Emerald Ash Borer Research and Technology Development Meeting, October 20-21, 2009

FHTET-2010-01

Forest Health Technology
Enterprise Team

TECHNOLOGY
TRANSFER

Emerald Ash Borer

EMERALD ASH BORER RESEARCH AND TECHNOLOGY DEVELOPMENT MEETING

October 20-21, 2009
Pittsburgh, Pennsylvania

David Lance, James Buck, Denise Binion,
Richard Reardon and Victor Mastro, Compilers

Forest Health Technology Enterprise Team - Morgantown, WV

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Compiled by:

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FOREWORD

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is one of those invasive species that has shaped, and will continue to shape, the composition and richness of natural and urban forests in North America. All species of North American ash appear to be susceptible to attack by this beetle, and all succumb and die within a few years of colonization. Like chestnut blight (*Cryphonectria parasitica*) and Dutch elm disease (*Ophiostoma ulmi*), EAB has the potential to effectively eliminate entire tree species from our forests. In the case of EAB, it could potentially eliminate all of the species in an entire genus.

The speed that EAB has spread and caused damage is surprising compared with many other invaders. It was first discovered killing ash trees in 2002 near Detroit, MI, though it was likely introduced and established in the early to mid 1990's (N. Siegert et al.). Since then, EAB has been found in 15 states, including Michigan, Ohio, Indiana, Illinois, Maryland, Virginia, New York, Pennsylvania, West Virginia, Wisconsin, Minnesota, Missouri, Iowa, Kentucky, Tennessee, and also in the Canadian provinces of Ontario and Quebec.

Many of the infestations found in new areas are three or more years old when discovered. A newly infested tree does not display many symptoms of attack in the first year of colonization. In subsequent years, as insect density increases and the tree's phloem is consumed, outward symptoms become apparent, such as crown thinning and die back, bark splits, epicormic branching, and wood pecker feeding sites. The current management approach of the USDA and affected states remains unchanged. A major thrust of that approach is to limit artificial spread of EAB through an aggressive outreach program and regulations to control movement of ash trees and wood. To date, most newly discovered infestations have been determined to have been established prior to the time that those regulations were put into place. At this point, however, it is still too early to judge how effective these efforts are. There is simply no "control area" for comparison.

The EAB program and other forestry related programs, such as the Asian longhorned beetle program, have highlighted the risk associated with movement of firewood and other unprocessed wood. Several studies have revealed the huge volume and large distances that firewood moves, both commercially and privately. The recognition of this risk has led several states to adopt firewood movement regulations. A national firewood standard is now under discussion. On an international level, a wood packing standard (ISPM-15) was adopted in 2005 in response to the recognized risks associated with this material. Work continues on treatments to allow safe movement of wood under this standard.

As part of its management strategy, the USDA-APHIS has recently invested in the construction of a facility to produce EAB parasitoids. Currently, three species of parasitic wasps (two that attack EAB larvae and one that attacks the eggs) are being reared in this facility. Operational releases of three species will begin in 2010. Several experimental releases of parasites have already been conducted and sites of those release are being evaluated for establishment and impact. In addition, several other species of parasites are being held in insect containment facilities, where they are being evaluated for possible colonization and release. It is hoped that, as parasites become established, they will be able to decrease EAB levels to the point that tree mortality can be slowed or even prevented.

This biological control program is just one of many fruits of an intensive research program that was initiated almost at the time of the original discovery of EAB in Detroit. An attractant-baited trap is another. Originally, the program relied on visual survey to determine presence or absence of EAB.

As was mentioned earlier, symptoms of early EAB attack are not apparent and as the shortcomings of visual survey became apparent, research pointed the way to the use of "trap trees." Trap trees were simply girdled 5" to 8" diameter ash trees that were provisioned with a sticky band. The trees were then felled in the fall, post EAB flight, and their bark was stripped to locate any larvae or feeding damage. Survey on a national scale with trap trees presented a number of challenges, including financial, logistical, and safety-related issues involved with locating, felling, and bark-stripping trees across a wide area. Research, however, developed an attractant-baited trap in a short period of time. The trap and a lure combine visual and olfactory cues into an effective survey tool. Nationally, surveys conducted with this tool have identified a number of previously unknown infestations. Research and development will continue to make incremental improvements of trap and lures.

Other products of research may have greater impacts in the future. Findings from research on the mechanism for resistance to EAB in some Asian and North American ash species could help preserve North American ash resources. Products of regulatory treatment research have already improved our treatments for wood and wood products. Systemic insecticides and delivery methods have been further developed within this and the ALB programs.

At this writing, no EAB research review is planned for 2010, but one will be scheduled for 2011. Even though no meeting is planned, a robust research effort continues and will yield more products that can be applied directly to EAB or to other invasive pests.

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Emerald Ash Borer Program Reports

PENNSYLVANIA 2009 EMERALD ASH BORER PROGRAM UPDATE

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ABSTRACT

By the end of 2008, emerald ash borer (EAB) was known from four counties in Pennsylvania: Allegheny, Beaver, Butler, and Mercer. These four counties along with Lawrence County were placed under quarantine for EAB. In 2009 EAB was detected in an additional seven counties: Mifflin, Lawrence, Westmoreland, Armstrong, Washington, Indiana, and Juniata. The existing quarantine was expanded to include all eleven counties. Pennsylvania employed 43 seasonal workers to deploy over 4,000 purple panel taps as part of a grid based and high risk site survey. Positives in Mifflin, Lawrence, and Juniata counties were obtained via destructive sampling of symptomatic trees. Positives in Westmoreland and Indiana counties were obtained by the capture of adults specimens on ash foliage. Positives in Armstrong and Washington counties were obtained on purple panel traps.

