, where the distribution of these two parasitoids overlap, we estimate their combined impact resulted in ca. 74% reduction in local *A. planipennis* populations during 2005.

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is a solitary egg parasitoid discovered parasitizing *A. planipennis* eggs on ash trees in Jilin province during 2004. Field studies in 2005 showed *O. agrili* completed at least two generations per year, and parasitism rates peaked during July and August at 56.3 percent and 61.5 percent, respectively. *O. agrili* is parthenogenic, and its sex ratio is female-biased at 14.5:1 (female:male). A portion of the *O. agrili* population undergoes diapause within *A. planipennis* eggs during the winter and emerges the following summer, providing synchrony with its host life cycle.

We developed a standard rearing protocol for *O. agrili* in our containment room in Michigan using laboratory-reared *A. planipennis* eggs. In the laboratory, *O. agrili* completes its life cycle in 20-25 days at 25°C, and females fed honey and water have an average adult longevity of 14 days. *O. agrili* oviposit in *A. planipennis* eggs ranging from newly laid to 9 days old. Each female produces an average of 24 eggs in her life time, with a daily maximum of five and lifetime maximum of 62.

We evaluated the host specificity of *O. agrili* using laboratory assays. In no-choice assays, *O. agrili* were exposed to eggs of six buprestids (*Agrilus anxius*, *A. bilineatus*, *A. cyanescens, A. egenus*, *A. ruficollis,* and *A. subcinctus,*), two cerambycids (*Megacyllene robiniae* and *Neoclytus acuminatus*) on their respective host plants, and the eggs of four lepidopterans (*Bombyx mori, Choristoneura rosaceana, Manduca sexta,* and *Pieris rapae*) on small ash branches. *Oobius agrili* did not parasitize eggs of cerambycids, lepidopterans, or *A. cyanescens, A. subcinctus, A. egenus*, whereas eggs of the larger *Agrilus* spp. (*A. anxius*, *A. bilineatus*, and *A. ruficollis*) were parasitized. Eggs from *Agrilus* spp. unacceptable to *O. agrili* are approximately half the size of eggs from *Agrilus* spp. accepted by this parasitoid, suggesting *Agrilus* egg size may limit acceptance. In the choice assays, *O. agrili* were exposed to eggs of *A. planipennis* and eggs of the three larger *Agrilus* spp. accepted during no-choice assays (*A. anxius*, *A. bilineatis,* or *A. ruficollis*). When given a choice, *O. agrili* demonstrated a strong preference for *A. planipennis* eggs on ash versus the other three species on their respective host plants (birch, oak, or raspberry, respectively). These results suggest that *O. agrili* is a good candidate for the biological control of *A. planipennis* in North America.

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HOST SPECIFICITY OF *SPATHIUS AGRILI* YANG, A PARASITOID OF THE EMERALD ASH BORER

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ABSTRACT

Spathius agrili Yang is a gregarious ectoparasitoid that was found attacking emerald ash borer (EAB) in Tianjin China. Females search ash bark and respond to the noise or vibration of feeding EAB to lay clusters of eggs on the outside of EAB larvae. The parasitoid larvae develop on the outside of the EAB, form cocoons, and emerge as adults to complete one of the three generations per year. *Spathius agrili* has several characteristics that enhance the likelihood of successful biological control: more than one generation per year, female-biased sex ratio, and oviposition on host. Monophagy is another characteristic that enhances the success of biological control, and *Spathius agrili* has never been described from any other wood borer, but this study was designed to determine the potential physiological and ecological host ranges of *S. agrili*.

No choice tests were conducted in the United States and China to determine whether *S. agrili* would attack different species if given no other option. Test larvae were collected in the field by removing them from infested logs. Twigs of the larvae's primary host plant were split longitudinally and a chamber was drilled through to the bark from the inside out. A single test larva was placed in each chamber and the twig was held together with rubber bands. Thirty twigs of each species were presented individually to mated, naïve *S. agrili* females.

From 2003-2005, eight species of wood borers that occur in the same ash forest as EAB were tested. Thirty-three percent of the EAB were attacked, but no other species were parasitized. In 2006, two *Agrilus* species native to the United States were tested, as were five *Agrilus* and two other species in China. *Spathius agrili* did attack five of the *Agrilus* species, but percentage parasitism was significantly lower than attacks on EAB (Fisher's exact test). No adult females emerged from the two U.S. species, although adults of both sexes were produced for two of the Chinese *Agrilus* tested.

Y-tube olfactometer tests were conducted to determine if *S. agrili* females preferred test plants over clean air. In China, leaves attached to twigs were tested, and in the U.S., bark with *Agrilus* frass was tested. In China, *S. agrili* was only attracted to *Fraxinus pennsylvanica*, *F. velutina,* and *Salix babylonica*. *Spathius* females were not attracted to 11 other species, including crabapple, tangerine, and Chinese prickly ash, in spite of the fact that this parasitoid did attack larvae in these plants in no-choice tests. Testing is just beginning on the response of *S. agrili* to bark contaminated with *Agrilus* frass, but so far, five out of seven parasitoids were attracted to ash bark containing EAB frass, and attraction to oak and birch was less pronounced.

In conclusion: 1) *Spathius agrili* did not attack any wood boring larvae outside the genus *Agrilus*, 2) *Spathius agrili* attacked significantly more EAB than non-target *Agrilus* in no-choice tests, 3) Olfactometer tests showed that *S. agrili* is attracted to leaves and twigs of ash and willow, and 4) *Spathius agrili* was not attracted to leaves and twigs of crabapple, tangerine, or prickly ash even though it attacked borer larvae in these host plants in no-choice tests.

Choice tests and further olfactometer tests are planned to further elucidate the host specificity of *S. agrili*.

EXPLORATIONS FOR NATURAL ENEMIES OF EMERALD ASH BORER IN CHINA IN 2006

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ABSTRACT

An exploratory survey for the emerald ash borer (EAB), *Agrilus planipennis*, and its natural enemies was made in China during July-August, 2006. We visited 11 field plots in four provinces. We visually inspected living *Fraxinus mandshurica, F. chinensis* var. *rhynchophylla, F. velutina, F. pennsylvanica,* and *F. americana*, then peeled or chipped off the bark in search of EAB and associated natural enemies. We found active EAB infestations in five of the 11 field plots, and collected material from all four provinces: Hebei, Jilin, Liaoning, and Tianjin. Signs of earlier EAB infestations were found at the other six sites where no active infestations were present. The abundance of EAB was highly variable, and larva densities range was 0-50 larvae/meter^2 of bark surface. We found EAB on *F. mandshurica*, *F. velutina, F. pennsylvanica,* and *F. americana*, but not *F.chinensis* var. *rhynchophylla*. Ash trees growing along streets, highways, in plantations, and city parks seemed to be more susceptible to EAB infestation than those growing in forests. We recovered two species of parasitoids during this survey, both of which were previously recorded from EAB. The first species was *Spathius agrili* Yang (Hymenoptera: Braconidae), a gregarious ectoparasitoid reported in Jilin and Tianjin Provinces (Liu et al. 2003, Yang et al. 2005). Parasitism by this species ranged from 0 to 4 percent and averaged 1 percent. The second species was *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), a gregarious endoparasitoid that occurs in Liaoning and Jilin (Liu et al. 2003) and Heilongjiang Provinces (Yang et al. 2006). EAB larva parasitism by *T. planipennisi* in our 2006 samples ranged from 0 to 36 percent and averaged 8 percent.

Some of the sites visited in 2006 were the same or near those visited by Liu et al. (2003) in their preliminary survey for EAB and its natural enemies in China, so any additional explorations should be focused in different areas. Perhaps the most promising areas to look for specialized natural enemies of EAB would be the region in Heilongjiang Province straddling the 130th meridian and adjacent areas of Russia. This region has deciduous and pine-deciduous forests containing substantial components of ash (*Fraxinus* spp.). Other potential exploration areas include the Shandong Province of China, South Korea, and Japan.

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EMERALD ASH BORER SURVEY

THREE YEARS OF A RISK-BASED EMERALD ASH BORER DETECTION SURVEY AND FIREWOOD SURVEY IN MICHIGAN AND WISCONSIN

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ABSTRACT

A risk based detection survey was initiated in Michigan in 2004 to detect outlier populations of the exotic emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). This survey was expanded to include northern Wisconsin in 2005 and to include all of Wisconsin in 2006. The number of sites used in this survey has increased from 116 in 2004 to approximately 190 in 2006. Survey sites are concentrated in campgrounds under various ownerships due to the risk of the inadvertent movement of this insect in firewood. This survey utilizes girdled ash trap trees (*Fraxinus* spp.) to detect adults and larvae of emerald ash borer, firewood inspections to detect potential introductions of emerald ash borer and observations of declining trees to detect existing emerald ash borer populations. Where possible, a subset of trap trees established each year are left in place, as opposed to being cut and peeled at the end of the field season to look for larvae, and reused the following year to supplement newly established trap trees. In 2005, the first emerald ash borers from the Upper Peninsula of Michigan were collected as early instar larvae from a trap tree at a survey site that had been established at Brimley State Park in Chippewa County in 2004.

Data were collected in the middle of the field season to characterize the condition of the trees in the survey based on standard forest health monitoring protocols. Data collected includes crown light exposure (scale: 1-5), percent dieback of the canopy (5% classes), and tree vigor rating (scale: 1-6). These data will enable us to characterize trees that are useful for a second year of trapping—i.e. which characteristics of each of the three ash species used are most likely to be associated with a tree that will reflush in a second year of trapping.

In 2004, emerald ash borers were found in firewood at three locations in Lower Michigan but not found on trap trees. In 2005, emerald ash borers were detected on trap trees established in 2004 and 2005 at six sites in Lower Michigan and one site (Brimley State Park) in Upper Michigan. Two additional detections in 2005 came from firewood and declining tree inspections. In 2006, emerald ash borers were detected on trap trees established in 2004, 2005, and 2006 at over 14 sites that were previously not known to be infested. No detections were made in firewood or declining trees in 2006.

A total of 7,025 firewood piles were inspected in 2006, and 3.2 percent contained ash firewood (ranging from 1.4 percent to 6.2percent, depending on state and ownership). This was a slightly lower incidence of ash in firewood than in 2005, when of 5,649 firewood piles, 3.9 percent contained ash (ranging from 1.8 percent to 10.8percent, depending on state and ownership). Hence, ash is still being used as firewood, and this continues to present a risk for the spread of emerald ash borer in Michigan, Wisconsin, and other states.

Trap trees remain among the most effective detection tools we have available to detect populations of emerald ash borer, and techniques are being refined so as to optimize the selection of trees as trap trees. Detection of emerald ash borer from this and other surveys is essential for management activities, including those that aim to eradicate localized outlier populations and those that implement silvicultural treatments such as ash reduction to reduce damage from emerald ash borer.

DEVELOPING SURVEY TECHNIQUES FOR EMERALD ASH BORER: THE ROLE OF TRAP HEIGHT AND DESIGN

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ABSTRACT

The objective of this ongoing research is to develop a trap that can improve the sensitivity and efficiency of emerald ash borer (EAB) surveys. In 2006, we studied the effect of trap silhouette and trap size on EAB capture. Four trap silhouettes were compared for effectiveness: three flat-paneled traps (a four-sided box, 35cm x 60cm, with 0.84m^2 of trapping surface area; a three-sided prism, 35cm x 60cm, with 0.63m^2 of trapping surface; and a two-sided, single panel, 35cm x 60cm, with 0.42m^2 of trapping area) and a four-sided crossvane, 45cm x 60cm, with 1.06m^2 of trapping area. There was no significant difference in total catch among any of the traps, but the prism and single-panel trap caught significantly more EAB per m^2 than the crossvane trap. There was no significant difference in catch per m^2 among the flat-paneled traps. We also compared five sizes of our three-sided prism trap: standard (see above), narrow (17.5cm x 60cm, 0.32m^2 area), long and narrow (17.5cm x 120cm, 0.63m^2 area), wide (55cm x 60cm, 0.99m^2 area), and long (35cm x 120cm, 1.26m^2 area). There was no significant difference in total catch or catch per m^2 among the sizes.

In previous years, as well as in 2006, we studied the effect that trap height plays in EAB catch. Ten trap lines consisting of a purple prism trap were placed at three heights (13.0m, 6.5m, and 1.5m) either along an ash-lined driveway or a windbreak in Howell, Michigan. The 13.0m trap was hung via a rope and pulley from a branch in the upper canopy of an ash tree. The 6.5m trap was hung via a rope from the bottom of the 13.0m trap, and the 1.5m trap was hung from an iron rebar pole near the base of the tree. The highest total catch was observed during the week of June 19 for traps placed at 13.0m and 6.5m and during the week of June 26 for traps at 1.5m. We caught 65 percent, 21 percent, and 14 percent of the total EAB on traps in this study at 13.0m, 6.5m, and 1.5m, respectively. The difference in catch between the 13.0m traps and those at the other two heights was significant, but the 6.5m and 1.5m traps

were not significantly different from each other. The ratios of females to males were 1.8:1, 2.1:1, and 1.2:1 for traps at 13.0m, 6.5m, and 1.5m, respectively. This was significant for the traps at 13.0m and 6.5m, but not for the traps at 1.5m. Based on these results, it appears that placing the traps in the mid-canopy of the tree increased their effectiveness, especially early in the EAB flight period.

A MULTISTATE COMPARISON OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS* FAIRMAIRE) (COLEOPTERA: BUPRESTIDAE) DETECTION TOOLS

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ABSTRACT

Emerald ash borer (EAB) is an exotic wood-boring pest that was first discovered in southeastern Michigan in 2002. Since that time, it has spread to and been detected in other areas of Michigan, as well as in a number of nearby states, including Ohio and Indiana. Detection of EAB in areas of low population density is problematic. To date, the most effective trapping tool has involved the use of girdled trap trees. Research to develop effective detection tools for EAB has been conducted since the beetle was first found in Michigan.

This study aims to compare trapping technologies developed by a number of collaborating research groups at sites with a range of both ash densities and EAB population densities. In the 2006 field season, the study included 62 sites distributed throughout Michigan,

Indiana and Ohio. Between eight and ten potential survey tools (treatments) were tested at each site. Treatments included ash trap trees girdled in the lower 1.5m of the bole with either a clear sticky band or a purple sticky band wrapped above the wound, ungirdled ash trap trees, ash trap trees girdled at 3m above the ground, ungirdled ash trap trees baited with Manuka oil release devices at 3m above the ground, purple prism traps baited with Manuka oil, and non-ash ungirdled trap trees. Where available, ash trap trees girdled in the lower 1.5m of the bole in 2004 and 2005 with a clear sticky band around them were used. Sites used represented locations with high or low ash density and high, low, or very low emerald ash borer density. Traps were established in late spring (May-June) 2006 and were monitored for adult EAB throughout the summer flight season. In the fall, trap trees were cut and peeled at each site to evaluate EAB larva density in the ash trap tree treatments.

Preliminary analyses of the numbers of adult beetles caught on the traps suggest that the effectiveness of different trap designs varies according to the density of ash at a site and the density of EAB. This study will be continued during the 2007 field season.

EVALUATION OF A MULTICOMPONENT TRAP FOR EMERALD ASH BORER INCORPORATING COLOR, SILHOUETTE, HEIGHT, TEXTURE, AND ASH LEAF AND BARK VOLATILES

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ABSTRACT

Since the 2002 discovery of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in southeastern Michigan and Windsor, Ontario, there have been considerable efforts to develop improved means to monitor and detect infestations. Studies have found that EAB is attracted to girdled ash trees (Poland et al. 2004, 2005; McCullough et al. 2006), the color purple (Francese et al. 2005), and purple panel traps baited with various blends of host volatiles, including certain ash leaf blends (Poland et al. 2004, 2005) and manuka oil, a steam distillate from the New Zealand tea tree that contains similar volatile compounds as ash bark and wood (Crook et al. 2006). In addition, EAB attack densities appear to be consistently higher on rough-barked trees (Anulewicz et al. 2006) and on open-grown or edge trees, compared to trees in a closed canopy (Poland et al. 2005, McCullough et al. 2006).

We developed a multi-component trap, incorporating all of the known attractive stimuli for EAB, and tested it at six field sites with varying EAB density in southern Michigan. Each multi-component trap consisted of a 10 ft tall polyvinyl chloride (PCVC) pipe (4 inches in diameter) that was painted purple. The height of the PVC pipe was intended to produce a "silhouette" similar to the tree bole of an open-grown tree that would be readily discovered by EAB adults. A three-sided panel trap constructed from purple, corrugated plastic, roughly 24 inches long by 15 inches wide on each side, was attached to the top of the PVC pipe, creating a broad trapping surface and silhouette similar to the tree crown of a small (4 inch dbh) ash tree. A second three-sided trap was attached to the pipe roughly 2 feet below the top trap. Both purple panel traps were coated with clear Pestick. The PVC pipe between the two traps was wrapped with plastic wrap and coated with Pestick. Traps were installed by sliding the PVC pipe over a T-post that had been set into the ground to provide support.