EMERALD ASH BORER FIRST DETECTOR TRAINING IN MINNESOTA

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ABSTRACT

Emerald ash borer (EAB) (*Agrilus planipennis*) is a serious forest pest in the United States and its range is expanding. University of Minnesota Extension partnered with Minnesota Department of Agriculture (MDA), Minnesota Department of Natural Resources (MDNR), and the United States Department of Agriculture Federal National Plant Diagnostic Network (NPDN), to develop the EAB First Detector Program to help identify the first incidence of EAB in Minnesota and each Minnesota county. EAB First Detector workshops were organized in 2008, targeting a wide variety of professional landscape workers as well as Master volunteer groups (e.g. Master Gardeners, Master Naturalists). Six, day-long workshops were conducted throughout the state during March and April. These workshops included information on the Federal and Minnesota First Detector programs and EAB biology, identification, symptoms, and management. A total of 184 people attended these workshops with 158 becoming First Detectors. In 2009, First Detector workshops were expanded to include three additional forest pests: gypsy moth (*Lymantria* dispar), Asian longhorned beetle (*Anoplophora glabripennis*), and Sirex woodwasp (*Sirex noctilio*). One hundred seventy-two people (including 30 people who became First Detectors in 2008) attended five workshops taught at four different sites with 65 becoming First Detectors. First Detector training played a small role in the first detection of EAB in Minnesota in May, 2009

Behavior, Biology, and Ecology

SURVEY OF EMERALD ASH BORER DISTRIBUTION IN RUSSIA

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ABSTRACT

The emerald ash borer, *Agrilus planipennis* Fairmaire (EAB), is a beetle native to East Asia and the Russian Far East where it is considered a minor pest, preferably attacking weakened or dying ash trees. In 2006, EAB was found to be responsible for enormous damage of ash species in Moscow, Russian capital, which causes serious concern for Europe. Recently we reviewed the situation with the pest in Russia and made recommendations for research and management strategies in Europe (Baranchikov et al. 2008). Here we report the results of 2008-2009 survey of further EAB distribution in Russian Federation.

In the Eastern part of the country, the beetle is distributed through nearly all regions occupied by native ash species *F. chinensis* and *F. mandshurica*. It was found from Vladivostok at the South to the village of Dzhonka on Amur river in Khabarovskiy Kray (N 49°40'04.4", E 136°56'51.9") in the North. There were no signs of EAB at South Sakhalin Island, although there are native stands of F.mandshurica.

In the Russian Far East, the beetle completes its life cycle in one (Southern Primoriye) or two years depending on the climate. Larvae are exclusively found in live cambium where they compete with bark beetles *Hylesinus eos*, *H. singulatus*, *H. laticollis*, and, in particular, with *H. chlodkovskyi*; in last case, the bark beetle was found to be about five times more abundant than *A. planipennis* (Yurchenko et al. 2008). Two braconid species (*Spathius depressithorax* Belokobylskiy and *S. generosus* Wilkinson) and an undetermined eulophid (*Tetrastichus* sp.) were found parasitizing EAB in the Far East.

Surveys showed that the beetle is extremely rare on *F. chinensis* and *F. mandshurica* occurring in natural stands of various ages. It is more common, albeit not a serious pest, in sparse plantations near cities or villages or ornamental trees along street or in parks. In mixed oak-ash forests, it was found infesting mature trees stressed by fire damage and infested by root rusts. No obvious difference was observed in the level of attack between the two native ash species but EAB eliminated all trees of *F. pennsylvanica* planted in cities.

From 2003 to 2006, several entomologists independently collected about 20 beetles of an unknown *Agrilus* species from several areas within Moscow. It was only in January 2007 that these beetles were determined to be *A. planipennis* (Baranchikov 2007). The association was found to be linked to increased mortality in ash stands in the streets and parks of Moscow, observed during the previous 2-3 years (Izhevskiy 2007). Intensive surveys were initiated in spring 2007 to assess the distribution of *A. planipennis* and its association with ash dieback in the Moscow region. These surveys showed that the pest was more or less equally distributed over the entire city, destroying ash trees in parks and streets of the Russian capital. Hundreds of dead trees were cut as a result of the *A. planipennis* outbreak. In Moscow, ash trees play an important role in urban forestry. It is the 6th most abundant tree genus planted in the city. The introduced American species *F. pennsylvanica* largely dominates, with European ash *F. excelsior* being occasionally planted. Majority of infestations of *A. planipennis* were observed on *F. pennsylvanica*, but *F. excelsior* is obviously also very susceptible.

In Moscow, *A. planipennis* was often found infesting ash trees along with the bark beetle *Hylesinus fraxini*. No parasitoid was found associated with the buprestid beetle, and the woodpecker *Dendrocopus major* was the only observed predator of larvae. The diameter of infested ash stems varied from 5 to 46 cm at breast height, and the number of trees with emergence holes varied from 35 to 100%. Attacked trees contained both exit holes and larvae of various stages, suggesting that the same trees were attacked during at least two successive years. On the infested trees, the average density of exit holes at breast height varied from 1 to 2.4 per square decimeter. In Moscow, EAB completes its life cycle during one year.

In 2009, numerous site surveys were performed using visual inspection and dissection of infested trees in the area within a ~150-km radius from Moscow. It was found that the EAB distribution area is limited by the cities of Mytiszhi from the North (Stroitel' railway station, N 55°56'00.5", E 37°46'56.7"; 20 km from Moscow); Bykovo from the East, N 55°37'54.8", E 38°05'28.9"; 30 km from Moscow; Serpukhov from the South, N 54°55'14.2", E 37°25'00.8"; 90 km from Moscow; and Mozhaisk from the West, N 55°30'17.3", E 36°02'00.8"; 95 km from Moscow. If we'll agree that EAB was introduced to Moscow approximately 10 years before it was registered (Izhevskiy 2007), the speed of its distribution was ≤4 km per year; this is in agreement with experimental estimations of the spread of EAB in the U.S. (Kovacs et al. 2009). Spread of the pest in firewood should be minimal in Russia because (a) wood for camping fires as a rule is prepared just at places around camping and (b) people do not use ash wood to heat houses preferring conifers and birch.

In 2008 and 2009, surveys were also made on *F. pennsylvanica* stands in streets and parks of South Siberian cities of Tomsk, Novosibirsk, Krasnoyarsk, Abakan, Ulan-Ude and Yekaterinburg (Central Urals), but no sign of *A. planipennis* infestation was found.

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REGULATION OF CARBOHYDRATE INTAKE AS A POSSIBLE MECHANISM OF EMERALD ASH BORER ADULT FEEDING PREFERENCE UNDER HIGH NITROGEN AVAILABILITY

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ABSTRACT

Nitrogen is important and in many cases is a limiting nutrient for living organism (Mattson, 1908). Some recent studies conducted mainly in the laboratory using artificial diets, however, indicated that nutrient balance, in particular, the protein to carbohydrate ratio, may play an important role in feeding preference of many herbivores (Behmer, 2009). Here, we report some preliminary evidence that emerald ash borer (EAB), Agrilus planipennis Fairmaire (Coleoptera: Buprestidae), adults might be regulating balanced nutrients by choosing high carbohydrate host plants under high nitrogen conditions.

In a study examining the influence of leaf age, light availability, and girdling on green ash (Fraxinus pennsylvanica Marsh) foliar nutrition and defense, three dual-choice tests (Mature vs. Young; Sunny vs. Shady; and Girdled vs. Ungirdled) were conducted. Mature foliage was found to be preferred by EAB females over young foliage. Foliage collected from ash seedlings grown in the sun or girdled in the trunk was preferred over foliage from ash seedlings grown in the shade or ungirdled, respectively. Nutritional indeces [total protein, amino acids, total non-structural carbohydrate (TNC), protein to TNC ratio] and two groups of potential defensive compounds (protease inhibitors and total phenolics) were then determined to examine their correlation with feeding preference. In the Mature vs. Young test, amino acid levels in mature foliage were higher than in young foliage, while the levels of chymotrypsin inhibitors and total phenolics in mature foliage were lower. This might indicate that these three variables contribute to EAB's feeding preference in this test. In Sunny vs. Shady and Girdled vs. Ungirdled tests, only TNC levels were found to correlate well with EAB female feeding preference. That is, TNC levels in ash foliage grown in the sun were greater than in foliage grown in the shade. Similarly, TNC levels in ash foliage from girdled seedlings was greater than in foliage from ungirdled seedlings.

In a second study comparing EAB female adult feeding preference among green, black (F. nigro), and white (F. americana) ash, it was observed that EAB females consumed more foliage (leaf area in cm2) of green or white ash, compared to black ash within 48 h. The foliage consumption between green ash and white ash did not differ statistically over the same period of time. Differences in total proteins among the ash species could not explain EAB females' feeding preference. Similarly, the amount of total phenolics, trypsin or chymotrypsin inhibitors did not explain female choices because (1) the amounts these inhibitory compounds in green and white ash foliage were either equal to or greater than amounts in black ash foliage; and (2) large quantities of phenolics were excreted by EAB females. Frass of EAB females contained approximately or more than twice the amount of phenolics

compared to the ash foliage they consumed, regardless of ash species. Furthermore, total foliar nitrogen of ash seedlings used in both studies was almost or more than twice the normal nitrogen level found in the field. It's known that the negative effects of some defensive compounds disappear under high nitrogen (or balanced nutrient) conditions. The TNC level in green ash foliage was the highest, and that of black was the lowest. Although the difference in TNC levels between black ash and white ash was not statistically significant, white ash had nearly 20% greater TNC than black ash. Overall, the TNC pattern among the three ash species corresponded to EAB female feeding preference.

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COLD TOLERANCE AND OVERWINTERING PLASTICITY OF EMERALD ASH BORER PREPUPAE (*AGRILUS PLANIPENNIS*)

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ABSTRACT

The geographic range of invasive species like the emerald ash borer (EAB) could potentially be limited by temperature constraints on survival, but estimating the potential geographic range of the EAB from macroclimatic records alone is difficult. In the sapwood of ash trees, the prepupae and larvae of the EAB are not directly exposed to the ambient temperature, but subject to microclimatic conditions that might vary with orientation of the tree, light exposure, and snow cover. Climate change effects such as sudden warm spells in mid-winter, where temperatures can rise well above 0°C for an extended period of time, or below 0°C during late cold snaps in spring, are common under current North American climatic conditions. We investigate the cold tolerance and overwintering plasticity in EAB prepupae with regard to seasonal and climate change induced temperature variability. The main overwintering stage of *A. planipennis* is freeze avoiding, with mean supercooling points being lowest in January (-30.5°C) and increasing to -23.5°C in April. Supercooling points are lowered using a combination of antifreeze proteins and increased hemolymph osmolality. The SCP is likely to be the lower lethal temperature, since survival after prolonged chilling at -10 and -20°C for up to 12 h did not differ from survival in unexposed controls if cooling rates similar to those observed in nature were applied. Simulated mid-winter warm spells at 15°C and 10°C resulted in deacclimation to cold, and the SCP increased significantly within seven days of exposure. Subsequent reacclimation to a colder temperature (-10°C) was incomplete, and the supercooling points did not decrease again to the low values observed prior to the warm exposure. Our experimental data will be combined with measurements of bark microclimate from different locations in Alberta, Saskatchewan and Ontario (Canada), and eventually used to model the potential spread of the EAB and other invasive wood-boring species in North America.

INDIRECT EFFECTS OF THE EMERALD ASH BORER ON AMPHIBIAN POPULATIONS IN SOUTHEASTERN MICHIGAN

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ABSTRACT

The infestation of the emerald ash borer has led to changes in forest canopy cover throughout southeastern Michigan, thereby altering forest habitat conditions in general. Amphibians are extremely sensitive to any habitat changes that occur and rely on ephemeral ponds in forests for breeding. Ash trees are often ubiquitous in these ephemeral pond areas. We examined the indirect effects of the emerald ash borer infestation on amphibian populations. Study sites were chosen based on a chronosequence of the emerald ash borer infestation to account for time. Study sites consisted of an ephemeral pond area with ash mortality and an ephemeral pond area with no ash mortality. Amphibian surveys were performed, in addition to vegetation and water analysis. No significant differences were found in amphibian richness or abundance when comparing open ephemeral ponds with ash mortality to closed ponds with no mortality. No significant differences were found in pH or dissolved oxygen content in open versus closed canopy ponds. There was a larger difference in amphibian abundance when a pond area with extreme ash mortality was compared to a closed canopy site. The lack of statistically significant results may be due to the patchy nature of ash trees throughout the forests of southeastern Michigan.