Factors that we evaluated included (A) a blend of ash foliar volatile compounds (*cis*-3-hexenol, *trans*-2-hexenol, *trans*-2-hexenal, and hexanal); (B) manuka oil; and (C) texture on the panel traps. The texture was achieved by mixing kitty litter with purple paint and applying it to the panels. The traps were set out in randomized complete blocks at six sites located in Ingham, Livingston, Washtenaw, Isabella, and Genessee counties. Each block was comprised of four traps. One trap in each block included the foliar lure attached to the top panel, vials of manuka oil on the lower panel, and texture on both panels. A second trap in each block included the foliar lure and the manuka oil, but texture was not applied to the panels. The remaining traps in each block had textured panels plus either the foliar blend or the manuka oil but not both. We installed four to six blocks of traps (16 to 24 traps) at each site on 15 and 16 June 2006. Traps were checked weekly until no EAB were captured for two weeks in a row (early September).

Overall, we captured 4,060 EAB on the 140 traps. The pattern of responses to the different treatments was similar at all trapping sites, but differences between treatments were not significant at every site. Therefore, results from all sites were pooled and summarized. For all sites combined, traps baited with both the leaf blend and manuka oil but without texture captured the most EAB. Traps baited with the manuka oil alone and coated with the texture caught significantly fewer EAB than traps baited with both manuka oil and the leaf blend without texture. Traps coated with texture and baited with either the leaf blend alone or the leaf blend and manuka oil caught an intermediate number of EAB. The combined effect of omitting the leaf blend and including texture significantly reduced attraction compared to traps with both the leaf blend and manuka oil but no texture. Therefore, it appears that leaf volatiles are an important factor in EAB attraction. The texture that was applied to the traps may have interfered with EAB capture, due to reduced adhesion of the beetles to the rough surface, which was difficult to coat with Pestick. Thus, bark roughness is probably not an important factor in long range attraction of EAB, but is more likely involved in post-landing acceptance of oviposition sites.

At all sites and for all treatments, the peak number of EAB captured occurred during the week of 26 June 2006. The number of beetles captured increased from Week 1 (20 June) to Week 2 (27 June) and then tapered off. A similar number of beetles were captured on the upper and lower panels during the first two weeks; however, more beetles were captured on the lower panels compared to the upper panels during the three subsequent weeks (6, 12, and

19 July), after which the number of EAB captured decreased rapidly. The relative increase in EAB captured on lower compared to upper panels in later weeks may reflect a preference of beetles for bark volatiles and lower portions of the tree later in the season. When EAB adults first emerge, they must feed for 5-7 days before mating begins, and females feed for an additional 5-7 days before beginning to lay eggs (Bauer et al. 2004). Thus beetles may be more attracted to leaf volatiles and upper canopy positions initially during the time that they complete maturation feeding and begin mating. Later, as they become sexually mature, beetles may prefer bark volatiles and lower portions of trees as they seek out sites for oviposition.

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ACTIVITY AND MICROHABITAT-SELECTION PATTERNS FOR EMERALD ASH BORER AND THEIR IMPLICATIONS FOR THE DEVELOPMENT OF TRAPPING SYSTEMS

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ABSTRACT

Behavior of adult emerald ash borers (EAB), *Agrilus planipennis* Fairmaire, was observed on green ash, *Fraxinus americana* L., at field edges during 8 days in late June 2005 and 2006. At hourly intervals, beetles were counted on 100 leaflets at each of two sides and three levels (low, mid, and top) of an ash tree's canopy (total of 500 leaflets per period in 2005 and 600 in 2006). In addition, beetles were counted flying within the visual field of binoculars (fixed observation point) during five (2005) or six (2006) 1-minute observations on fixed areas of canopy (same areas used in leaflet count), and during 5-minute searches of (a) stems from approximately 1 cm diameter to bole and (b) the understory beneath trees.

Individual EAB were observed during 15-minute periods at hourly intervals from 0930 and 1600 hours; observations were recorded on audio tape, and the data were subsequently transcribed onto spreadsheets. Individuals were observed an average of only 8 minutes 52 seconds out of the 15-min periods, in large part because 48 of the 115 beetles we studied flew out of view before the end of the observation period. Overall, beetles averaged 0.70 ± 0.92 flights per observation period (includes flights into and out of the observation area as well as flights within the area). Beetles typically flew from leaf to leaf or even among leaflets of individual leaves. Overall, only about 5 percent of beetles were mating when observed during all phases of this study. Observations of oviposition and beetles on stems in general were also less frequent than perhaps expected.

Beetle density on trees averaged approximately one beetle per 50 leaflets during both years of the study and did not appear to vary with height in the canopy. Beetles per 5-min count on stems averaged well less than 1 per observation period, suggesting that adult EAB spend much higher proportions of their time on foliage rather than stems. On a day in 2005 that following several consecutive cool, cloudy days, beetles were seen mostly in the understory. Beetles moved into the trees the next day once direct sunlight hit their resting sites. They may simply have fallen from trees and accumulated during several days that weren't conducive to flight; large numbers of beetles in the understory were not observed again.

Individual adult EAB that we observed on leaves spent nearly three-quarters of their time resting, 15 percent feeding, 10 percent walking on surfaces of leaflets, and 2 percent flying. Counts of flights indicated that flight activity was concentrated in the tops of trees. We counted nearly four flights per minute near tops of trees (or just over two when corrected for the larger visual field of the binoculars at tree-top level vs. low in the canopy) as opposed to a half (corrected) and a quarter of a flight per minute at mid- and lower-canopy levels, respectively.

The high landing rates of beetles on foliage at tops of trees suggested that it could perhaps be possible to enhance trap catch by placing traps high in trees at the tops of canopies. We constructed small prism-shaped sticky traps (17 x 30 cm h on each of 3 sides) of Coroplast® (corrugated plastic sheeting) and hung them at ends of branches toward the top and bottom of the canopy of eight ash trees. An additional trap was placed at the top-center of the canopy by attaching it to a 5-m fiberglass “banner pole” that was strapped to an upper branch. Traps on half of the trees were purple; traps on the other half were green. A standard purple EAB prism trap (35 x 60 cm h per side) was hung on a rebar pole at the base of each tree. For a variety of reasons, traps were not in place until the latter portion of the trapping season.

Small traps at the top of the canopy caught roughly 10 adult emerald ash borers each, whereas those near the base of the canopy caught half that many. The standard (rebar-based) traps also caught about 10 adults each, although the surface area of these traps is 4 times that of the traps in the canopy. The larger standard traps caught similar numbers of males and females, but the traps in the canopy, at all levels, caught primarily males (approaching 90 percent). This finding is consistent with observations from other studies that males seek mates through active flight while visually searching foliage.

Several times more beetles were captured on green traps rather than purple traps in the canopy. This was a surprise, as purple has outperformed other trap colors in numerous tests; this may be an indication that beetles at the edge of the canopy seek foliage for landing sites. Despite this, casual observations indicate that beetles still tend to land on leaves much more readily than on the traps, so it should be possible to improve the efficiency of these traps by improving their visual (and perhaps chemical) characteristics. Overall, placement of traps at the periphery of the canopy high in trees merits further investigation for its potential in enhancing sensitivity of trapping systems for detection of emerald ash borer populations.

CHEMICAL ECOLOGY OF EMERALD ASH BORER

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ABSTRACT

During 2005, bark volatiles from green ash *Fraxinus pennsylvanica* were tested for electrophysiological activity by *Agrilus planipennis* using gas chromatographic-electroantennographic detection (GC-EAD) and for behavioral activity using baited purple traps in Michigan. GC-EAD analysis of the headspace volatiles of bark tissue samples from non-girdled (healthy) and 24-hour-old fully girdled (stressed) ash trees revealed that the latter had elevated sesquiterpene levels. Six of the elevated compounds in stressed bark samples consistently elicited antennal responses by both male and female *A. planipennis*. Five of the EAD-active compounds were identified as α-cubebene, α-copaene, 7-*epi*-sesquithujene, trans-β-caryophyllene and α-caryophyllene (humulene). The sixth EAD-active compound remains unidentified. Purple glue traps containing Manuka oil (a distillate from the Manuka tree, *Leptospermum scoparium*, containing α-cubebene, α-copaene, trans-β-caryophyllene and α-caryophyllene) caught significantly higher numbers of beetles than other treatments. We therefore demonstrated that bark sesquiterpenes play an important role in host location for both male and female *Agrilus planipennis* and show potential for use in semiochemical based monitoring programs.

During 2006, individual EAD-active compounds were tested in the field, but results showed no significant differences compared to control purple traps. Phoebe oil, an oil distillate from the Brazilian walnut tree, *Phoebe porosa*, was discovered to have high amounts of 7-*epi*-sesquithujene as well as α-cubebene, α-copaene, trans-β-caryophyllene, and α-caryophyllene. Phoebe oil was tested in the field alongside manuka oil. Fractions of both oils were also made in an attempt to concentrate ash-like EAD-active compounds and remove non-ash like compounds. In 2006 field tests, a low dose (non-fraction) release of Phoebe oil and Manuka oil containing all fives identified EAD-active compounds caught the most beetles, although no treatment was significantly different after pairwise analysis.

FIELD ATTRACTION OF EMERALD ASH BORER TO ANTENNALLY AND BEHAVIORALLY ACTIVE ASH VOLATILES

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ABSTRACT

Early detection of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), remains a major challenge for regulatory officials due to the delay in onset of visual symptoms of attack. Currently, regulatory and resource management agencies rely on girdled trap trees for statewide survey and detection programs. However, locating suitable detection trees can be difficult, and felling and debarking trees to locate galleries is labor intensive and costly. Development of effective traps and attractants is a high priority for the EAB management program. Understanding EAB host location behavior and attraction to host volatiles can lead to the development of attractive traps and lures. Our objectives were to compare responses of EAB to visual and olfactory cues in host location, identify ash leaf volatiles associated with EAB attraction to different ash species, and test EAB attraction to various ash leaf and bark volatiles in traps in the field.

To compare EAB attraction to visual and olfactory cues associated with host location, we conducted an experiment using live potted ash trees and artificial trees (approximately 1 m tall) in an outdoor arena. Artificial nursery trees were created using 2.5-cm diameter PVC pipe painted taupe for the stem, into which branch and leaf material from an artificial indoor fig tree was inserted. The amount and size of the branch material was similar to the live trees. The outdoor arena was constructed using T-posts set out in two concentric rings (approximately 5 m and 7 m in diameter). Rolled paper (1.5 m wide) was used to encircle the inner ring of T-posts, forming a low “wall.” The paper wall was ventilated by cutting holes (approximately 10 cm in diameter) in the paper. Clear plastic sheeting encircled the outer ring of T-posts and extended over the top to create an enclosed arena. Small holes were cut in the plastic sheeting to provide ventilation and prevent over-heating inside the arena. Live and artificial ash trees were set in a ring just inside the paper wall of the arena or between the paper wall and the plastic sheeting for “hidden” trees.

Treatments included: a) live ash nursery tree (visual and olfactory cue), b) artificial ash tree (visual cue only), c) live ash nursery tree hidden behind the ventilated paper wall (olfactory cue only), d) artificial ash tree baited with manuka oil (visual and olfactory cue), and e) the paper wall (control). Manuka oil is a steam distillate from the New Zealand tea tree that has a similar volatile profile as ash bark and has been shown to be attractive to EAB.

We released 100-120 EAB adults at a time in the center of the arena and then recorded the number of beetles on each tree (real or artificial) and on the paper every 20 min for 4 hours. A total of 20 replicates were conducted. We found that significantly more beetles landed on the real trees, exposed or hidden behind the paper, than on the artificial trees with or without the manuka oil. The number of beetles that landed anywhere on the paper wall was not significantly different from the number of beetles that landed on the real trees. However, the numbers were not adjusted for surface area (i.e., there was not adjustment for the surface area of the paper wall, which was much greater than that of the trees). The results indicate that olfactory cues from live ash trees are important in host location by EAB.

To identify particular volatiles that may be associated with host preference by EAB, volatiles were collected from green, white, black, blue, European, and Manchurian ash seedlings. We quantified the amounts of several volatile compounds in the different ash species, including hexanal, *E*-2-hexenal, *E*-2-hexenol, *Z*-3-hexenol, butoxyethanol, *Z*-3-hexenyl acetate, hexenyl acetate, *E*- -ocimene, nonanal/linalool, nonatriene, and *Z*-*E*- -farnasene. The amounts and proportions of the different volatile compounds varied by ash species. For instance, *Z*-3-hexenol was present in significantly higher quantities in European ash than in any of the other ash species, while *Z*-3-hexenyl acetate was significantly less prevalent in green ash than in any of the other ash species. Differences in volatile profiles between the ash species may be related to differences in preference of EAB for different species of ash. The results may lead t

We conducted several field trapping experiments comparing EAB attraction to three-sided purple "prism" traps baited with various ash volatiles. Overall, we found that traps baited with a blend of green leaf volatiles, including hexanol, *E*-2-hexenol, and *Z*-3-hexenol, captured significantly more EAB than unbaited traps. Traps baited with *Z*-3-hexenol at a high or low release rate captured significantly more EAB males than unbaited control traps; however, the number of EAB captured in traps baited with low or high release rates of *E*-2-hexenal was the same as that for control traps. Several individual compounds present in ash that have been found to have behavioral activity in other forest insects were also tested. None of these compounds, including chalcogran, myrtenol, nonanal, octenol, pityol, verbenone, *exo*-brevicomin, heptanol, phenyl ethanol, or *trans*-conophthorin, captured more EAB than unbaited control traps. However, traps baited with manuka oil or a four-component leaf blend comprised of hexanal, hexanol, *E*-2-hexenal, and *Z*-3-hexenol, captured significantly more EAB than unbaited control traps. To date, the most promising lures for EAB are manuka oil and a blend of green leaf volatiles.

EMERALD ASH BORER ATTRACTION TO GIRDLED TREES: EFFECT OF PLACEMENT AND TIMING ON ATTRACTION

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ABSTRACT

Attraction of emerald ash borer to girdled ash trees has been demonstrated in prior trapping studies. This study, which began in the spring of 2005, was designed to investigate whether the timing or placement of girdling affects the attractiveness of the trap tree. We placed ten replications at a location with a moderate beetle density where the trees were still in fairly good health. Trap trees were at least 15 meters apart. There were a total of five treatments, all involving ash trees and clear shrink wrap coated with Pestick placed in a band a little above 4.5 feet. The treatments were: an ungirdled tree, a tree girdled at chest height on April 2 (before sap began to flow), a tree girdled at chest height on May 3 (sap flowing, but before major leaf out), a tree girdled at chest height on May 25 (after leaf out), and another tree girdled on May 25. The last girdle was placed 10 feet from the base of the tree and the sticky band was place in the same position as those of the other treatment types, but in this instance, below the girdle.

Through the adult flight season we collected the beetles caught on the sticky bands once a week. The treatment with the girdle located 10 ft from the base of the tree caught significantly more EAB than the treatment with an ungirdled tree. This was the only statistically significant difference.

During the following winter, we peeled nine of the ten replications to find all of the EAB larvae in each tree. The diameter and height of each larva's location was recorded. All portions of the tree, from the base of the tree to branches 5 cm in diameter, were peeled. We used a mixed model ANOVA (random block effect) to analyze EAB larvae density by treatment, gallery height (as a proportion of total tree height, divided into ten sections), and treatment by gallery height. Treatment had a significant effect on larvae density ($p<0.01$), and within-tree gallery height varied significantly ($p<0.01$). However, the treatment type did not effect the gallery height of larvae within the trees. Larvae density varied significantly by treatment type, with the control and May 3 girdle containing the fewest larvae, although not statistically significantly fewer that April 2 or May 24 girdles. The trees girdled at 10 feet contained significantly more larvae than ungirdled and May 3-girdled trees (Tukey HSD a=0.05). We divided the trees' heights into ten sections to analyze the density of larvae within each section. We found that the highest within-tree concentration of larvae was in the middle of the tree (Tukey HSD a=0.05).

ATTRACTION OF EMERALD ASH BORER TO TRAP TREES: CAN MEJA OR MANUKA OIL COMPETE WITH GIRDLING?

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ABSTRACT

To date, 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, however, 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 caused changes in foliar volatiles similar to those induced by physical stress such as girdling. 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 at least somewhat effective in attracting EAB to traps. We assessed the relative effectiveness of methyl jasmonate, Manuka oil, and physical stresses for EAB attraction.

In 2005, we estimated the larva densities from adult EAB captured on trees that were girdled, treated with Garlon 4 herbicide, exposed to methyl jasmonate, or left as untreated controls. We used a randomized block design with 18 replicates of each treatment at three sites varying in EAB density. We found that significantly more EAB adults were captured on sticky bands (placed at 1.5 m aboveground) on girdled ash trees than on trees treated with methyl jasmonate or on healthy, untreated trees (control). The number of EAB captured on trees treated with Garlon 4 herbicide did not differ significantly from the girdled or methyl jasmonate-treated trees. We felled and debarked 10 trees of each treatment to quantify larva densities in the winter. Significantly more galleries per m^2 were found on the girdled trees than on the MeJA, herbicide, or control trees. Results from this study and related work in 2004 demonstrated that herbicide-treated trees are generally dead or dying by mid- to late-summer and are either unattractive to ovipositing EAB females or unsuitable for larva development.