LINGERING EFFECTS OF EAB: INDIRECT EFFECTS OF CANOPY GAPS ON INTERACTIONS BETWEEN PRICKLY-ASH AND GIANT SWALLOWTAIL BUTTERFLY LARVAE

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ABSTRACT

Widespread canopy gap formation resulting from EAB-induced ash mortality could trigger a cascade of direct and indirect effects on forest communities and ecosystems. Canopy resulting from tree mortality increases light availability in the understory, which can influence several plant processes including photosynthesis, growth, and chemical defense. In turn, these properties can effect interactions between plants and insect herbivores. Prickly-ash, *Zanthoxylum americanum*, a dioecious understory shrub, contains furanocoumarins that, when photoactivated in the presence of UV radiation, interfere with DNA replication and transcription, thereby deterring most insect herbivores. Furthermore, biosynthesis of furanocoumarins requires an immense energy investment and other studies have shown their concentration to be lower in the shade where low photosynthetic rates decrease the energy budget of the plant. Therefore, we predict that prickly-ash plants growing in canopy gaps created by ash mortality, and thus exposed to increased UV and photosynthetically-active radiation, will have higher concentrations of more bioactive furanocoumarins.

Although furanocoumarins deter most insect herbivory, the giant swallowtail caterpillar, *Papilio cresphontes*, feeds exclusively on plants containing furanocoumarins including prickly-ash, which it detoxifies via cytochrome P450 mediated metabolism. Detoxification of diets with high furanocoumarin concentrations incurs immense energy costs, thereby reducing available energy for growth. Hence, increased concentrations of photoactivated furanocoumarins could induce physiological trade-offs in P. cresphontes when feeding on hosts growing in EAB-induced canopy gaps, with more resources allocated to detoxification and less to growth and development.

Increased concentrations of photoactivated furanocoumarins could also affect natural enemies of *P. cresphontes*. For example, several parasitoids attack *P. cresphontes* pupae.

Increased concentrations of photoactivated furanocoumarins could also affect natural enemies of *P. cresphontes*. For example, several parasitoids attack *P. cresphontes* pupae. Caterpillars with growth slowed by high furanocoumarin concentrations may experience extended vulnerability to parasitoids ("slow growth – high mortality hypothesis"). Alternatively, adapted larvae may sequester the higher concentrations furanocoumarins in their own tissues as chemical defenses against parasitoids, which are unable to detoxify these metabolites, thereby increasing their survival in canopy gaps.

The objectives of this research, which was initiated in 2009, are to quantify the effects of increased light in EAB-induced canopy gaps on concentration and toxicity of furanocoumarin expression in prickly-ash, and the effects of furanocoumarins on development giant swallowtail larvae. Additionally, we will examine the effects of furanocoumarins on parasitoids of swallowtail pupae.

NATIVE AND INVASIVE PLANTS IN THE UNDERSTORY OF FORESTS IMPACTED BY EMERALD ASH BORER

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ABSTRACT

Plant biodiversity is an important component for the sustainability of native forest ecosystems. Non-native invasive plants may alter the native biodiversity and affect the integrity and function of native forest communities. In the wake of emerald ash borer (EAB), many forested areas contain large numbers of canopy gaps caused by ash tree mortality. Such disturbances may facilitate the growth and spread of exotic plants that are already present in the understory of many forests. Since 2008, we have been studying the impacts of ash mortality on the diversity and abundance of native and invasive plant species in the understory of 129 plots established in large forest tracts in the Huron River watershed in southeast Michigan. Within every plot we established four 4-m^2 microplots in which we have indentified and counted all native and invasive woody plant species present. In a subsample of plots, we located and tagged individuals of ten different woody invasive species (*Berberis thunbergii*, *Celastrus orbiculatus*, *Elaeagnus umbellata*, *Euonymus alatus*, *Lonicera japonica*, *Lonicera* spp, *Ligustrum vulgare*, *Rhamnus cathartica*, *R. frangula*, and *Rosa multiflora*) and measured the volume (length × width × height) of each. We also located, tagged, and measured a native woody plant species within 1 m of the invasive species. All plants were remeasured in 2009 in order to compare relative growth rates of the native and invasive species. After one season, we have yet to see any clear trends in relative growth rate of native relative to paired invasive plant species. The most frequent exotics encountered were *R. cathartica*, *R. frangula*, and *C. orbiculatus*. The most frequent native species in the understory were *Fraxinus* spp. followed distantly by Carpinus caroliniana. Volume measurement and understory woody plant diversity surveys will be repeated for two more seasons.

ASH REGENERATION IN THE WAKE OF EMERALD ASH BORER: WILL IT RESTORE ASH OR SUSTAIN THE OUTBREAK?

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ABSTRACT

In the Huron River Watershed of southeast Michigan, emerald ash borer (EAB, *Agrilus planipennis*) has killed more than 99% of ash (*Fraxinus* spp.) with stem diameters at breast height (DBH) > 2.5 cm. To investigate the ecology of the EAB invasion in these forests, we have established 38 transects, each of which consists of three replicate plots (0.1 ha) Our previous studies have shown that ash are the most common woody species in the seedling and sapling layers of these forests, which has led us to pose these questions: (1) will this regeneration restore ash if EAB is locally extirpated due to depletion of its food resource, or (2) can ash regeneration maintain an EAB population indefinitely as the supply of susceptible saplings is continually replenished?

Within monitoring plots in Michigan and Ohio, we have quantified (1) ash regeneration by sampling the ash seed bank, as well as ash seedling and sapling dynamics, and (2) EAB populations in relation to ash density through annual sampling with purple panel traps. We measured densities of four demographic classes of ash: newly germinated seedlings (cotyledons present), established seedlings (at least one-year-old but less than 25 cm tall), saplings (25 cm tall to DBH of 2.5 cm), and trees large enough to support EAB (DBH > 2.5 cm).

Four years of intensive soil sampling (432 samples/yr) suggests that the ash seed bank in these stands is rapidly depleted as overstory trees die. Small numbers of seeds were found in 2005 and 2006;

however, no ash seeds were found in the soil or on the soil surface in 2007 or 2008. Our observations also indicate, contrary to some earlier speculation, that ash trees do not increase seed production as they become stressed by EAB. Patterns of ash demography were consistent with conclusions reached from seed bank sampling. Density of new ash seedlings was 0.5 and 0.1 plant/ha in the Michigan plots in 2008 and 2009, respectively. In contrast, density of new ash seedlings routinely exceeded 800 plants /ha in Ohio plots where EAB mortality was still low or nonexistent, and exceeded 20,000 plants/ha in some plots in 2009 following mast seed production on a regional scale in 2008. Established ash seedlings (no cotyledons but less than 25 cm tall) were far more abundant than new seedlings in the Michigan plots, averaging 76 and 191 plants/ha in 2008 and 2009, respectively. However, this pattern was reversed in Ohio plots, where new seedlings greatly exceeded established seedlings, except in stands where mortality of mature ash approached 100%.