In 2006, we conducted a similar study to compare adult EAB capture rates and larva densities on trees treated with different stress agents—girdling, MeJA, and Manuka oil—and control trees. We used a randomized complete block design and implemented the study at five sites with EAB densities categorized as high (more than 35 adults captured/tree attacks/m^2 on

control trees), moderate (10-15 EAB/tree), or low (fewer than three EAB/tree). At four sites all treatments were included (n=40). Three of the four treatments (girdle, MeJA, and control) were included at the fifth site (n=30). Blocks of trees were selected to represent a range of sun exposure conditions, including, open-grown, full exposure, edge settings, densely wooded, and closed-canopy settings. Exposure of each individual tree was ranked as (1) all sides open, (2) super dominant/above canopy, (3) two or three sides open, (4) one side open/edge or (5) closed canopy/shaded.

All EAB on sticky bands were removed 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 the 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, but differences were not significant. At two sites where trees in all canopy exposure rankings were represented, more than 90 percent of the EAB captured were on trees that were fully exposed or had two or three sides exposed to sunlight. We plan to fell and dissect half of the blocks of trees during the winter to quantify larva density among treatments.

APPLICATION OF REMOTE SENSING TECHNOLOGY FOR DETECTION AND MAPPING OF HARDWOOD TREE SPECIES AND EMERALD ASH BORER-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, most of Southeastern Michigan is now considered generally infested, along with portions of Indiana and Ohio. Currently, detection methods rely heavily on visual surveys and labor-intensive trap trees. 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 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. Our objectives were to determine (1) whether ash trees can be differentiated from other northern hardwood species, (2) whether stressed ash trees can be differentiated from healthy ash trees, and (3) what time during the growing season is optimal for discriminating ash trees and their health status.

Ground-based and airborne studies were started late in 2003 and have been ongoing in an effort to prove the technology. Results from the ground-based studies indicate that differentiation between ash and other northern hardwood tree species is possible at a leaf level. Ash stressed by girdling can also be differentiated from non-girdled ash two months after the girdling event. Analysis of the leaf-level data is still in progress as there are many different analysis methodologies being investigated. Results from the airborne data sets are limited because of the many problems in collecting the data. After failed collection attempts in 2003 and 2005 and a corrupted data set in 2004, we finally have good-quality airborne data for the 2006 season. In 2006, we were able to collect hyperspectral imagery in both early June and late August. We also collected LIDAR data and high-resolution panchromatic imagery during early June. Data were collected over 150 square kilometers at locations in Northern Michigan (Lower Peninsula), Southern Michigan, and Northern Ohio.

Analysis of the 2006 airborne data set is in progress, and we have four different groups investigating the data with diverse approaches. RMS Inc. (Houston, Texas) is looking at the data with a proprietary analysis that separates the geologic and biologic components employing a fusion of the hyperspectral imagery with the LIDAR and panchromatic datasets. Clark Labs at Clark University (Worcester, Massachusetts) is classifying the images using traditional hyperspectral analysis techniques. The USDA Forest Service (Durham, New Hampshire) is taking a chemical ecology approach. Finally, ITT Space Sciences Division (Rochester, New York) is looking at sub-band engineering approaches to classification. Our objective in comparing the different analytical methodologies is to identify a system that can process hyperspectral data and deliver results in a timely fashion in an effort to make remote sensing technology operationally useful in program management.

THE BIOLOGY OF *CERCERIS FUMIPENNIS* (HYMENOPTERA: CRABRONIDAE) IN SOUTHERN ONTARIO AND ITS POTENTIAL FOR MONITORING THE DISTRIBUTION OF *AGRILUS PLANNIPENNIS* (COLEOPTERA: BUPRESTIDAE)

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ABSTRACT

We present a novel solution for a complex monitoring problem: using wasps to find emerald ash borer (EAB). The current monitoring methods for EAB are costly, labour-intensive, and at times destructive or impractical. Rather than using the current inefficient traps, why not use a biological agent that actively searches the forest for EAB, and after catching the beetle, returns with it to a waiting surveyor? Such a tool exists in the form of a native crabronid wasp, *Cerceris fumipennis* Say, which ranges from southern Ontario to Florida and west to Texas.

A ground nesting solitary wasp, *C. fumipennis* nests in loose "colonies," an aggregation of independent nests. In Ontario these colonies can contain between 10 and 500 nests. Each nest is maintained by a single female who spends the summer provisioning her subterranean burrow with paralyzed adult metallic wood-boring beetles (Buprestidae). During 2004, *C. fumipennis* in the Windsor area were observed provisioning their nests with EAB and other buprestids. Although the wasps are not sufficiently abundant or specialized to serve as a biocontrol agent, they can be used to monitor for the invasive EAB beetles.

In early July, a large *Cerceris* colony can collect upwards of 250 buprestids an hour; EAB have been observed to represent as much as 80% of a wasp's daily catch. EAB can be monitored by checking returning female *C. fumipennis* for the invasive beetle and then releasing the wasps to continue provisioning their nests. This monitoring technique requires minimal effort; it is also sustainable and already in operation.

We are investigating five aspects of the wasp's biology related to monitoring for EAB:

1. Defining the biotic and abiotic characters of known *Cerceris* colonies in Ontario and then using these characters to assist in locating "undiscovered" colonies in southwestern Ontario;

2. Identifying the daily and seasonal phenology of *C. fumipennis* activity and foraging with regards to both EAB and other buprestids;
3. Quantifying the effect that researcher interference has on the wasps' behaviour. We need to ensure that activities associated with using the wasp as a monitoring tool do not negatively impact the wasp's behaviour. Results from 2006 suggest that researcher interference has little effect on the continued provisioning of a nest.
4. Estimating the foraging range of *C. fumipennis* and, consequently, the area effectively sampled by a wasp colony;
5. Time permitting, experimenting with developing both transplant and mobile colonies. This would address the limitation that monitoring is currently restricted to areas near naturally established colonies.

Cerceris colonies are found in all states/provinces currently infested with EAB. They can often be located by checking the locality labels on pinned wasp specimens in local insect collections. Upon visiting the localities, check for areas of disturbed, bare, hard-packed sandy soils that are exposed to full sun. In Ontario, the wasps are commonly associated with campsites, old baseball diamonds, areas around fire pits, roadsides, parking areas, and foot paths. The nest entrances resemble small ant mounds with a 7mm-diameter hole running straight down through its center.

We strongly encourage the various agencies responsible for managing EAB to take advantage of this available, simple, reliable, and inexpensive monitoring tool.

EMERALD ASH BORER REGULATIONS AND OUTREACH

ESTIMATING EMERALD ASH BORER DENSITY AT LOCAL, LANDSCAPE, OR REGIONAL SCALES

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ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, an Asian phloem-feeding pest, was discovered in 2002 in areas of southeast Michigan and Essex County, Ontario. Since then, numerous localized “outlier” populations have been identified in other areas of Michigan, Ohio, Indiana, Illinois, and Maryland. An estimated 15 to 20 million ash (*Fraxinus* sp.) trees in urban and forested areas of southeast Michigan have been killed by EAB and all North American ash species are threatened by this exotic pest.

Federal and state regulatory agencies launched major programs in 2002 to contain EAB and to reduce the economic and ecological damage likely to occur if this invasive pest continues to spread. To date, containment activities at outlier sites have involved felling, removal, and destruction of all ash trees within a 200- to 800-m radius of trees known to be infested to ensure that non-symptomatic but infested trees are removed. Limited funding and an abundance of outliers, however, have required officials to prioritize sites and determine how to allocate limited resources most efficiently while achieving a desired reduction in EAB density. In addition, forest managers and property owners in areas with established EAB populations need a means to compare costs and benefits of alternative silvicultural options for stands with a substantial ash component.

Our objectives were to:

1. Develop a model to predict ash phloem area using tree diameter;
2. Determine the number of EAB adults likely to emerge per m^2 of ash surface area; and
3. Apply models to real-life ash tree inventory data to assess effects of alternative tree removal strategies on potential production of EAB.

We measured surface area, as a surrogate for phloem area, on 38 green ash (*F. pennsylvanica*) and 110 white ash (*F. americana*) trees, ranging from 5.2 to 65.0 cm in diameter at breast height (DBH). After felling, trees were sectioned and length and diameter of sections ≥6 cm diameter were measured. Surface area measurements from all sections (estimated as a conical frustum) were summed to estimate the area of phloem available to EAB larvae. A second order polynomial model [$Y=2.63-0.307(x) + 0.024(x^2)$] provided the best fit to the relationship between DBH and tree surface area ($r^2 = 0.94$). There was no significant difference between green and white ash trees in the relationship between DBH and area.

Potential production of EAB per m^2 was estimated by counting exit holes left by emerging EAB adults and woodpecker attacks on 71 ash trees (38 green ash; 33 white ash) recently killed by EAB. Five to 13 areas (depending on tree size) at 1 to 3 m aboveground were intensively examined on each tree; total area examined ranged from 0.17 to 3.73 m^2 per tree. Number of EAB adults produced per tree ranged from 17 to 170 per m^2 of phloem. Overall, an average of 89 ± 4.6 beetles could be produced per m^2. Beetle production was roughly 20 percent lower in trees less than 14 cm (4 inches) DBH than in larger trees. When the smallest trees (> 14 cm DBH) were excluded, an average of 110.3 ± 6.25 EAB developed per m^2 of phloem.

We applied our models to the ash inventory data from two outlier sites in Michigan (Brimley and St. Joseph) to estimate potential EAB production by tree size class at 200 to 800 m radii around the epicenter of the infestations. To eradicate these populations, regulatory officials delineated an area with a radius slightly greater than 800 m for ash tree removal. Using a 100 x 100 m grid overlaid on the affected area, survey crews measured and marked all ash trees, then tallied the trees by diameter class in each grid cell. Contractors felled, removed and destroyed the ash trees at Brimley in October 2005 and at St. Joseph in April 2004.

In the Brimley outlier site, less than 900 ash trees were in the 2 km^2 area designated for eradication (800 m radius around known infested trees). There was roughly 2,772 m^2 of ash phloem in the eradication area, which could have potentially produced more than 246,000 EAB. More than 75 percent of the ash trees, however, were small and less than 14 cm (5 inches) DBH. The small trees would have contributed only about 11% of the potential EAB production. In contrast, 6% of the ash trees were of merchantable size (> 25 cm), but they would have produced more than 50 percent of the EAB population.

In the St. Joseph outlier, more than 21,000 ash trees were removed from the 2.2 km^2 eradication area. Roughly 80 percent of these trees were small (< 14 cm or 5 inches DBH). Despite their abundance, small trees accounted for less than 10 percent of the total number of EAB that could have been produced in the area. In contrast, trees with commercial value (> 26 cm or 10 inches DBH) comprised only 6% of the ash trees in the treated area, but would have produced at least 65% of the beetles.

Data from both the Brimley and St. Joseph outlier sites also illustrate the importance of ash tree distribution. Removing ash trees only within the first 200 m of the Brimley epicenter, which included some very large ash trees, would have reduced EAB production by roughly 50%. In contrast, removing ash trees within the first 200 m at the St. Joseph site would have reduced potential EAB production by less than 2 percent.

Our results show that effectiveness of EAB containment options such as removing trees within areas of varying size or removing only large trees will be determined by the abundance, size and spatial distribution of ash trees in relation to the source or epicenter of an outlier infestation. More broadly, our models can be used to estimate potential EAB production for individual ash trees or for any area of interest where the abundance and size of ash trees is known. For example, our models have recently been applied to estimate "beetle pressure" in residential areas to help predict the likelihood that insecticides will effectively protect ash shade trees. Results of this study, in combination with ongoing studies of EAB dynamics, can provide a baseline for estimating EAB population density and spread over time. This in turn can serve as a tool to help regulatory officials and foresters more accurately evaluate costs and benefits of EAB management options including selective harvest or biological control.

SINKS, BARK, AND GARLON: APPLIED STUDIES FOR EMERALD ASH BORER MANAGEMENT

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ABSTRACT

Localized outlier populations of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, continue to be discovered across lower Michigan and in Ohio, Indiana, Illinois and Maryland. To date, operational programs have attempted to eradicate emerald ash borer (EAB) populations in high priority outlier sites by felling, removing, and destroying all ash trees within a 0.5 mile radius of known infested trees to ensure that infested but non-symptomatic trees are eliminated. These projects are costly, however, and funding to contain, eradicate, or otherwise treat such sites is increasingly limited. Alternative options and strategies that are less expensive should be considered.

CUT-AND-LEAVE

Strategies such as a cut-and-leave protocol for all ash trees or a combination of selective harvest of large trees and a cut-and-leave strategy for small trees at a given site could reduce costs considerably. As the phloem in the cut trees dries, EAB larvae will not be able to complete development or emerge. The rate at which phloem and cambium in cut trees or logs dries will vary depending on the size of the cut pieces, exposure to sun, and weather. The effectiveness of a cut-and-leave approach will depend on the proportion of larvae in the trees at the time of cutting that would complete development and emerge and whether down trees would be re-infested and produce viable adults the year after felling.

HERBICIDE

Another option could involve herbicide application to standing ash trees. Garlon 4, for example, can be applied as a spray to the basal trunk of ash trees efficiently and without the need for specialized equipment or vehicles. Such a treatment might be especially appropriate in outlier areas with sensitive soils or wet conditions. We found in previous trap trees studies that trees will become infested following a June application of Garlon. Although the trees generally decline or die during the summer, at least some EAB were able to emerge as adults the following year. The proportion of larvae that completed development is not yet known. In addition, we have not previously determined whether applying herbicides earlier in the year would cause trees to be less suitable for EAB colonization or development.

We initiated a study in 2006 to assess the effect of felling infested ash trees or treating infested ash trees with herbicide in spring on the proportion of EAB that successfully emerge. We also determined whether EAB adults would oviposit on cut and down ash trees or trees killed by herbicide, and if so, would larvae be able to complete development. The study was replicated at two sites with low EAB density and two sites with high EAB density. At each site, two to three small trees (< 13 cm DBH) and large trees (> 18 cm DBH) were cut or treated with Garlon 4 in early March, early April, and mid-June. Analysis of these data is in progress, and we plan to monitor emergence from samples of trees in 2007. A report with final results will be provided to operational programs and will also be posted on the website *www.emeraldashborer.info.*

SINKS FOR EAB

Eradication efforts to date have not been consistently successful. This likely stems from the difficulty of accurately delimiting low-density EAB populations and ensuring removal of all potentially infested ash. In addition, simply finding and removing all ash trees and all pieces of cut trees can be difficult, especially in thickly wooded or riparian areas. If EAB adult beetles emerge and find no readily available ash foliage for maturation feeding, they are forced to disperse to find suitable hosts. Thus, incomplete eradication of a population effectively drives the beetles to colonize hosts beyond the perimeter of the action area.

The idea of creating "sinks" to attract or "mop-up" remaining beetles in an outlier site arose from our observations and previous studies. We hypothesize that clusters of ash trees left in eradication areas are likely to be highly apparent and readily found by EAB adults. Girdling some or all of the ash trees within the cluster would substantially increase their ability to attract EAB adults. Ideally, therefore, beetles emerging from ash material would be attracted back to the designated ash trees that remain within the action area and would be less likely to colonize ash beyond the perimeter of the action area.. Those trees could then be debarked for monitoring purposes and would be removed before the next generation of adult beetles emerged. We refer to these trees as "sinks" for EAB.

ALCONA – EVALUATION OF SINKS FOR EAB

An outlier EAB infestation site in Alcona County in northeastern lower Michigan was discovered in January 2005. Preliminary surveys were conducted to assess the extent of the infestation, but no trees were removed, nor were any other actions taken at this site.

The Alcona site consisted primarily of low, wet bogs dominated by black ash, bounded by Lake Huron on the east and Cedar Lake on the west. The infestation was likely caused by transportation of infested firewood into a small community east of Cedar Lake. We observed an obvious North/South gradient in EAB infestation level, with attack densities decreasing in both directions from the apparent epicenter. With the assistance of Michigan Department of Agriculture personnel, we selected six clusters consisting of 10 to 12 ash trees, each 4 to 10 inches in DBH. Three clusters were north of the apparent epicenter, and the other three clusters were south of the epicenter. Distance between the northernmost and southernmost cluster was roughly 2 miles. Half of the ash trees in each cluster were girdled in June 2005 to create "sinks" for EAB.

We also selected six additional clusters of ash trees as controls, located at similar latitudes and distances from the epicenter as each sink, but at least 0.25 miles to the east or west of their paired sites. The trees in the control clusters were similar in size and spacing to trees in the sinks, but the trees were not girdled. In January 2006, we felled six trees in each sink and six trees in the corresponding control cluster to quantify density of EAB larvae. We examined at least three bark windows on every tree; more than 0.7 m^2 of area was sampled per tree.

In the northernmost sinks and paired clusters, we found no EAB galleries, indicating that the population had not yet spread to this area. In the sink and control cluster nearest the epicenter, all trees had been colonized and previously killed by EAB and provided no useful data. In the southernmost sink and control cluster, EAB larva density was very low, and we found only a few galleries.