Density of ash saplings was much lower (6.1 plants/ha in 2009) than that of established seedlings, possibly due to self-thinning. Density of trees large enough to be colonized by EAB (> 2.5 cm DBH) was less than 1.0 plant/ha in both 2008 and 2009. Numbers of EAB captured on purple panel traps were correlated with percent survival of mature ash, and declined from 2008 to 2009 as ash mortality increased. However, EAB continued to persist at low levels in all plots, suggesting that ash saplings may be sustaining low density populations.

In summary, EAB-induced mortality of trees with DBH > 2.5 cm now exceeds 99% in the Huron River Watershed in southeast Michigan. Our evidence suggests the ash seed bank does not persist after overstory trees succumb. Ash regeneration has ceased in these plots, as new seedlings were virtually nonexistent in 2008 and 2009, averaging less than one plant/ha. Established seedlings and saplings with DBH < 2.5 cm are the only demographic classes of ash remaining in these plots. EAB continues to persist at low levels even in plots where ash mortality exceeds 99%, suggesting that an EAB population might be sustained, at least in the short-term, as established seedlings and saplings become large enough to be colonized. Ultimately, in the absence of ash regeneration, EAB may become locally extirpated as this orphaned cohort of juvenile ash is gradually depleted by EAB mortality.

FURTHER STUDIES ON EMERALD ASH BORER FLIGHT BEHAVIOR

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ABSTRACT

Using computer-monitored flight mills to record the flight performance of 177 emerald ash borers, we found that emerald ash borers fly in bouts of several minutes, separated by non-flight periods ranging in duration from minutes to hours. Mated females flew further per day and longer than unmated females or males. Furthermore, the median distance flown by mated females that were allowed to feed between flight periods was >1 km/day for four days with 10% flying more than 7 km in four days. Calibration experiments showed free-flight speeds are three times the tethered-flight speeds, suggesting that 20% of mated females could fly >10 km and 1% flying >20 km (Taylor et al. 2010). We report here on a more detailed study of EAB flight behavior. We examined the distribution of flight and rest bouts, the change in speed during a bout, the relationship between the change in flight speed with the duration of flight and rest bouts, and the change in pattern of flight speed with time.

Flights consist of a series of bouts lasting from a few seconds to many minutes, separated by varying periods of inactivity, denoted as rest periods. The duration of rest bouts increases with the length of prior flight bout, whereas the duration of flight bouts is not correlated with the length of the previous rest bout (Fig. 1). This suggests that the more active a flight, the longer it takes to recover, but once the insect is ready to fly, the duration of the flight bout is determined by other factors. In general the earlier flight bouts are faster and longer than later bouts (Fig. 2). They are characterized by a rapid acceleration lasting 2-4 seconds, a leveling off, followed by a steady decline, terminating in a deceleration period of 5-10 seconds. In general the faster the initial peak flight sped, the more rapid the decline; thus slower bouts tend to be at relatively constant speed. Within a flight, the decline in flight speed of a bout (Fig. 3a) is positively correlated with the time since flight began (Fig. 3b). This relationship differs between individuals and may vary between days for an individual.

We have also identified two distinctly different flight modes we are denoting continuous and burst (Fig. 4a & b, respectively). The two examples, from different mated females, are approximately the same duration and average speed (~1 revolution/sec). Both the continuous and burst mode flight bouts occurred within the first hour of flight. Although our sample size is small at this stage (we have

analyzed only 15 of 240 flights in detail), it appears that females engage in burst flying more often than males, and that mated females more than unmated females. It seems possible that the two modes of flight may be connected to energy utilization which is expected to be different for mated females that must balance their energy consumption with the demands of embryonation.

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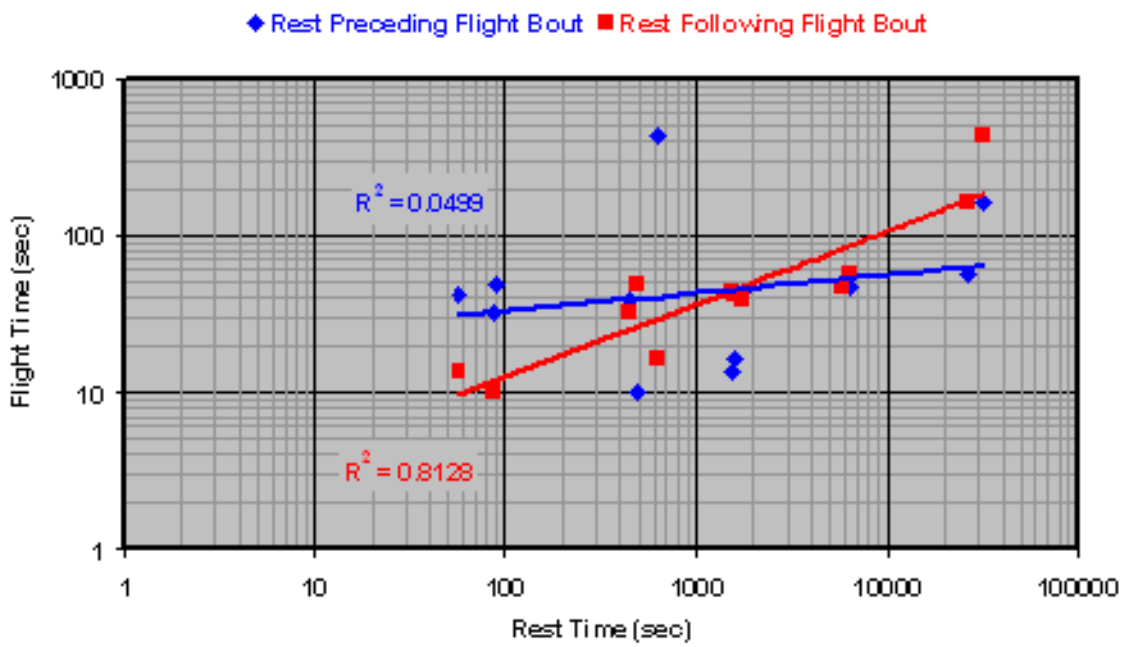

Fig. 1: The duration of flight bouts is not correlated with the preceding rest bout, however, the duration of rest periods following flight are correlated.

Fig. 2: Flight bout tends to get shorter and slower with time.

Fig. 3: The decline in flight speed is correlated with the time from the beginning of the flight experiment.

Fig. 4: Two distinctly different modes of flight, termed continuous and burst, have been identified.

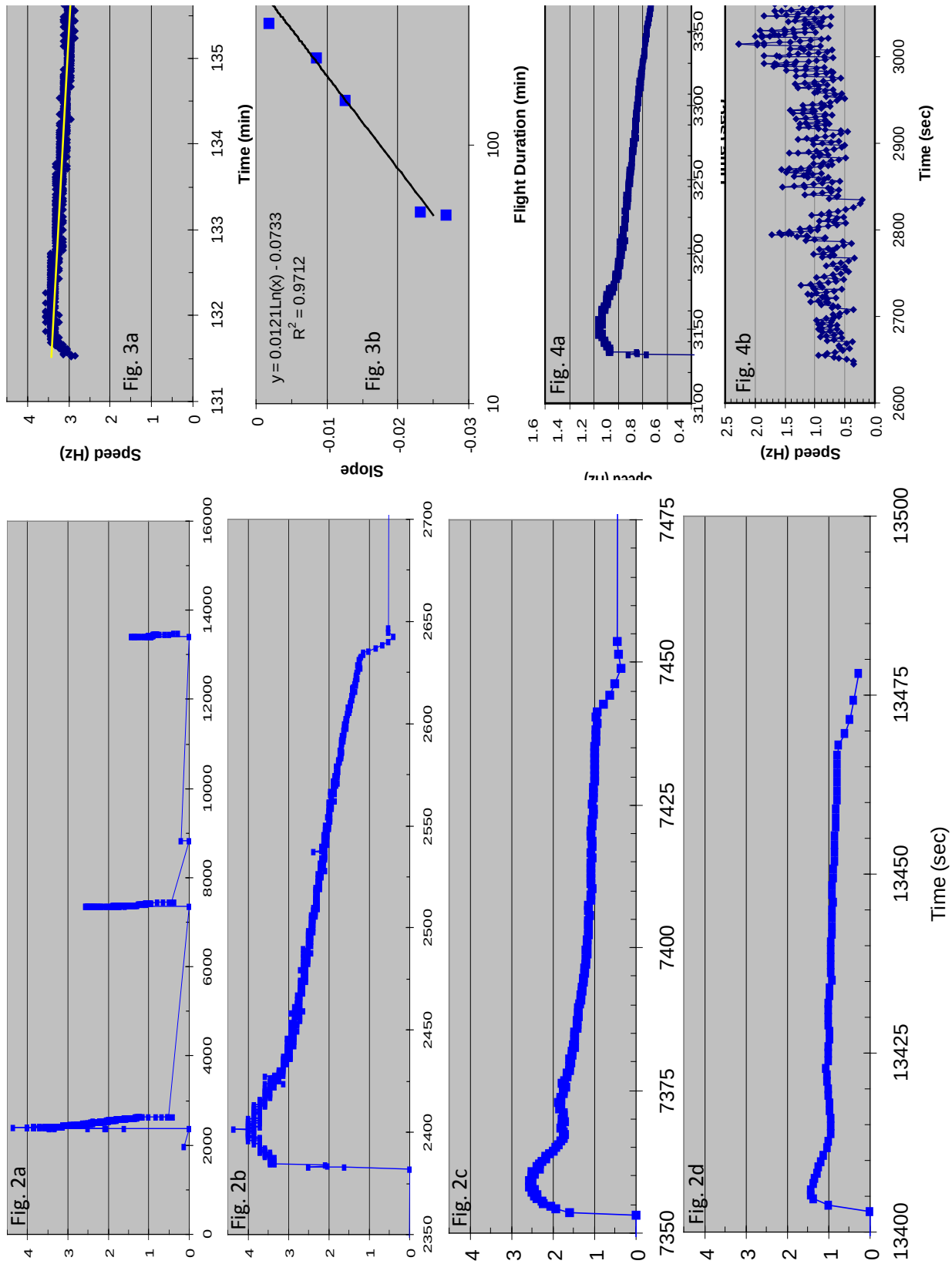
Fig. 2a
Fig. 2b
Fig. 2c
Fig. 2d
Time (sec)
Fig. 3a
Speed (Hz)
Fig. 3b
Time (min)
Slope
y = 0.0121Ln(x) - 0.0733
R2 = 0.9712
Fig. 4a
Flight Duration (min)
Fig. 4b
Speed (Hz)
Time (sec)

DISPERSAL OF EMERALD ASH BORER AT NEWLY COLONIZED SITES

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ABSTRACT

Emerald ash borer has become one of the most destructive forest pests in North America. Since it was first identified in 2002 in southeast Michigan and Windsor, Ontario, dozens of isolated *A. planipennis* populations have been discovered throughout Michigan and Ontario, and in 12 other states and the province of Quebec. We assessed realized *A. planipennis* dispersal at two discrete outlier sites that originated 1 yr and 3 yr earlier from infested nursery trees. We systematically sampled ash trees within an 800 m radius of the origin of each infestation to locate galleries constructed by the progeny of dispersing *A. planipennis* adults. Our sampling identified eight trees at the 1 yr site infested with a mean ± SE of 11.6 ± 8.4 *A. planipennis* larvae and 12 trees at the 3 yr site with 25.8 ± 11.1 larvae per m^2. Dendroentomological analysis indicated that *A. planipennis* populations were predominantly undergoing a 2 yr (semivoltine) life cycle at both sites. Colonized trees were found out to 638 and 540 m from the epicenters at the 1 yr and 3 yr sites, respectively. Logistic regression was used to determine whether the likelihood of *A. planipennis* colonization was affected by wind direction, ash phloem abundance, distance from the epicenter, or land-use type (i.e., wooded, residential, agricultural, or urban). Results show that the probability of *A. planipennis* colonization was significantly affected by ash phloem abundance and decreased with distance from the epicenter.