We were able to quantify EAB density in two sinks and their paired control clusters. Overall density of galleries was more than twice as high on the girdled trees in the sinks as on the paired, ungirdled trees in the control clusters. There was an average of roughly 81 galleries per m^2 on girdled trees in the sink south of the epicenter compared to three galleries per m^2 on the paired control trees. To the north of the epicenter, there was an average of roughly 24 galleries per m^2 on girdled trees in the sink compared to one gallery per m^2 on the paired trees in the control cluster.

These results are preliminary but do suggest that the sinks, e.g., the clusters of girdled trees, were highly attractive to EAB adults despite the abundance of ash trees throughout the area. Presumably, clusters of girdled trees could be even more effective if other ash trees were not readily available to feeding or ovipositing adult beetles.

Based on these results and other observations, we hypothesized that using sinks to attract EAB in outlier sites could be expanded into lethal "islands of attraction." Islands of attraction would be similar to the sinks we tested at Alcona: clusters of ash trees would be left to attract and "mop-up" any remaining EAB in the action area. However, trees in the islands would be treated with a systemic insecticide (e.g., trunk or soil injection) in spring, then girdled a few weeks later after the insecticide had been translocated to the canopy. These trees would presumably still attract EAB adults but the insecticide treatment would further reduce the risk that adult beetles would disperse beyond the action area. Trees would subsequently be removed before any surviving larvae could develop and emerge.

Whether such islands of attraction will be effective is not yet known. The Michigan Department of Agriculture created such islands in two outlier sites in lower Michigan in 2006. They selected four to six clusters of ash in each area, treated trees with a trunk injection of imidacloprid, then girdled trees roughly three weeks later. We plan to fell, debark, and sample trees from islands at each site in 2007. It will be difficult, however, to assess the success of the island concept because there are no true controls for this kind of treatment. Nevertheless, integrating options such as sinks or islands of attraction with selective removal of large ash trees may warrant consideration, at least for some outliers. This type of integrated approach could be especially appropriate in areas where small ash trees would be cut and left on site or where ash trees would be treated with herbicide. Such strategies could perhaps be useful in reducing EAB density and rate of spread, particularly in multi-year containment efforts.

EVALUATION OF PUBLIC AWARENESS OF ISSUES RELATING TO FIREWOOD MOVEMENT AND THE EXOTIC EMERALD ASH BORER IN MICHIGAN

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ABSTRACT

The exotic emerald ash borer (*Agrilus planipennis*) was first identified in Michigan in 2002, and has killed millions of ash trees (*Fraxinus* spp.) in Michigan and surrounding states. A key goal in management of emerald ash borer is to reduce artificial spread through the movement of the insect to new locations in firewood. The movement of firewood is now regulated in some infested, as well as in some uninfested states. Inspections of firewood in state and federally operated campgrounds have revealed that ash firewood is still being used and is likely being moved around the state. Public education campaigns have been implemented to inform people about emerald ash borer and the associated firewood regulations. These educational programs use media that include fliers, billboards, radio and television advertisements, newspaper articles, and television documentaries.

During the summer of 2006 two types of questionnaire based surveys where conducted at State Park campgrounds throughout Michigan to:

1. determine public awareness of the regulations associated with the movement of firewood,
2. determine any demographics that influence a patron's knowledge about the firewood regulations, and
3. identify the components of the educational program that are reaching the most campground patrons. The first type of questionnaire was distributed at selected State Parks and self administered by campground patrons. The second type of questionnaire was administered by a researcher at selected state parks.

Based on the results of the researcher-administered questionnaires, approximately one quarter of campground patrons bring their own firewood with them, and of these, about one quarter travel over 200 miles to camp. Most campground patrons (95%) are aware that there are regulations relating to the movement of firewood, and when asked if they had heard of emerald ash borer, almost 91% indicated that they had. The researcher-administered surveys also revealed that patrons are hearing about emerald ash borer and the firewood regulations through educational outreach programs. The results of these questionnaire based surveys will help future emerald ash borer education efforts to focus on the methods of educational outreach that are apparently the most effective.

ASIAN LONGHORNED BEETLE PROGRAM REPORTS

U.S. ERADICATION PROGRAM UPDATE

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ABSTRACT

NEW YORK

Asian longhorned beetles (ALB) were first detected in New York State in 1996. As of 7 October 2006, 7,964 trees (6,092 of which were infested) have been removed and destroyed in New York due to the ALB infestation. In 2006, a total of 57 infested trees were detected in the infested areas New York City (Manhattan, Brooklyn, and Queens) and in central Long Island. No detections were found in the infested area of Islip, Long Island. The last infested tree found in Islip occurred in June 2002. The total New York quarantined area remains at 132 square miles. The last quarantine expansion occurred in 2003, when 10 square miles were added to the regulated area in Brooklyn and Queens, New York City, as a result of infested trees found on the southern boundary of the Brooklyn infestation. Surveys of host trees are conducted daily with federal and state personnel.

Regulatory activities occur daily, and there are now 910 compliance agreements in place. Over 78,000 trees have been surveyed this year, and 50,698 trees were treated in New York this year. Basal soil injection was used as the primary application method. As a result, about 87 percent of the host trees received soil injection treatment. In areas where soil injection could not be used, pressurized trunk injection using an Arborjet trunk injection application device was used.

ILLINOIS

Asian longhorned beetle was first detected in Illinois in July of 1998. 1,771 trees were removed and destroyed due to the ALB infestation. In 2006, 4,195 trees were treated.

In 2006, the final 9 square miles of the Illinois quarantine area were de-regulated for ALB. The last ALB detected in Illinois was in 2003 in the Oz Park area of Chicago. However, the ALB Eradication protocols require four consecutive years of annual surveys with negative results before complete eradication can be declared. Until this is achieved, the area is designated "infested." The Illinois project is currently in its third consecutive year of negative survey results. Over 83,000 trees have been surveyed as of September 30, 2006, and 61 square miles are still considered infested in Illinois. An aggressive public outreach program continued this year to augment our eradication strategies. ALB eradication in Illinois is projected for 2008.

NEW JERSEY

Jersey City/Hoboken ALB Infestation

Asian longhorned beetle was first detected in New Jersey in Jersey City in October of 2002. One hundred and thirteen infested trees were detected in this area, and 461 host trees were removed and destroyed. Three years of consecutive annual chemical treatments were applied to all host trees located within one-half mile of the infestation, and no new infested trees have been detected since 2002. As a result, this 4 square-mile area was de-regulated in 2005. Surveys will continue in order to confirm the eradication of ALB from Jersey City, New Jersey. Eradication is projected for 2008.

Middlesex/Union Counties ALB Infestation

In August of 2004, a second ALB infestation was detected in New Jersey. Through DNA analysis, this infestation was determined to be a separate introduction from previous U.S. infestations. To date, 616 infested trees have been detected, 89 of which were found in 2006. In order to quickly eradicate ALB from New Jersey, all host trees within a quarter-mile radius of an infested tree are removed and destroyed, resulting in the removal of 22,318 high risk trees since 2004. The total quarantine area is 25 square miles. There are 130 compliance agreements in order to regulate the movement of ALB host material. In 2006, over 22,000 trees were surveyed, and 31,391 host trees were chemically treated. All host trees located within a half-mile radius of an infested tree will receive annual chemical treatment for a minimum of three consecutive years. Eradication is projected for the Middlesex/Union Counties infestation in 2012. New Jersey State Forestry began replanting the trees lost to the ALB infestation, and all property owners and municipalities that lost trees are eligible for a replacement tree that is not a known host to ALB. To date, 2,112 trees have been replanted.

TORONTO ASIAN LONGHORNED BEETLE ERADICATION PROGRAM UPDATE

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ABSTRACT

The Asian longhorned beetle (ALHB) is an invasive alien species that is a threat to Canada's hardwood resources. It was first detected in an industrial/commercial area bordering on the cities of Toronto and Vaughan in September of 2003. Detection and delimitation surveys utilizing ground and aerial inspection techniques were conducted. Science, operation, and communication sub-committees were established, including partners from the federal, provincial and municipal governments as well as representatives from the United States Department of Agriculture. A Regulated Area covering approximately 169 square kilometres was established in February 2004 to control the movement of host material and firewood.

The eradication program lead by the Canadian Food Inspection Agency resulted in the removal of 15,000 trees in 2003/2004. Those trees removed included all infested trees and all suitable host trees within 400 m.

The monitoring phase started on June 30, 2004, the earliest possible date of emergence of ALHB in our climate. During the monitoring phase of 2004/2005, additional infested trees were detected within the Regulated Area and were determined to be part of the original infestation. An additional 10,000 trees were removed as a result. All infested material was chipped and ground to five-eighths of an inch or less to prevent survival of any insects in the wood.

In Canada, we consider the following genera to be suitable hosts for ALHB development: *Acer* spp., *Aesculus* spp., *Salix* spp., *Ulmus* spp., *Betula* spp., *Platanus* spp., *Celtis* spp., *Populus* spp., *Sorbus* spp., *and Albizia* spp. A special survey was conducted to study other hardwood species listed as hosts in other parts of the world. Analysis of the data compiled over three years indicates that there was no evidence of beetle attack on any of these additional tree species.

Survey results in 2006 revealed no new infestation, and monitoring will be on-going for an additional four years in order to declare the Regulated Area pest-free. The Canadian Food Inspection Agency continues to collaborate with partners and promote research on better detection and control methods for this very serious pest.

RESEARCH ON ASIAN LONGHORNED BEETLE IN CANADA

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ABSTRACT

An established population of the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), was discovered in 2003 in an industrial park in the Greater Toronto Area, on the border between Toronto and Vaughan, Ontario, Canada. The purpose of this presentation was to provide an overview of the research activities undertaken and type of information collected since that discovery. This presentation provided an overview of the research program that was initiated during the rapid response phase of the eradication programme against the Asian longhorned beetle. The overall objective of that research was to fill up knowledge gaps, to formulate local and site specific treatment recommendations, and to improve the mitigation strategy. Also presented was information on the research that has been initiated recently with a formulation of the systemic insecticide imidacloprid® to mitigate the increased risk of dispersal to uninfested areas by residual populations within the infested area.

The research program was conducted concurrently with the operational programme to eradicate the Asian longhorned beetle. Eradication of this beetle in Canada consisted of the removal and chipping of all trees considered suitable for infestation and located within 400 m of an infested tree. A tree were considered suitable if there was visual evidence (i.e., exit holes) that the beetle could complete its entire life cycle there under field conditions. All suitable trees were identified to species, assigned a unique identifier and geo-referenced prior to removal. Infested trees were brought back to a laboratory and cut into 50 cm bolts. Each bolt was examined, and the intensity of attack (e.g., number of oviposition pits and exit holes) and characteristics of the attack (e.g., size of oviposition pits and exit holes) were recorded. Also, live specimens (i.e., eggs and larvae) were collected for molecular analyses. The infested bolts were subsequently cut into smaller pieces (about 1.5 - 2.0 cm thick), and the year in which each exit hole had been created was determined. The entrance of each feeding gallery leading to an exit hole was located, and the year it had been created was also determined so that the number of years larvae spent in the tree could be calculated. Also, all species located around trees with exit holes created by the Asian longhorned beetle were surveyed to assess their suitability as hosts for the beetle. In a nutshell, information on host suitability, larva behaviour, within-tree colonization patterns, dispersal patterns among trees in the landscape, and molecular relatedness of the beetle was collected and is expected to lead to improved detection and sampling methods, as well as increased survey accuracy. Over 500 trees were completely dissected to collect the above information.

The second portion of this presentation reported on the status of three studies currently underway involving the insecticide imidacloprid®. The objective of the first study was to perform a comparative assessment of the uptake and fate of imidacloprid following soil or stem injection to high-value maple trees for control of Asian longhorned beetle. The high pressure (Echo) soil injections took place on 6 June 2006 on silver maples (four trees; avg DBH = 30 cm) and sugar maples (four trees; avg DBH = 26 cm) using Merit (75% a.i.) at 0.56 g a.i./cm DBH. For each tree, there were eight injection sites on two circles at the half and full drip-lines, for a total of 16 injection sites/tree. Stems were injected either with Confidor® (200 g/L) or EcoPrid® (50 g/L) at a rate of 0.25 g a.i./cm DBH between 7 and 9 June 2006 using the EcoJect® system. Four silver maple (avg DBH = 31 cm) and four sugar maple (avg DBH = 26 cm) were treated with each formulation. The residues of imidacloprid from soil, foliage, and cortical tissue samples collected at regular intervals from the treated trees are being quantitatively determined by HPLC-DAD analysis.

The objectives of the second study were to assess the operational feasibility of Confidor®/EcoJect® injections and to estimate the potential of trunk injections of Confidor® for controlling Asian longhorned beetle by relating foliar residue levels in each species with existing LC values from adult feeding bioassays (Wang et al. 2005). Fifteen tree species were injected: Norway maple, silver maple, Manitoba maple, sugar maple, red maple, Amur maple, white birch, willow, poplar, American elm, Siberian elm, Sycamore, mountain ash, horsechestnut, and hackberry. The DBH of these trees varied between 18 and 44 cm. Five trees of each species were treated between 26 and 29 June 2006 at a rate of 0.25 g a.i./cm DBH. Two leaves were sampled from each quadrant of the mid and upper crown (total of 16 leaves per tree) at 1, 3, 6, 9, 12, and 15 weeks after treatment. For most species, the injection of the whole amount was completed in less than 1 hour. The foliar residues of imidacloprid are being quantitatively determined by HPLC-DAD analysis.

The objectives of the third study, conducted in accordance with OECD guidelines for GLP compliance and currently ongoing, was to determine whether imidacloprid contained in leaves that fall from systemically-injected trees pose a risk of harm to non-target decomposer organisms or processes. Comparisons to risk resulting from direct exposure to imidacloprid concentrations (i.e., soil injection and leaching) were also incorporated in the study.

REFERENCE

Wang, B., R. Gao, V.C. Mastro and R.C. Reardon. 2005. Toxicity of Four Systemic Neonicotinoids to Adults of *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Journal of *Economic Entomology* 98(6):2292-2300.

ASIAN LONGHORNED BEETLE SURVEY, REGULATORY, AND CONTROL

DETECTION AND MONITORING OF THE ASIAN LONGHORNED BEETLE: UPDATE ON SENTINEL TREE, ATTRACT-AND-KILL, AND ARTIFICIAL LURE STUDIES

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ABSTRACT

Survey for trees infested by the Asian longhorned beetle (ALB), *Anoplophora glabripennis*, is based upon visual inspection of known host trees within a specified radius from trees showing signs of attack. However, visual surveys have been reported by USDA-Animal and Plant Health Inspection Service (APHIS) to be 33 to 60 percent effective, depending upon the method of visual survey (i.e. ground survey, bucket-truck survey, or tree-climber survey). There are currently no methods designed specifically to detect and monitor adult *A. glabripennis*, such as sentinel trees or attractants. Therefore, the objectives of the research reported here are: 1) to develop sentinel trees for detection of adult ALB, 2) to develop an attract-and-kill strategy for monitoring adult ALB, and 3) to develop of an artificial lure for detection and monitoring of adult ALB.

SENTINEL TREES STUDIES

The objectives of the studies reported here were to: (1) evaluate the relative attractancy of ALB to five key tree genera utilized by ALB as hosts in China (*Tilia*, *Eleagnus*, *Salix*, *Populus*, and *Acer*), (2) to evaluate the effects of wounding on the attractancy of ALB to *Acer mono* and *Acer negundo*, (3) to evaluate the relative attractancy of ALB to *Acer mono*, *Acer platanoides*, and *Acer truncatum*, and (4) to evaluate the efficacy of *Acer mono* to attract ALB from ALB-infested *Acer negundo* landscape trees under varying ALB population levels. Results from replicated field studies showed, to date, that: (1) ALB are significantly more attracted to *A. mono* than to *Tilia paucicospapa*, *Eleagnus agustifolia*, *Salix babylonica*, and *Acer negundo*, (2) ALB are significantly more attracted to *A. mono* than to *Acer platanoides*, the key maple species attacked in the U.S., and (3) ALB are significantly more attracted to *A. mono* than to *Acer truncatum*, a sister species of *A. mono* in China. Results also showed that wounding *A. mono*, by adult feeding or artificial methods, significantly enhanced ALB attraction, particularly of female beetles, indicative of response to host odors. Studies also showed that ALB attraction to *A. mono* occurs during both peak and declining ALB population levels and that *A. mono* is capable of attracting adult beetles out of large *A. negundo* landscape trees. These results provide the basis for using *A. mono* for detection and/or monitoring of adult ALB.

ATTRACT-AND-KILL STUDIES

The objective of the studies reported here was to determine if potted *A. mono* trees treated with Scimitar® (an encapsulate pyrethroid) altered the attractancy of ALB to *A. mono.* Results from studies initiated in 2006 showed that ALB attraction, particularly of female ALB, was not altered by treating potted *A. mono* with Scimitar® at either 300mg a.i./L or 450mg a.i./L. Although studies will continue in 2007, these results provide the preliminary basis for using *A. mono* for monitoring of adult ALB.