EFFECTS OF CLUSTERS OF GIRDLED TREES ON THE SPREAD OF EMERALD ASH BORER IN LOW-DENSITY INFESTATIONS

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ABSTRACT

The ability to manipulate the spread of an invasive species could potentially be integrated into an effective management strategy to delay dispersal to un-infested areas while concentrating the population in an area where suppression activities could be applied. Here we examined the influence of clusters of girdled ash trees on the spread of isolated emerald ash borer populations in a forested area. Two 16 ha (40 ac) forested sites with low-density emerald ash borer populations were selected for this study. Each site was divided into sixty-four 50×50 m cells and complete ash inventories by diameter class were conducted. The amount of ash phloem in each 50×50 m cell was calculated and two 4 ha (10 ac) blocks with similar ash density and distribution of total ash phloem were selected per site. At each site, four clusters of girdled ash trees (3 trees per cluster; n = 12 total girdled trees per site) were established in one of the randomly-selected 4 ha blocks. Beetles were released from infested logs placed between the two blocks in Summer 2007. Girdled and non-girdled ash trees were sampled in Winter 2007-8 (n = 234 trees) and Winter 2008-9 (n = 232 trees) to assess spread of the populations. Preliminary results indicate that clusters of girdled trees strongly influenced the spread of the low-density emerald ash borer populations at both sites. Overall, emerald ash borer densities were significantly greater on the 4 ha with clusters of girdled trees compared to the 4 ha without girdled trees at the two sites, respectively. Girdled trees had significantly higher emerald ash borer densities than adjacent non-girdled trees and non-girdled trees in the control blocks at the two sites each year.

DISPERSAL OF EMERALD ASH BORER WITHIN AN INTENSIVELY MANAGED QUARANTINE ZONE

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ABSTRACT

The emerald ash borer (EAB) was introduced into Maryland April 2003 via infested nursery stock shipped to a nursery in southern Prince George's County. A Maryland Department of Agriculture (MDA) nursery inspector noticed EAB infested trees at the site in August 2003. A 0.5 mile buffer zone was established around the nursery, and an aggressive eradication program was initiated to destroy all ash trees within the quarantine zone. By March 2004, approximately 1,100 ash trees were cut down and destroyed. From 2004 to 2006, sentinel trees were established and monitored, and surveys to locate ashes in the area were initiated. EAB were detected again in August 2006. Consequently, buffer zones were expanded 1.5 miles to account for the potential movement of the pest over the previous 3 years. Between 2006 and 2008, however, thousands of new EAB detections were made and 25,000 trees were removed on over 14,000 acres. In August 2008, EAB was detected over 4.5 miles south of the original infestation site from five years earlier.

The situation in Maryland provided a unique opportunity to study dispersal of this pest within well defined spatial and temporal dimensions in an area under federal quarantine with an intensively managed eradication program. The objective of this research was to determine the rate of spread of EAB in a quarantine zone where aggressive intervention tactics, including host tree destruction, extensive surveillance, and public education limited the dispersal of this pest, with the goal of helping arborists and urban foresters understand the rate of spread of EAB, and to provide empirical information to help them develop effective EAB management strategies.

We examined historical records from the MDA to determine the rate of spread of EAB in Maryland within the quarantine zone. The nursery infested in 2003 provided the point of origin for the EAB infestation, and MDA records of EAB detections for 2006, 2007 and 2008 were used to determine the rate of dispersal. The 4 most distinct detection points occurring furthest from the introduction site were determined for each year using GIS survey data gathered by MDA. The maximum distances (1 site in each of 3 years) and average distances of 12 sub-samples (4 sites in each of 3 years) were regressed against time to determine the average rate of spread for the 5 year period. Despite attempts at eradication and public education, EAB persisted and the leading edge of beetles moved away from the central infestation point at an average annual rate of 0.76 miles per year and a maximum annual rate of 0.9 miles per year between 2003 and 2008.

Our results provide insight into the relative merits of an aggressive quarantine and eradication program for EAB. Although the extent to which dispersal in Maryland is natural or anthropogenic is unknown, we believe that the concentration of detections in relatively close proximity to the initial site of infestation and the rather uniform annual rate of spread are consistent with the notion of aerial dispersal as a primary mode of movement by the beetle. We suggest that the maximum rate of spread is the more informative of the two estimates in assisting planning decisions by urban foresters, and we proffer a caveat for future attempts at eradication programs for EAB: if destruction of hosts is considered, the eradication zone must exceed the 0.5 miles employed in the Maryland infestation.

EMERALD ASH BORER IN PENNSYLVANIA: SEASONAL ABUNDANCE AND DISPERSAL POTENTIAL

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ABSTRACT

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), a destructive wood borer native to northeastern Asia, was first discovered attacking ash trees (*Fraxinus* spp.) in southeastern Michigan in 2002. Since then, it has spread to Ohio, Maryland, Virginia, Indiana, Illinois, West Virginia, Pennsylvania, Wisconsin, Missouri, Minnesota, Kentucky, and New York in the U. S. and Ontario, Quebec in Canada. Tens of millions of ash trees have been lost to this pest.

In Pennsylvania, emerald ash borer was first detected in Cranberry Township in Butler County in June 2007 when live adults were collected from infested ash trees. Subsequent detection surveys revealed that a few townships in Butler and Allegheny counties were also infested. As a result, both Butler and Allegheny counties, along with neighboring Beaver and Lawrence counties were placed under quarantine. This quarantine was expanded to Mercer County in summer 2008 as additional emerald ash borer infested trees were found in Wheatland Borough.