ARTIFICIAL LURE STUDIES

The objectives of the studies reported here were: (1) to isolate and identify the volatiles emitted by *A. mono* that are electroantennographically active and (2) to identify blend(s) of *A. mono* host volatiles that are attractive to ALB in an olfactometer bioassay. Results from GC-EAD studies have identified a group of antennally active *A. mono* host volatiles. Additionally, results from initial olfactometer studies conducted in 2006 have identified blend(s) of host volatiles that are significantly attractive to adult female ALB. Olfactometer studies are continuing, and field studies will be conducted in 2007.

INCIDENCE OF ASIAN LONGHORNED BEETLE INFESTATION AMONG TREATED TREES IN NEW YORK

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ABSTRACT

Treatment of uninfested host trees with the systemic insecticide imidicloprid is an important component of the eradication program for Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Coleoptera: Cerambycidae). From the year 2000 to 2006, nearly 700,000 treatments have been applied to almost 250,000 individual trees in Illinois, New York, and New Jersey (about half of these in New York). To gauge success of the treatment program and to be alert to potential problems, we have been keeping track of the treatment history of all trees found with ALB damage. Infested treated trees are dissected, the number of ALB egg sites and exit holes are counted, and the date of origin and developmental status of the life stages are determined in the laboratory. Growth-ring analysis is used to date the injury.

Of 8,372 infested trees discovered and removed over the course of the program, only 104 had been treated (101 of them in New York). Removal of trees despite treatment is not a cause for concern, however.: we know that uptake and distribution of the chemical within a tree can be problematic, so 100 percent protection after a single application is not really expected. Nonetheless, signs of treatment problems would be: (1) an adult emerging the year after treatment (indicating that an egg laid within a few months of treatment had survived), (2) a live larva found several months after treatment (for the same reason), (3) infestation after two or more consecutive years of treatment (which should have left higher, more uniform residues of imidicloprid), or (4) substantial numbers of insects surviving a treatment. An adult emerging in the same year as a tree's first treatment is not a concern because the egg was laid well before treatment, and the larva was probably in a late stage of development, protected in the xylem, at the time of application. In fact, only an emergence hole provides concrete evidence that an ALB has survived treatment, because a larva found alive when a tree is removed would not necessarily have completed development. Among 62 treated trees in New York bearing one or more exit holes, in 31 cases, the emergence occurred either before or within a few months of the first treatment or at least two years after the last treatment, leaving just 31 examples of post-treatment survival that might reflect a problem.

These are not all clear-cut examples of treatment failure. Treatment records for New York from 2001 and 2002 are unreliable because they do not include specific addresses. Although maps of the treated areas do exist, many individual properties were skipped for treatment because of access issues. In addition, ALB damage was not precisely dated by growth-ring analysis until late 2003.

In all, there were just six (6) cases with concrete evidence of adult emergence during the year after treatment. Five of these were large maples (DBH ≥ 51-66 cm = 20-26 in.) treated in 2004 by soil injection at a single location (Mt. Olivet Cemetery, Queens). Soil conditions, application problems or insufficient dosage may have been responsible for the incomplete protection. Still, only six ALB adults emerged from these five trees. Five trees in Brooklyn, all treated by soil injection in 2005, were found with live larvae in June 2006 (before that year's emergence period). Given the late date, it is quite possible, though not certain, that these larvae would have completed development to adulthood. The sixth tree, a 25 cm (10 in.)-diameter maple in Queens treated in 2003 and 2004 by the Mauget trunk injection system, had three emergence holes dated to 2003 and 2004.

Program-wide, only 11 out of nearly 250,000 at-risk, treated trees in New York, Illinios, and New Jersey (0.004%) have been found with strong evidence that some ALB may have escaped the effects of chemical treatment, and these produced only nine adult ALB. Such a low rate of survival testifies to the outstanding success of the treatment program. It is almost certain that the imminent eradication of ALB in Chicago (to be declared in 2008, pending four years of negative survey results) can be attributed in large part to this strategy.

NATURAL ENEMIES OF NATIVE WOODBORERS: POTENTIAL AS BIOLOGICAL CONTROL AGENTS FOR THE ASIAN LONGHORNED BEETLE

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ABSTRACT

Asian longhorned beetle (ALB), *Anoplophora glabripennis* (ALB), is among the high-risk invasive species that recently invaded the U.S. from China. ALB has attacked 25 deciduous tree species in 13 genera in Northeast, most notable seven maple species. Biological control efforts for ALB have been limited in China. Thus, the objectives of studies reported here include: 1) identifying the native cerambycid wood borers and associated North American native natural

enemies found infesting red maple (*Acer rubrum*), pignut hickory (*Carya glabra*), mockernut hickory (*Carya tomentosa*), and Virginia pine (*Pinus virginiana*) stressed by felling, full-girdling, and half-girdling; and 2) evaluating the efficacy of the native natural enemies to parasitize ALB within infested bolts in quarantine. To date, numerous cerambycids and parasitoids, including braconids, ichneumonids, and chalcidoids, have emerged from all four tree species treated by each of the three methods. Most similar to ALB, cerambycids have been recovered from the girdled and half-girdled red maple trees. While most of the cerambycids, braconids and ichneumonids have been identified to genus, they are awaiting taxonomic identification. To date, individuals of most parasitoid species have been caged with the ALB-infested bolts. Most importantly, at least two braconid species (one of which is an *Atanycolus* sp.) and one ichneumonid species appear to parasitize ALB in infested bolts. Furthermore, parasitization by one of the braconid (not *Atanycolus*) species resulted in successful parasitization, development, and emergence of F1 offspring from the ALB-infested bolts. This is significant in that it provides the first concrete evidence of a native natural enemy successfully parasitizing ALB outside of Asia.

EFFICACY OF LAMBDA-CYHALOTHRIN FOR CONTROL OF THE ASIAN LONGHORNED BEETLE

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ABSTRACT

Asian longhorned beetle (ALB), *Anoplophora glabripennis*, is among high risk invasive species that recently invaded the U.S. from China. The methods used to eradicate ALB within North American infestations have thus far included: visual survey for ALB-infested trees, removal of ALB-infested trees, removal of all host trees within a given radius (e.g., 400 meters) of known ALB-infested trees, and/or treatment with a systemic insecticide (e.g., trunk injection or soil injection) of all host trees within a given radius (e.g., 400 meters) of known ALB-infested trees. To date, over 32,000 and 23,000 high value shade trees have been removed

in the US and Canada, respectively, in an effort to eradicate ALB and prevent its permanent establishment.

The objective of the research reported here was to investigate the potential development of an alternative control method based upon selective application of the pyrethroid, Lambda-Cyhalothrin, as an encapsulated insecticide under the trade names of Demand®CS or Scimitar® CS. More specifically, the objectives of the initial studies include: (1) to determine the Lethal dose (24hr) and Knockdown dose (1 minute) of Lambda-Cyhalothrin, the active ingredient of Demand® and Scimitar®, applied topically to adult ALB, and (2) to determine the residual activity of Demand® by exposing adult ALB to treated bands (Bands: 600 X 300 Denier, 7 Mil. PVC backed polyester fabric; Source: American Home and Habitat, Inc.; Product # FPV600B; Contact: www.ahh.biz). The objectives of the subsequent studies include: (1) to determine the residual activity of Demand® by exposing adult ALB to treated caged Acer mono trees, and (2) to determine the efficacy of Demand® and Scimitar® by spraying ALB-infested Acer negundo street trees.

LETHAL AND KNOCKDOWN DOSE OF LAMBDA-CYHALOTHRIN

Results from the 24-hour lethal dose (LD) studies showed that the: (a) LD_{50} = 0.13639µg/beetle (CI = 0.04717, 0.21372), and (b) LD_{90} = 0.78461µg/beetle (CI = 0.47376, 3.03056). Results from the knockdown (KT) studies showed that the: (a) KT_{50} = 69.28298 sec (CI = 58.87043, 84.27864), and (b) KT_{90} =282.78445 sec (CI = 187.77320, 624.53467).

RESIDUAL ACTIVITY OF DEMAND®TREATED DENIER BANDS

Demand®CS provided 100 percent mortality for 90 days when applied to bands at 450mg a.i./L and 600mg a.i./L. Additional field studies in which Demand®CS is applied to bands are needed. Exposure of adult *A. glabripennis* to a lethal dose of Demand®CS is based upon several factors, including: (1) the willingness of adult beetles to walk onto and across treated bands, and (2) the number and position of bands wrapped around branches in trees at risk. We recently evaluated the willingness of adult beetles to walk onto and across different materials. Results showed that that, among the materials tested, adult *A. glabripennis* most readily walked onto and across denier, while they hesitate to walk onto burlap. We have been evaluating where within different tree species adult *A. glabripennis* most commonly reside, particularly adult female *A. glabripennis* as they lay eggs during the first year of colonization. These studies will pin point where bands should be placed within trees so that they have the highest probability of killing adult beetles and preventing colonization.

RESIDUAL ACTIVITY OF DEMAND® -TREATED POTTED *ACER MONO* TREES

Demand®CS, prepared in tap water at dosages of 94.0mg a.i./L, 204.24mg a.i../L, and 315.19mg a.i../L, was applied to each of 10 potted *Acer mono* trees. Tap water was applied to 10 control trees. Each tree was then individually caged using hardware cloth. On the 1st, 8th, 15th, 22nd, 29th, and 36th day post-treatment (DPT), two male and two female field-collected ALB were randomly introduced into each of the forty cages. Adult beetle mortality was assessed after 24 hours. Beetles failing to exhibit leg movement when prodded with a fine bush were scored as dead. Results from the cage study indicate that Demand®CS can cause 95% and 90% 24-hour mortality for 29 days when applied to potted trees at 204.24mg a.i../L and 315.19mg a.i../L, respectively. However, because adult beetles were commonly found seeking refuge in cooler areas of the cage (e.g., holes or cracking in the soil surface or underneath the lip of the pots) that had not been treated with Demand® CS, these results likely **underestimate** the mortality that would occur on large landscape treated-trees and on treated potted trees that are adequately shaded and/or on which all surfaces are treated. Additional field studies where Demand®CS is applied to potted sentinel trees for monitoring the relative seasonal abundance of adult *A. glabripennis* are planned. Most importantly, these additional studies will determine if Demand®CS alters the attractiveness of the sentinel trees. However, where potted sentinel trees are strictly used for detection of adult *A. glabripennis*, treating sentinel trees with Demand®CS or any other insecticide is **not necessary** since the unique signs of feeding left by adult beetles are sufficient for detection.

EFFICACY OF DEMAND® CS- AND SCIMITAR® CS-TREATED *ACER NEGUNDO* URBAN-LANDSCAPE TREES

Results showed that exposure to 300mg a.i../L and 600 mg a.i../L Demand®CS provided overall population control of 99.0% (27 live/2,765 dead) and 98.4% (43 live/2,717 dead), respectively, over the 58-day test period in 2005 (14 July to 9 September). Results also showed that exposure to 300mg a.i../L and 600 mg a.i../L Scimitar®CS provided overall population control of 98.4% (15 live/926 dead) and 98.4% (13 live/791 dead), respectively, over the 67-day test period in 2006 (13 July to 17 September). This shows that Demand® is highly effective at controlling adult ALB. However, it is important to note that the treated and check (control) plots were spatially very close to one another, and as a consequence, the live ALB that continued to be found within the treated trees were largely immigrants from the untreated check plots. We are confident that, had the treated and check plots been farther apart and/or a treated buffer been included between the treated and check plots, it highly probable that percent control would have been consistently maintained at ca. 100 percent. To obtain a direct measure of immigration, additional data analysis is currently in progress at this time, comparing the number of exit holes/tree for 2005 and 2006. This will aid in determining the relative proportion of ALB within trees that resulted from emergence versus immigration. Furthermore, because our goal is to prevent oviposition by live female ALB and because ALB in Yanji, Jilin, have a 24-month life cycle, exit holes/tree will be evaluated in 2007 and 2008. This data will provide a measure of the efficacy of Demand® CS and Scimitar® CS to prevent attack by ALB.

RESEARCH UPDATE ON THE SYSTEMIC INSECTICIDES FOR THE CONTROL OF THE ASIAN LONGHORNED BEETLE

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ABSTRACT

Evaluation of systemic neonicotinyl insecticides were continued in 2005 and 2006. The objective was to develop effective chemical control techniques to be integrated into the eradication program of the Asian longhorned beetle, *Anoplophora glabripennis* Motschulsky. **Note:** All results are preliminary. Mentioning of a product, or a trade name or company does not imply the endorsement of the authors or their affiliated governmental agencies or institutions.

METHODS

Two sites were selected, one in Gansu Province, China, in 2005 with white poplar (average DBH=10cm) and another in Henan Province in 2006 with willow (DBH=15-20cm). We tested imidacloprid, dinotefuran, thiamethoxam, clothianidin, acetamiprid, and thiacloprid. In addition, two biopesticides, spinosad and emamectin were also tested. Delivery methods for each year were listed in Table 1(for 2005) and Table 2 (for 2006)

To assess the efficacy of insecticide treatment, we checked adult mortality in flight season, mortality of caged beetles, the number of exit holes per tree, number of egg sites, and mortality of all stages of the beetle in tree in October. Residual effects of the insecticide treatment were also evaluated by checking the number of dead beetles under treated trees and counting number of exit holes on treated trees one to two years after insecticide application.

RESULTS

For 2005 treatment, within approximately three months after the application, more dead beetles were found under treated trees for all treatments than the untreated, especially under trees trunk injected with imidacloprid, acetamiprid, thiacloprid and dinotefuran (Figure 1a). The last 2-week counts of the dead beetles under trees (Figure 1b) were comparatively higher for soil injected imidacloprid than it was for the whole period (52 days), probably because it takes time for a significant amount of soil applied insecticide to be translocated into different parts such as twigs and leaves of the tree where ALB adults feed.

Dissection of treated trees in October 2005 found more egg sites with the untreated trees than with treated trees, especially trees trunk injected with imidacloprid, acetamiprid, and thiacloprid. The mortality of larvae was significantly higher for ALB in imidacloprid-treated trees and thiacloprid through trunk injection than in the untreated trees. All stages combined, mortality was higher for imidacloprid through soil drench and trunk injection.

For the 2005 application, dead beetle counts under treated trees in July and August 2006 were higher for trees treated with imidacloprid through soil application (drench and basal injection), acetamiprid though trunk injection, and dinotefuran through trunk injection than for the untreated trees.

In addition, when the trees treated in June 2004 were dissected in October 2005, we found that the number of new exit holes was much lower in all insecticide treatments than that in the untreated trees.

For trees treated in June 2006, the number of dead beetles under insecticide treated trees where higher than under the untreatedtrees, especially for imidacloprid applied thorough trunk or soil basal injection and for thiamethoxam and dinotefuran through soil basal injection (Figure 2). More results will be available when all data analyses for the 2006 insecticide test are completed.

ACKNOWLEDGEMENTS

We thank the following people and companies for providing help or products: Dr. Phillip Lewis, Mr. David Cowan (USDA APHIS Otis Lab), Dr. Tonghai Zhao (Chinese Academy of Forestry), Jingtai Forestry Protection Station, Kaifeng Forestry Protection Station; Valent U.S.A. Co., Creative Sales Inc., J.J. Mauget Co., Arborjet Inc., Syngenta Crop Protection Inc., and Veyong Bio-chemical Co. Ltd.

Table 1. ALB Insecticide treatment in June 2005

Insecticide	Delivery Method (abbreviations in parentheses)	Formulation
Imidacloprid	Soil Injection: Soil Drench (ImidaSoilDrench)	Merit 75 WP
Imidacloprid	Soil Injection: Basal Injection (ImidaSoilbasal)	Merit 75 WP
Imidacloprid	Trunk Injection: Mauget Gen II Capsule (ImidaMauget)	10%, 4 ml Solution
Dinotefuran	Trunk Injection: Mauget Gen II Capsule (DinoMauget)	10%, 4 ml Solution
Thiacloprid	Trunk Injection: Modified Arborjet with USDA Tip (ThiacTrunk)	10% solution
Acetamiprid	Trunk Injection: Modified Arborjet with USDA Tip (AcetamiTrunk)	9.2% solution
Spinosad	Cover spray (SpintoCover)	SpinTor® 2 SC Naturalyte®
Control	Not treated (CK)	

Table 2. ALB Insecticide treatment in June 2006

Insecticide	Delivery Method (abbreviations in parentheses)	Formulation
Imidacloprid	Soil Injection: Basal Injection (Imida_BSoil)	Merit 75 WP
Imidacloprid	Trunk Injection: Mauget Gen II Capsule (Imid_M)	10%, 4 ml Solution
Thiamethoxam	Soil Injection: Basal Injection (Thiam_BSoil)	Flagship 25WG
Dinotefuran	Soil Injection: Basal Injection (Dino_BSoil)	Safari 20 SG%
Emamectin	Trunk injection (Emem_T)	62.4% WP by Veyong, China
Emamectin	Cover spray (Emem_Spray)	1% EC by Veyong, China
Control	Not treated (CK)	

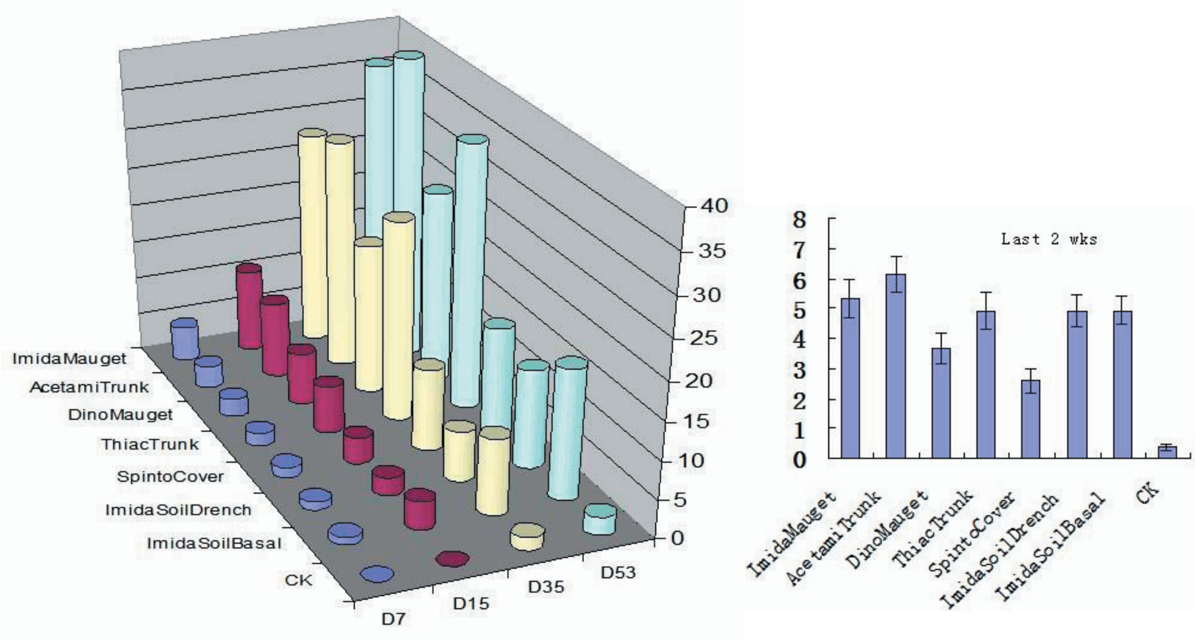

Figure 1. The number of dead ALB adults on ground under trees treated in 2005.
1a: total for each tree from June 25 to Aug 16, 2005 (52 days);
1b: total for each tree from August 5 to Aug 16, 2005 (3 weeks)

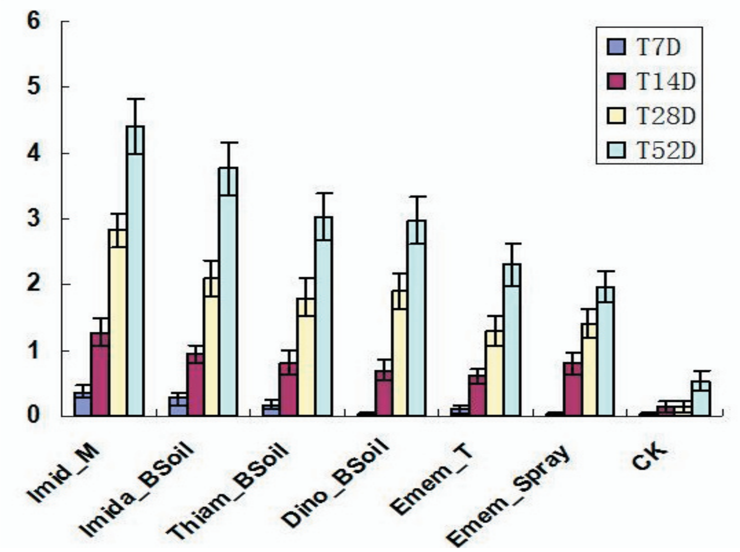

Figure 2. The number of dead ALB adults under trees treated in 2006 (from 7/2/06 to 8/23/06). T7D to T52D: total for 7, 14, 28, and 52 days, respectively.

PESTICIDE DISTRIBUTION, SAMPLING, AND RESIDUE ANALYSIS: EMPLOYMENT OF ELISA FOR IMIDACLOPRID DETECTION

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ABSTRACT

The Asian longhorned beetle (ALB), *Anoplophora glabripennis* Motschulsky, has established populations in the U.S. in New York and New Jersey, and there is a population in Chicago that is close to being declared eradicated. A key component in the success of the ALB eradication program is the chemical treatment of at-risk host trees with the systemic insecticide imidacloprid. Since this program began in 2000, almost 700,000 treatments have been applied to trees.

In order to identify and recommend the best treatment methods to the ALB program, we have designed research studies to characterize how imidacloprid spreads within a tree and how best to accurately sample and determine the residue levels present in treated trees.

Residues of imidacloprid in plant tissue are typically determined by extraction of the sample in an organic solvent followed by analysis of the residue by either High Pressure Liquid Chromatography (HPLC) or by Gas Chromatography-Mass Spectrometry (GC-MS). These methods are expensive and time-consuming, and therefore are not efficient for processing of large numbers of samples. Analysis by these methods can lose up to half of the imidacloprid in a sample, and a repeat analysis of the same sample can result in a 13 to 20 percent difference in estimated levels of insecticide. An Enzyme Linked Immunosorbent Assay (ELISA) is commercially available and can rapidly analyze large numbers of samples with good reliability, great sensitivity (up to 1,000-fold greater than standard methods), and at a lower cost.

Initial efforts by us at characterizing the imidacloprid content within hardwood trees utilized xylem sap collections, but this method resulted in extremely variable residue values among samples taken from a particular tree (mean variation of 75 percent for five samples/tree, n=151). A method was then developed and verified that could process and extract imidacloprid from leaves for use in the ELISA assay (up to 95 percent recovery). There is low variability with this method because the data generated are from a composite sample of leaves taken throughout the tree. The residue values generated by leaf extractions can also be correlated to the LC_{90} toxicity values for ALB, allowing us to directly determine if enough imidacloprid is present within treated trees. Experience in sampling thousands of trees has shown that typical residue values from sap extractions of treated trees three months post-injection range from 20 to 200 ppb, while values from leaf extractions of trunk and soil injected trees typically range from 50 to 200 ppm and 15 to 50 ppm, respectively.

Imidacloprid residues in leaves taken from the lower, mid-, and upper-canopy regions of a tree do not reveal any trends, suggesting that imidacloprid is distributed evenly throughout a tree (Figure 1). Eight trees were trunk injected with imidacloprid at a rate of 0.16g per centimeter DBH (diameter at breast height); London plane trees averaged 18.6” DBH and Norway maple trees averaged 15.4” DBH. The error bars displayed in Figure 1 represent the within-sample variation of three sub-samples of a particular sample. Within-sample variation in this study for most of the samples was very good (below 5 percent), indicating that our extraction method is reliable and reproducible. The study results suggest that lower canopy sampling of foliage from the ground is sufficient, saving the extra time and expense of bucket trucks and tree climbers for sample collection.

The Asian longhorned beetle will feed on leaves and leaf stems, but prefers to feed upon young twigs, especially during female egg maturation feeding. To determine the relationship of imidacloprid content between twigs and leaves, sixty samples were taken from ash trees treated by soil injection. Leaves were removed from the samples, and the associated twigs and small branches were finely ground and processed for extraction. Results show a significant correlation between leaf and twig imidacloprid content of about 1:6.3 ($r^2=0.62$, $P<0.001$; Figure 2). Adult ALB tend to feed on only the outer portion of twigs, so an extraction of just these tissues and not all the woody tissue is anticipated to result in a value closer to that seen in leaves.

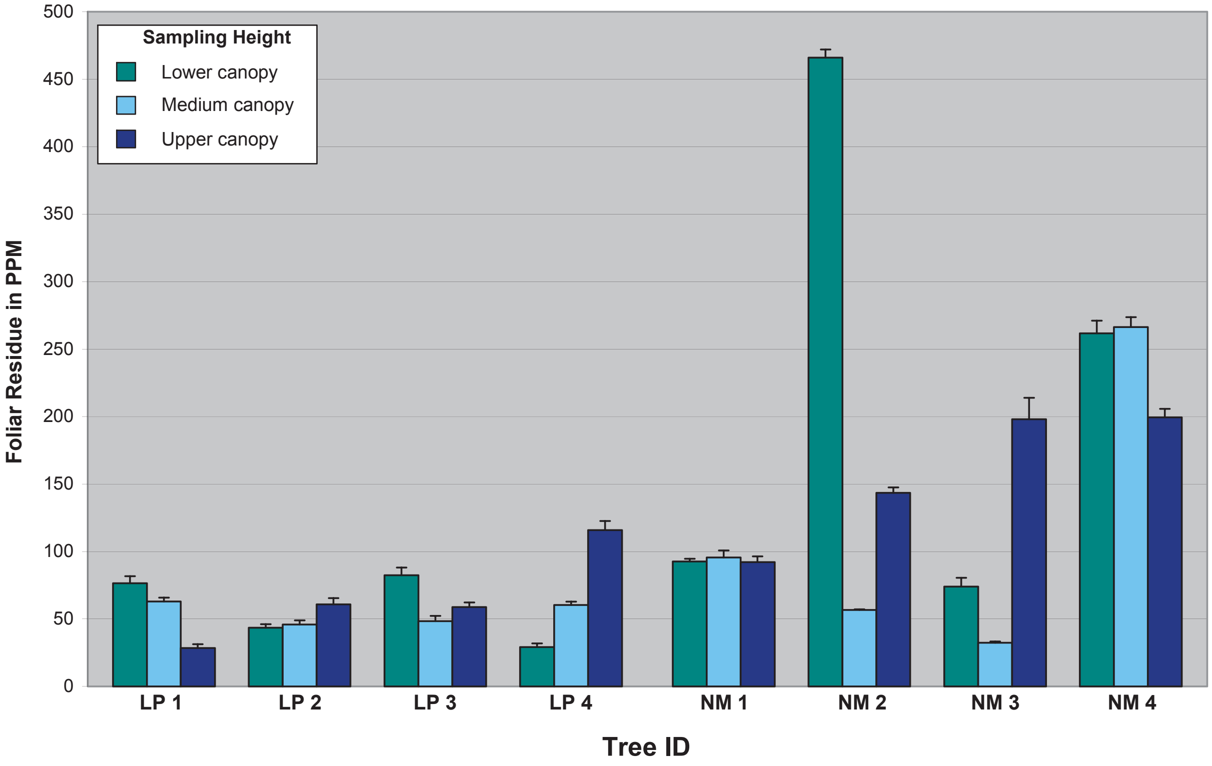

Figure1. Imidacloprid distribution at different canopy heights and within sample variation.

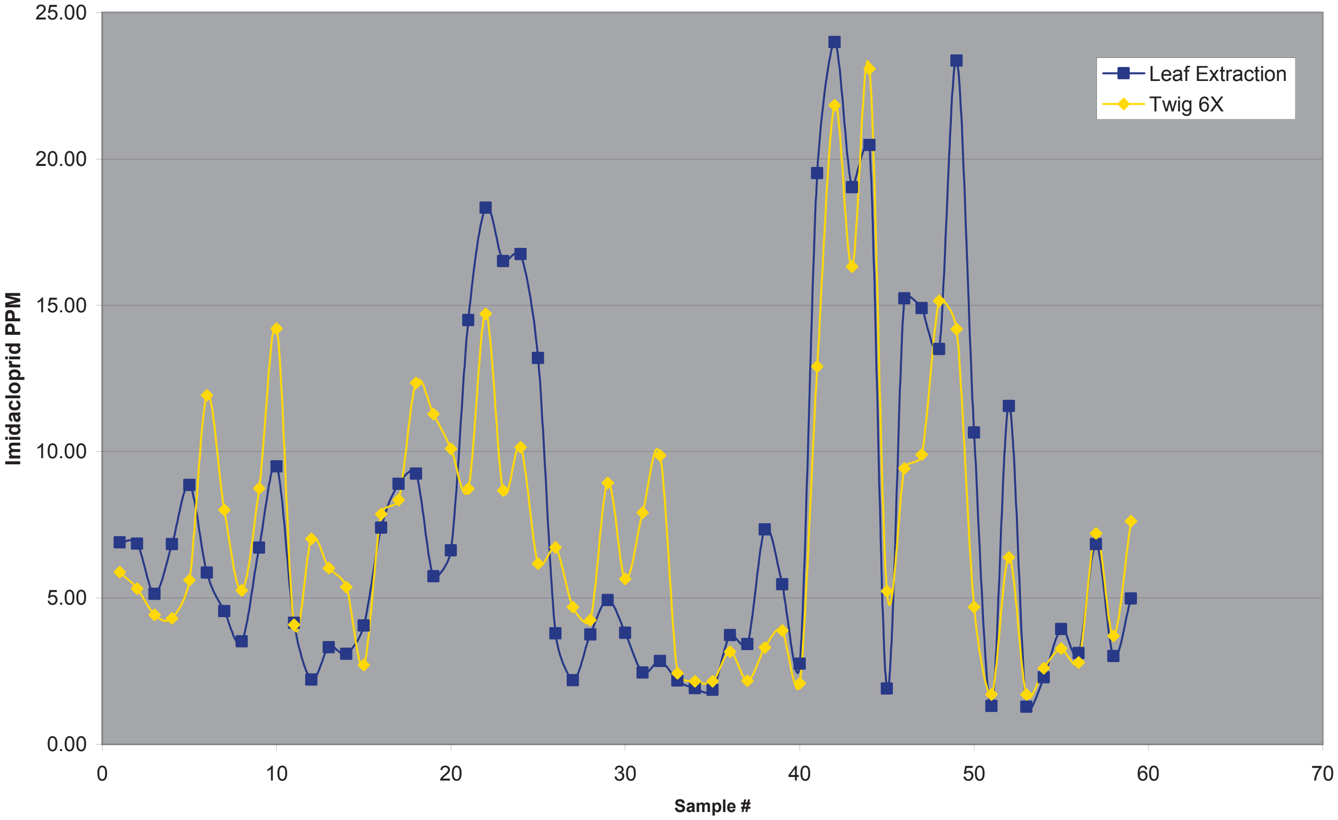

Figure 2. Correlation between imidacloprid residues found in leaf and twig samples.

POST-TREATMENT INSECTICIDE RESIDUE LEVELS IN TREES FOLLOWING TRUNK AND SOIL APPLICATIONS

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ABSTRACT

The Asian longhorned beetle (ALB), *Anoplophora glabripennis* Motschulsky, has established populations in the U.S. in New York and New Jersey, and there is a population in Chicago that is close to being declared eradicated. A key component to the success of the ALB eradication program is the chemical treatment of at-risk host trees with the systemic insecticide imidacloprid. Since this program began in 2000, almost 700,000 treatments have been made.

In order to meet the various needs of the ALB treatment program, research trials are regularly conducted to identify more efficient, economical treatment methods, as well as those methods best suited for special situations. One of these special situations is treatment wooded areas with a soil application of imidacloprid. Label restrictions severely limit this type of application to only a handful of trees per acre, resulting in the much more expensive and time-consuming trunk application in these environments (Table 1). A second study investigated the potential of treating trees every other year and sampled trees that had been treated by soil and trunk injection.

Imidacloprid was applied to maple trees growing in a wooded environment at the full labeled rate, one-half (low rate on the label), and one-fourth the maximum labeled dose. Each of the treatment groups contained two sets of trees: one set averaged 4.0 inches diameter at breast height (DBH) and the other 9.0 inches DBH. Tree foliage was sampled at 1 and 4 months post-application and analyzed in the laboratory for imidacloprid residue levels. Foliage was collected for residue analysis at 3, 12, and 14 months post-treatment for those trees treated only once in a two-year period.

Data on imidacloprid residues found in trees one year post-application was only available for one trunk injection study at the time of this meeting. This study used an Arborjet VIPER tree injection unit to apply the trunk injections at twice the amount of imidacloprid as currently used in the ALB program. Two tree species were used in the study; Norway maple trees (average 10 inches DBH, n=48) and London plane trees (average DBH 13.5 inches, n=49). Mean chemical residue levels in parts per million three months after treatment were 68.0 ± 7.56 (s.e.) and 157.9 ± 19.1 (s.e.) for London plane and Norway maple trees, respectively. Mean imidacloprid levels in these same trees 12 months post-application was less than 3 ppm, well below the desired level of 7 ppm, based on the calculated LC_{90} for ALB (Wang et. al. 2005; JEE 98:2292-2300).

Reducing the amount of soil applied imidacloprid results in a corresponding reduction in residue levels at one month and four months after application. Residue levels from soil applications are typically low at one month post-application, and for the groups in this study the average values were less than 2 ppm, but general trends for this sampling period were similar to the four-month data. The larger trees had lower mean average residue levels for each of the three treatment groups, but this difference was not statistically different when comparisons were made within a treatment group (Figure 1). For the small trees, the mean residue values at the full rate were statistically different than those trees in the ¼x rate group ($p<0.03$). For large-diameter trees, the mean residue values at the full rate were statistically different from the other two treatments ($p<0.001$). The full labeled rate (1x) for both small and large DBH trees resulted in imidacloprid residue levels that were well above the LC_{90} target level.

Reducing the amount of imidacloprid in soil treatments results in an increase in the incidence of marginal residue levels in the maple trees in this study, and is not recommended for the ALB treatment program.

REFERENCE

Wang, B., G. Ruitong, V.C. Mastro, and R.C. Reardon. 2005. Toxicity of four systemic neonicotinoids to adults of Anoplophora glabripennis (Coleoptera: Cerambycidae). *Journal of Economic Entomology* 98(6):2292-2300.

Table 1. Tree stand densities in New York City and imidacloprid needed for treatment.

					Max Labeled Rate	Low Labeled Rate
LOCATION	**# Trees**	**Approx DBH (in.)**	**Approx Acres**	**DBH/ Acre**	**Lbs Imidacloprid Needed (per acre)**	**Lbs Imidacloprid Needed (per acre)**
Queens, Golden And Crocheron Parks	3,306	29,818	32	932	2.92	1.46
Queens, Alley Pond Park Complex	10,043	72,605	120	605	1.89	0.95
Central Park, North Woods	1,800	23,400	90	260	0.81	0.41 *
Central Park, Ramble	1,328	14,066	38	370	1.16	0.58
Brooklyn, Evergreen Cemetery	629	13,905	225	62	0.19 *	0.10 *
Brooklyn, Highland Park	5,154	28,082	33	851	2.66	1.33
Brooklyn, Prospect Park	4,939	57,337	526	109	0.34 *	0.17 *

* falls within labeled rate

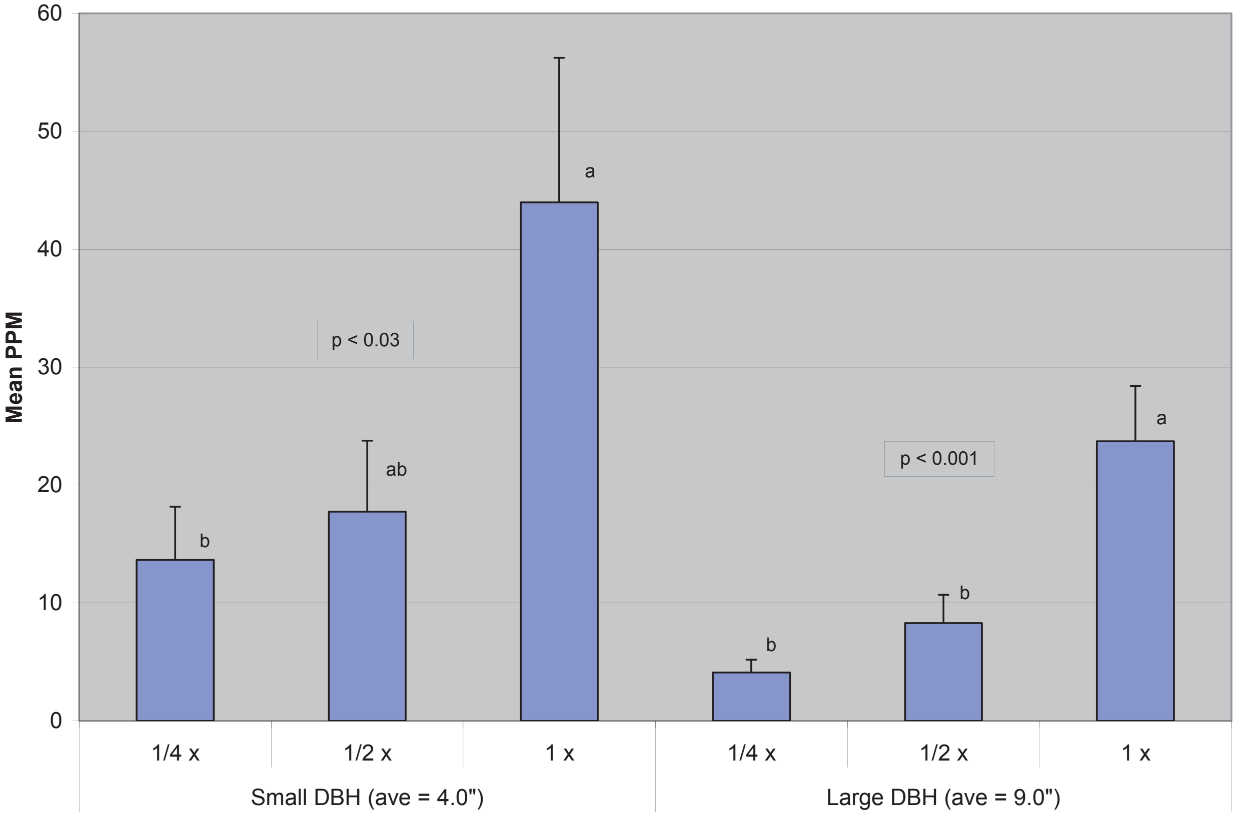

Figure 1. Four-month imidacloprid residue in foliage.

ASIAN LONGHORNED BEETLE BIOLOGY, REARING, AND PROGRAM MANAGEMENT

MICROBIAL COMMUNITY COMPOSITION AND WOOD DIGESTION IN THE GUT OF THE ASIAN LONGHORNED BEETLE

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ABSTRACT

Like other wood-feeding insects, Asian longhorned beetle (ALB) must acquire is nutrition through degrading cellulose. Based on other insect species, cellulolytic enzymes in the ALB may originate from gut symbionts, from the insect itself, or some combination of the two. We surveyed the gut of mid-instar larval ALB for bacteria and fungal species using culture-independent community analysis. PCR amplification of the total gut DNA for the 16S rDNA region for bacteria and elongation factor alpha-1 region for fungus coupled with cloning allowed us to screen the microbial community. In this study we show that the ALB gut harbors a rich variety of bacteria and fungal species including several unique genera (e.g., *Cellulosimicrobium* sp. and *Fusarium* sp.), known to play a role in wood decay in ALB fed on sugar maple and pin oak. Also, enzyme activity assays show activity of the complete suite of cellulolytic enzymes (endo- and exo-glucanases as well as beta-glucosidases) present in the ALB gut, demonstrating its ability to degrade wood. When the larvae were fed on different host trees with different host suitabilities, the enzyme activity level of these cellulose degrading enzymes changed, suggesting that enzyme activity may play a role in host tree suitability. We plan on furthering this research by investigating how the gut community changes through the insect's life history to understand better how the insect acquires and maintains the necessary gut community required to be able to feed on woody tissue.

REPRODUCTIVE BEHAVIORS OF ASIAN LONGHORNED BEETLE

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ABSTRACT

There is a critical need for information on the reproductive behavior of the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), to provide the biological basis for predicting population dynamics, especially as the population size declines due to eradication efforts. A series of four experiments using three strains (New York, Illinois, and China) was designed to document the following: 1) the reproductive behaviors (both mating and oviposition) of a single pair until natural separation or over 6 hours, whichever came first; 2) the number of females a male can successfully mate in its lifetime and how male age affects mating success; 3) the frequency and duration of matings associated with sustained female fertility; and 4) the interactions of all possible combinations (male and female) and sequences of three individuals(MMF, MFM, FFM, FMF).

The reproductive behaviors of *A. glabripennis* are typical of diurnally active species of the subfamily Lamiinae. When a male contacted a female with his antennae, he generally would quickly attempt to mount her and mate. If the female was receptive (did not fight the mounting and allowed access to her genital chamber), he would mate with her immediately after mounting and initiate a prolonged pair-bond. Non-receptive females would exhibit one or more of the following behaviors: run away, kick with hind legs, hit with antennae, make quick turns, fall, or fly. In these cases, the male might abandon his attempt and separate or perform a short antennal wagging courtship behavior. Non-receptive females would generally become receptive after further contacts. During the entire time, the male continuously grasped the female with his front tarsi or both front and middle tarsi. Individual copulation events lasted an average of 2.8 minutes, and 1-10 copulations occurred per event, followed by a male refractory period averaging 95 and 60 minutes, for the New York and Illinois males, respectively. During copulations, the female held her genital chamber open while stationary, walking, or chewing the host for oviposition (the latter occurred more often during later copulation events). Between copulations, the female most often walked or attempted oviposition in the pit she had chewed. The average total time *in copula* was 34 minutes, and this resulted in an average hatch of 56 percent of eggs females laid over their lifetime. Oviposition (0-5 eggs per female) lasted an average of 12 and 10 minutes, for the New York and Illinois females, respectively, on bolts with bark 1-2 mm thick. After a female chewed a pit, she rotated 180°, extended her ovipositor, and used it to find the pit. She then inserted her ovipositor under the bark and used the sclerites at the tip of her abdomen to pry the bark up while lifting her body. After laying an egg, she wiped excretions across the opening using the tip of her abdomen. Females abandoned some pits at various points in the process.

Male beetles at 11-104 days of age were able to copulate successfully (≥ 3 minutes in duration). A copulation was considered successful if the female laid eggs that hatched. Males generally had one or more unsuccessful copulations prior to the first success. Male fertility (as measured by percentage egg hatch of females he copulated with) and copulation success peaked for the three males tested at 3-5 weeks of age then slowly declined until copulation stopped at about week 15. When the males reached 12 weeks of age, they were less agile and less able to grasp and hold a female, which in an unconstrained environment could result in less receptive females escaping. The shortest interval between two successful copulations was 5 minutes (when a second virgin female was presented immediately after a copulation ended). The most times a female successfully copulated with one male was 27, resulting in a total of 1,366 progeny.

On average, females that had copulated 10 times or more (each ≥ 3 minutes in duration) had a significantly higher percentage of viable eggs than did females with only 1 to 5 copulations, though fecundity was unaffected. The number of copulation events did not significantly affect female longevity. These results suggest that over 1 hour of total time *in copula* (excluding mate guarding time) is needed for maximum sustained fertility, as measured by percentage hatch. In nature, this time requirement for copulation could be satisfied by one or more matings of longer duration (multiple copulations), rather than through a series of short encounters.

During the experiment where three beetles (male and female) were put together in different combinations and sequences, our observations suggested that several semiochemicals are likely involved in the mating process. We observed that, when males were added after a female was present, one-third of the males walked slowly, palpating the bark as if following a chemical trail directly to the female, then mounted the female after his antennae contacted her. We also observed that approximately one-third of the females located the males and made the first contact, but were not mounted until after the male contacted the female with his antennae. If an aggressive male to male encounter occurred first, then the percentage of opposite sexes finding each other declined dramatically. When a male mounted a female, he remained with her and fought off both males and females that made contact with them, usually without dismounting. When males dismounted, they were able to relocate the females easily. The loser of a male-to-male encounter flew away 21 percent of the time and ran away 75 percent of the time, which tended to disperse the males and thus ensured that all females would be found. Twice, we observed a male treating another male (that had recently been with a female) like a female, even initiating copulation attempts. These observations suggest that once the two sexes are on the same tree, they will find each other, that a male will stay with a female long enough to ensure she will remain fertile the rest of her life, and that males will disperse themselves to reduce aggressive encounters, thus increasing the likelihood of locating females even when populations are low.

FACTORS THAT INFLUENCE ASIAN LONGHORNED BEETLE PUPATION

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ABSTRACT

The ability to rear Asian longhorned beetle in quarantine continues to be critical to progress in developing/evaluating techniques for the exclusion, detection, and eradication of this serious pest. Understanding factors that influence pupation makes predicting adult emergence more reliable, both in the laboratory and the field. Survival and development of larvae from three populations (Ravenswood, Chicago, Illinois;, Bayside, Queens, New York; and Hohhot, Inner Mongolia, China) were compared for a variety of temperatures and nutritional conditions. The following factors were evaluated: 1) timing of the larva chill period, 2) length and temperature of the larva chill period, 3) larva nutrition and moisture content of diet, and 4) effects of constant and varing temperatures on pupation.

There were marked differences among the three beetle populations observed. Individuals from the China and Illinois populations weighed more than those from New York. Larvae from the Illinois population began pupation sooner than those from New York or China and were least affected by the timing of the larva chill period. The Chinese population was the most sensitive to the timing of chill, possibly indicating that larvae from this population tend to pupate at a later instar, that more individuals may require a chill to complete development, or that some individuals may require more than one year to complete development under some conditions. A larva chill period was not required by all the larvae, an observation consistent with earlier findings. Larvae that had not reached their critical weight for pupation prior to the chill period sometimes required a second chill period before they initiated pupation. The critical weight for pupation appears to vary both within and among populations, which could indicate a high degree of plasticity or genetic variation for this trait. Overall survival decreased when the developmental time decreased before the chill period. Artificially manipulating the timing of the larva chill appeared to be effective in synchronizing adult emergence and increasing pupation.

When larvae were chilled at 15°C, they required a longer chill period to cue them to pupate and in general, it took them longer to initiate pupation after the chill than those held at lower temperatures. Larvae chilled at 5°C had a higher mortality rate than those chilled at 10°C and pupated less synchronously after chill. Poor larva nutrition also lengthened the time from the end of chill to pupation and increased mortality. Drier larva diets seemed to cause larvae to pupate sooner and reduce the need for a larva chill prior to pupation. This would be a necessary survival tactic as larvae that are either in a dying tree or cut infested wood need to pupate as quickly as possible to ensure their survival.

Larvae did not pupate at constant temperatures ≥ 30°C or ≤ 10°C. The number of instars larvae went through before pupation varied from a minimum of five to more than nine depending on the temperature regime and larva nutrition. It took about two years for larvae to complete development at a constant 15°C or if eggs hatched too late to allow larvae to reach the critical weight/instar for pupation before chill in a varying temperature regime.

It appears that there are several factors that can interact to affect Asian longhorned beetle pupation. In the laboratory, temperature and larva diet can be manipulated to make the timing of pupation very predictable. These results improve our understanding of and ability to predict pupation in wood, but also emphasize the complexity of the process. In addition, these results may help explain why insects other than Asian longhorned beetles also appear to require one or two years to complete development as it relates to their geographic location or host quality.

A CONTROLLED STUDY OF THE HEALING RESPONSE OF HOST TREES TO SIMULATED ASIAN LONGHORNED BEETLE DAMAGE

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ABSTRACT

This study was undertaken to examine the healing response of selected genera of trees to simulated damage by the Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Coleoptera: Cerambycidae), while controlling certain key variables in the field. Results will be used to calibrate methods used to date actual ALB injury and to aid the survey program by documenting the appearance of damage inflicted at different times of the year after healing for various periods. Because tree bark is non-living and, in most species, is not shed, evidence of chewed oviposition pits and exit holes remains visible for years. However, over time, radial growth of the tree and the proliferation of callous tissue at the wound site usually distort the evidence of damage so that it becomes difficult to recognize. Exit holes grow completely closed and covered with new bark, sometimes within a year, leaving only a trace of the original injury on the bark surface. We see great variability in the rate of healing and the appearance of old damage, depending on the tree species and, apparently, on when and how long ago the damage occurred.

The experiment started in 2005 and will end in 2007. Thirty-two (32) specimens each of Norway maple (*Acer platanoides*), London plane (*Platanus acerifolia)* and elm (*Ulmus americana, U. pumila,* and *U. parvifolia*) were selected and tagged in the vicinity of Amityville (Long Island), New York. The species were chosen both for their importance as ALB host trees and to include a ring-porous species (elm), a diffuse-porous species (maple), and species that present challenges to surveyors because the bark is shed (*P. acerifolia* and *U. parvifolia*) or responds to injury with gnarled growth (*P. acerifolia*). On each tree, a single limb (approx. 8-10 cm diameter, or 3-4 in.) was chosen and marked with paint. Trees were mapped using GPS and photographed to help us find them later. On three occasions, in early June, late July, and mid-September 2005, damage resembling ALB egg sites and exit holes was applied to the limbs. These dates represent early-, mid-, and late-season points during the period of ALB adult emergence and oviposition. To economize on the number of trees involved and minimize the impact on them, the same limbs were used on all three occasions. A single "injury site" consisted of two simulated oviposition pits near each other and one simulated exit hole about 30 cm (12 in.) distad of the egg sites. The three injury sites (applied seven weeks apart) were separated from each other by approximately 1 m (40 in.) along the limb to avoid interactions among them. Half of the sites were applied to the upper (or adaxial) surface of the limb and the other half to the lower (or abaxial) surface to examine the responses of "tension wood" and "compression wood" and differences due to exposure to the elements. Application dates and locations (upper or lower surface) were assigned at random to the basal, middle, and terminal positions along the limb, but balanced so that two replicates of each species, destined to be sampled on a given date, received upper-surface damage and two received lower-surface damage. Simulated oviposition sites were created by gouging a pit in the bark with a Phillips screwdriver and then inserting a curved needle probe horizontally into the phloem/xylem interface beneath the bark. This was to simulate the mechanical damage caused by a female's ovipositor. The response to this injury includes characteristic dark staining and callous growth that remain visible in the xylem indefinitely. Exit holes were simulated by boring an 11 mm (7/16 in.) hole perpendicular to the limb surface to a depth of 2-3 cm (1-1½ in.) using a wood bit and cordless electric drill. Tools were sterilized with isopropyl alcohol between uses.

Four times a year (approx. March 15, June 15, September 15, and mid-winter), limbs are removed from four trees of each genus, selected at random. These sampling dates represent early-, mid-, and late-season times during a tree's growth period and a time of dormancy. The experiment continues for two years, so that healing is evaluated eight times post-injury. The total number of sites evaluated is thus: three genera of trees x three injury dates x eight sampling dates x four replicates = 288. Tree climbers working for the ALB program applied the simulated damage and collect the samples. When a limb is removed it is taken to the lab and cut into three sections, each including an injury site. The external appearance of the sites is documented by close-up digital photography. Cross sections are cut through each simulated egg site and exit hole and sanded to 320-grit smoothness. The cut surfaces are scanned at 1200 dpi on a flatbed scanner and the digital images are saved as jpeg files. Two rulers, placed at 90° to each other, are included in the image for scale and to aid quantitative analysis using computer software.

Results to date, halfway through the study, show that both the external and internal appearance of the healing wounds resemble actual ALB damage very closely. The external evidence shows that the rate of healing and the appearance of the injury after various periods do indeed depend on when the damage was inflicted (early, mid-season, or late). Injury done late in the season does not appear to be healing well, even after a year. Photographs showing how this appearance varies with host species and changes over time have already piqued the interest of program personnel. The cross sections confirm our interpretation of the timing of events based on the observed patterns of growth rings around the site of injury, thereby validating our dating methods. Based on the amount of incremental growth before and after the appearance of the injury, we will be able to place the time of oviposition or emergence to within a few weeks of its actual occurrence.

SPATIAL AND TEMPORAL DYNAMICS OF ASIAN LONGHORNED BEETLE INFESTATIONS IN CARTERET AND LINDEN, NEW JERSEY

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ABSTRACT

A major infestation of the exotic Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Coleoptera: Cerambycidae) was found in Carteret, New Jersey, in August 2004. DNA evidence indicates that this introduction was independent of those discovered earlier in Chicago, New York City, Long Island (New York), Jersey City (New Jersey), and Toronto (Ontario, Canada). In March 2006, another large infestation was found in Linden, New Jersey, about 4 km (2.5 mi.) from the center of the Carteret infestation. The work reported here, focusing on the Carteret and Linden cases, contributes to our long-term study of ALB populations in the U.S. Our objectives are to understand the spatial and temporal behavior of ALB infestations in general; to interpret the histories of specific infestations, including their origins, development and spread; and to use this understanding to advise program managers on strategies for survey and eradication. The spatial scale emphasized in these studies ranges from small (within-tree) to medium (1-2 km, within the range of natural dispersal).

We obtained data from the ALB eradication program, including survey, tree-removal, and treatment records, and collected more-detailed information through field studies. Data include the locations of approximately 400 host and non-host trees—including 250 infested trees—obtained with GPS equipment and aerial imagery (60-cm spatial resolution), species identifications, and estimates of tree size and health. We also estimated the numbers and vertical distribution of ALB egg sites and exit holes, inferred dates of attack from growth-ring

analyses and (with collaborator Maureen Carter at Cornell University) developed genetic profiles (mtDNa and microsatellite DNA) of over 100 larvae.

Primary questions to be answered in this research are: (1) Where and when did the infestations begin? (2) How did the infestations develop and spread over time? (3) What was the dispersal behavior of ALB adults from tree to tree and across the landscape? (4) Were the two populations (Carteret and Linden) related? (i.e., was one an offshoot of the other?) (5) Were the populations genetically distinct from each other and from the infestations discovered in other locations? (6) Can individuals from localized subpopulations within the Linden group be distinguished by their DNA? and (7) What were the sources of founders initiating several small satellite infestations discovered at distances ranging from 1.6 to 4 km (1 to 2.5 mi.) from the centers of the Carteret and Linden infestations?

Analyses are not yet complete, but the following conclusions may tentatively be offered. The infestation in Carteret probably dates back to 1996-1998 and originated at a warehouse where materials from China were received. The infestation was active for six to eight years before discovery. Only in the final two years did the infestation increase in radius from less than 250 m to more than 2.5 km and from the involvement of just a few dozen trees to more than 500—mostly red and silver maples. Five trees had more than 100 exit holes, and one large red maple, still doing well with only a few dead limbs, had more than 800. The infestation in Carteret (and neighboring Rahway and Avenel, to which it spread) affected trees in residential and commercial areas, a park, and the edges of woods.

The infestation in Linden appears to have been more recent by one or two years. The exact location and date of its origin are still uncertain, but it may have begun from materials stored at a staging area associated with the construction of a power plant around 2000. This is still under investigation. At the time of discovery, more than 100 trees were infested—mostly red maples and grey birches. Two maples had more than 100 exit holes (actually, more than 200), each. Many trees suffered heavy damage, with broken limbs and tops. This infestation occurred primarily on industrial wasteland bordering a salt marsh, spreading into sparse woods, a cemetery, and residential areas. At both Carteret and Linden, individual females (probably already mated) traveled more than 1.6 km (1 mi.) to establish several isolated satellite infestations. The broad expanses of open terrain in this area (wasteland, marshes, chemical tank farms, an airport, landfill, and commercial zones) probably facilitated or encouraged long-distance dispersal. Results from DNA analyses are not yet in, so many of the questions posed above remain unanswered for now.

Based on these preliminary results and information from other infestations, it appears that local populations of ALB remain highly focused near the point of introduction for many years. Spread, even to adjacent trees, is slow. Populations typically remain undiscovered for six or more years. Apparently, a point is reached when the local resource (a single tree or cluster of trees) is over-exploited, and then a rapid expansion of the infestation occurs over distances ranging from hundreds of meters to more than 1 km. Thus far, most infestations have been discovered after this spatial and numerical expansion, greatly increasing the difficulty and cost of surveys, regulatory actions, chemical treatments, pre-emptive removal of host trees, and ultimately, of eradication itself. Any tools or strategies leading to earlier detection would be of tremendous value.

MODELING THE SPREAD OF ASIAN LONGHORNED BEETLE IN NEW YORK CITY: INCORPORATING HOST SPECIES INFORMATION

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ABSTRACT

INTRODUCTION

This is an ongoing project to develop a spatially explicit model describing the local infestation dynamics of the Asian longhorned beetle (ALB), *Anoplophora glabripennis*, in Bayside, New York City. This model differs from other spread models in that it is designed to utilize operational data; in this case, using infested trees and uninfested host trees data from the New York Eradication Program. In most epidemiological data, there is uncertainty about the timing of events—such as the time a host was first infested, first contagious to others, or removed and no longer contagious—and this program data is no exception. In the case of ALB, timing uncertainty exists because of our inability to precisely 'backdate' the time of first exit from the level of damage observed on the infested tree. The use of a likelihood approach, combined with integration over timing uncertainty, allowed us to build models incorporating several factors that may affect the pattern of ALB spread. Information-based statistics, such as the Akaike Information Criterions (AIC), allowed us to directly compare and select the model best supported by the observed data. The selected model may then be used for both management and research applications (Russell & Lu in prep.).

THE EFFECT OF DISTANCE ON TRANSMISSION RATES

In 2005, we presented the first set of models created and compared using AIC (Lu & Russell 2005). These models examined the effect of distance (d_{ij}) on transmission rates (T_{ij}) (Figure 1). Three candidate models were considered and parameterized using infestation data from Bayside, Queens (Figure 2). Neighborhood scale simulations of the Bayside infestation using the selected model (the decay model, which had the lowest AIC value) and the simulated data were compared to the observed infestation data. Areas were found that were only infested in the simulations and not actually found infested in the field. These differences between model predictions and observations may be due to the role of other factors in dispersal, such as ALB host preference (Lu 2005), so our next step was to construct models that incorporated additional data.

INCORPORATING THE EFFECT OF HOST SPECIES PREFERENCE

Additional models were created in which ALB spread is based on both on the distance between trees (d_{ij}) and the species of the non-infested tree target tree (h_i) (Figure 3). In these models, h_i represents an ALB host preference index for different species of host trees, which was used to adjust the distance-based transmission rates (T_{ij}). The index was derived from published papers with data on ALB infestation, fecundity, and emergence rates for several tree species, factors which would have an effect on the likelihood a particular tree could pass an infestation to another. The validity of the index was established by comparing the index values to the host tree list in Nowak et al. (2001). Tree species classified as preferred host trees had index values greater than 0.2, whereas host trees with index values less than 0.2 were listed either as oviposition only or were not included in the Nowak et al. (2005) paper (Figure 4).

Incorporating the target tree host species data increased the likelihood of the observed ALB data for all three distance functions; the most parsimonious host model was found to be 10 times more likely to generate the infestation actually observed in Bayside. The decay function was chosen as the most parsimonious for both the distance only and the distance and target-host models (Figure 5). The host species index can be thought of as an “effective distance” affecting the probability of transmission between trees. Poor-quality hosts would have lower probabilities of becoming infested. These preliminary results tell us that the beetle’s dispersal patterns are more accurately described by a model that accounts for both the distance between trees and the “attractiveness” of different tree species—such that ALB adults actively seek out the trees they ultimately reproduce in.

FUTURE DIRECTIONS AND APPLICATIONS

Model II only considers the species of a target tree that is at risk of infestation – that is, that ALB adults do not disperse randomly, but instead search for better hosts. Additional models need to be developed that examine how the species of the infested, contagious tree affects the probability of transmission to an uninfested host—for example, by having increased rates of emergence. Models can also be constructed such that transmission rates are affected by the species of both the infested and uninfested trees. Comparision of these model variations using AIC will tell us whether or not spread patterns are due to beetles searching for hosts, differences in propagule pressure due to the quality of the infested host, or both. This modeling approach can readily be adapted as a management tool, such as an infestation risk map for New York City, using the parameterized decay model. Scenarios can also be tested using the best model. For example, the effects of operational treatments can be modeled by decreasing the probability that a treated tree would become infested. Program managers could then use the model simulations to compare the efficacy of different treatment strategies for decision-making.

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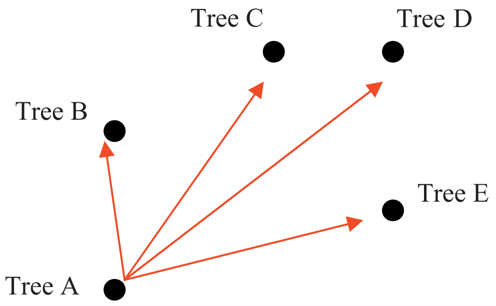


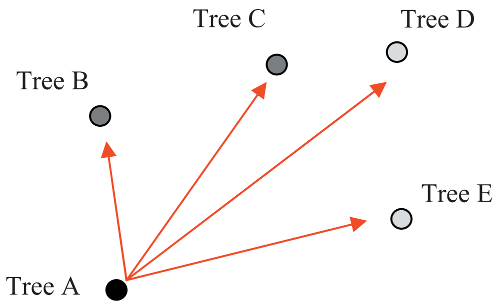
Distance Matrix **D**

Tree	A	B	C	D	E
A	-	1	2	2.5	2
B	1	-	1	1.5	1.5
C	2	1	-	.75	1.5
D	2.5	1.5	.75	-	1
E	2	1.5	1.5	1	-

Figure 1. Model I: Distance between infested and uninfested trees (represented in the matrix D) affects transmission rates Tij.

Model	$f(d_{ij})$	AIC
Dilution	a/d	181.7
Decay	$a*e^{-bd}$	148.9
Dilution & decay	$a*e^{-bd}/d$	150.4

Figure 2. AIC results for Model I (distance only). The decay model was most parsimonious, with the lowest AIC value.



Host Index Values for Target trees (**H**)

Distance Matrix **D**

Tree	A	B	C	D	E
A	-	1	2	2.5	2
B	1	-	1	1.5	1.5
C	2	1	-	.75	1.5
D	2.5	1.5	.75	-	1
E	2	1.5	1.5	1	-

Figure 3. Model II: Distance (dij) and target tree host species (hi) affect transmission rates (Tij). HI is represented as the vector H.

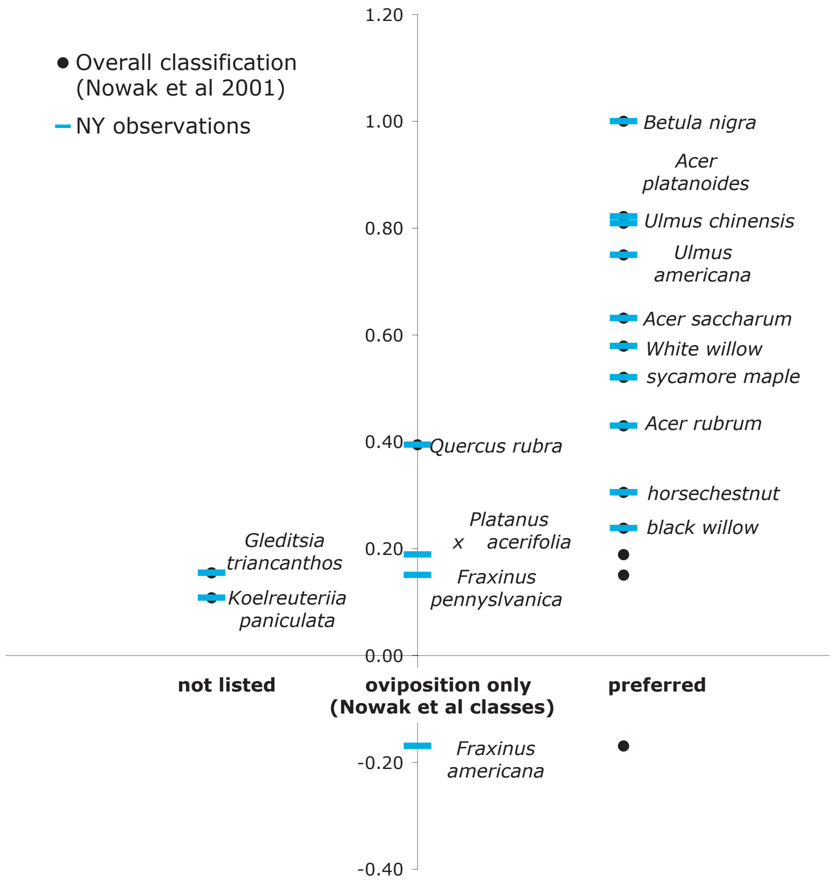


Figure 4. The ALB host preference index derived from published empirical data compared to the host species listing in Nowak et al. (2001). Tree species classified as preferred host trees had index values greater than 0.2, whereas host trees with index values less than 0.2 were listed either as oviposition only or were not listed in Nowak et al. (2001).

Model II	$f(d_{ij}) * h_{ij}$	AIC
Dilution	$a/d * h$	177.4
Decay	$a * e^{-bd} * h$	144.6
Dilution & decay	$a * e^{-bd}/d * h$	147.7

Figure 5. AIC results for Model II (distance and host species). Once again the decay model was most parsimonious, with the lowest AIC value.

UPDATE ON THE HOST RANGE STUDIES OF THE ASIAN LONGHORNED BEETLE IN A COMMON-GARDEN EXPERIMENT

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ABSTRACT

We continued the "common garden" experiment at two sites in China in 2006 to test preference of the Asian longhorned beetle (ALB), *Anoplophora glabripennis,* to important tree species, especially trees from different families whose status as a host of the beetle was not clear. Preference of trees by ALB were evaluated based on host feeding, oviposition, and suitability for the development of the beetle

Trees from North American were selected primarily based on results of previous laboratory tests with 57 tree species, including various species of maple, ash, birch, beach, alder, chestnut, hickory, horn beam, dogwood, catalpa, olive, larch, privet, sweet gum, poplar, apple, willow, oak, elm, cherry, locust, spruce, cottonwood, magnolia, etc. Other factors, such as abundance and importance of trees, feasibility of collection, and transplant feasibility in testing areas, were also considered. The two sites were evaluated, one in northwest China consisting of 34 tree species (e.g., more than 10 poplar species) mainly from within China and another in Beijing consisting of 26 tree species primarily from the United States.

Testing methods were the same in 2006 as in 2005—i.e., choice and no-choice tests. In the choice test, marked adult beetles were released in the field. Trees were checked for presence of released beetles, adult feeding, egg sites, and exit holes. In the no-choice test, a pair of adults (one male and one female) were caged on individual trees, which were later checked for evidence of adult feeding, oviposition sites, and exit holes. Adult longevity was recorded for all caged beetles.

Trees in the following tree species were found to have exit holes of adult beetles in 2005: *Acer mono*, *Acer platanoides*, *Acer saccharum*, *Betula papyrifera*, *Salix matsudana*, *Ulmus pumila*, *Alnus incana*, *Elaeagnus augustifolia*, and several species of poplar (*Populus* spp.). In 2006, we also found exit holes on green ash (*Fraxinus pennsylvanica*) and golden rain tree (*Koelreuteria paniculata*). However, no exit holes were found on some poplar species and hybrids, such as *Populus deltoids*, *Populus alba*, and *Populus glandulosa*. However, *Populus*

alba and *Populus alba* var. *pyramidalis*, which did not have exit holes in 2005, were found to have exit holes of ALB in 2006.

Egg sites and exit holes were not found on some of the testing species, such as *Rhus typhina*, *Syringa reticulate*, *Juglans regia*, *Ailanthus altissma*, or *Amorpha fruticosa*. There was almost no feeding on these species and a few other species tested, even under no-choice conditions.

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