Baseline information, such as the extent and size of these infestations, as well as pest population levels are needed before any management options can be considered. In 2009, the Pennsylvania Department of Conservation and Natural Resources (DCNR) conducted a study in the five quarantined counties to determine the seasonal abundance and dispersal potential of emerald ash borer. Two study areas were established at the beginning of 2009; an area with a 20-mile radius around the Cranberry infestation, and another area with a 10-mile radius around the Wheatland infestation. Public (state, county, and city/borough) land containing ash trees (single, small group, or entire stand) within the study areas were used. A total of 53 sites were selected for this study, including 38 sites in Cranberry and 15 sites in Wheatland. Site selection was based on diagonal directions at the interval of five miles from the initial infestation. For Cranberry, a total of 33 sites were selected from all eight diagonal directions, whereas only 11 sites were selected from five diagonal directions in Wheatland as half of the study area fell across the state border in Ohio. In addition, a total of five Cranberry sites and four Wheatland sites were selected outside the study areas to serve as outlier sites.

Standard purple panel traps baited with Manuka/Phoebe oil (80/20) lure was used to monitor adult emergence and seasonal abundance of emerald ash borer in the field. One live ash tree (infested or un-infested) was selected from each site for this study. Efforts were made to ensure that trees growing along forest edges or in the open with a diameter at breast height (DBH) greater than 8 inches were selected. A total of two traps were used at each site. Traps were placed in the lower canopy at about 6 m (20 ft) above the ground on the selected tree, with one each on the north and south side. The two traps were separated by at least 3 m (10 ft) from each other whenever possible. Traps were monitored

every other week from May 31 to September 26, with the lures replaced once in July 27-31. *Agrilus* (Coleoptera: Buprestidae) beetles captured on the traps were collected each time and returned to the laboratory in small plastic bottles for identification. Number of emerald ash borer adults from each trap was recorded and used in final analysis.

Results showed that emerald ash borer adults were found at the epicenter in the Wheatland study area, with two beetles captured throughout the season. No adults were found at any other sites. The removal of the previous infested ash trees at the epicenter prior to this study may have contributed to the low adult population recorded.

For the Cranberry study area, 17 out of the 38 sites were positive with emerald ash borer based on trap catch, including one outlier site at 21 miles away from the epicenter. Trap facing had no significant effect on number of adults captured. A total of 462 adults were collected from those sites, with the highest catch of 96 adults / site at New Sewickley in Beaver County. Emerald ash borer adults were active from June 01 to September 21, with the peak flight time occurring between mid- to late June. Significantly more adults were caught during the weeks of June 28 and July 14 compared with any other sample period, with an average of 9.2 and 7.6 adults per site, respectively. Negative correlation was found between trap distance from the epicenter and the total number of adults captured, with the majority (64%) of the adults found on traps placed between five to ten miles away from the epicenter. No significant effect was found on diagonal direction in reference to the epicenter.

In general, emerald ash borer population appears to be static at the Wheatland infestation, with low numbers detected at the epicenter. The infestation in Cranberry, however, is actively spreading beyond 20 miles from the initial introduction, with high populations found in the range of five to ten miles. Ash resource, rather than diagonal directions, is more important to emerald ash borer dispersal potential. Left alone, these two populations will advance to new and healthy ash resources in the region. New infestations found outside the initial quarantine by survey and detection efforts in 2009 will further complicate the population dynamics of this pest in Pennsylvania.

ASSESSMENT OF AN EMERALD ASH BORER INFESTATION IN HOUGHTON COUNTY, MICHIGAN: DEVELOPMENT OF AN APPARENTLY ISOLATED POPULATION

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ABSTRACT

The presence of EAB in Houghton County, Michigan was confirmed on August 5, 2008. This is the northernmost find of EAB in North America to date and was more than 250 miles from the closest known population at that time (Figure 1).

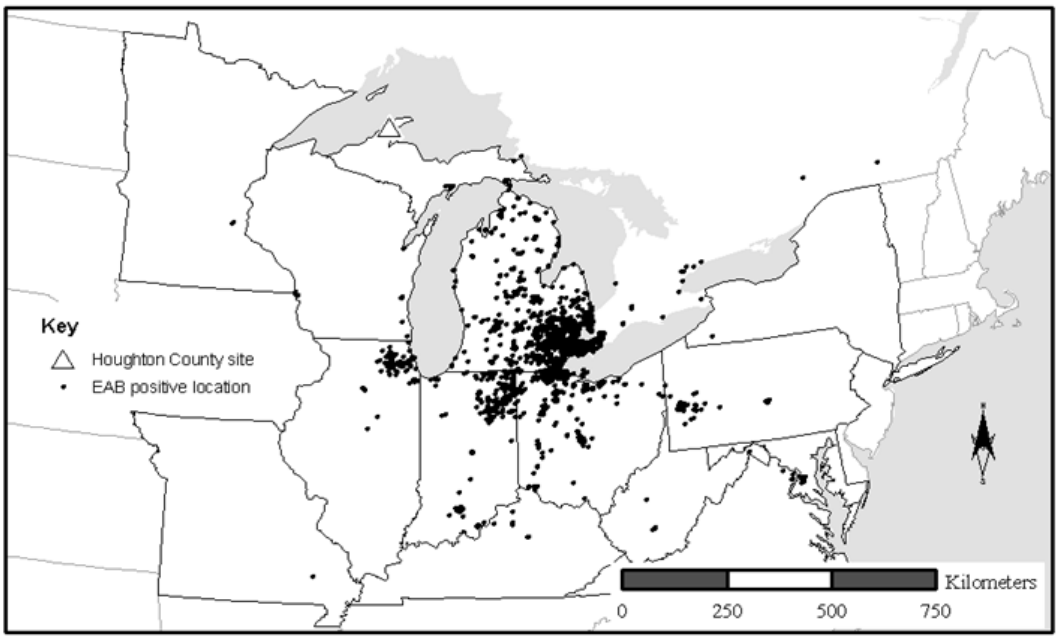

Figure 1. Map of known EAB locations (September 2009)

More than ninety ash trees have been destructively sampled to determine how EAB has spread in Houghton County. Larval density and exit holes were quantified on all sample trees. Preliminary dendrochronological analysis indicates EAB has been present in Houghton County for at least six years.

A ground survey was initiated to determine the extent of the ash resource and visual signs and symptoms of EAB around the infestation. Transects were run in the eight cardinal directions for 3.2 km radiating outward from the infestation. Basal area by species was assessed with a 10 BAF prism

every 161 meters, and every 805 meters a 0.04 ha fixed-radius plot was established. All trees larger than 5 cm DBH were tallied, and any ash tree found was assessed for vigor, dieback, crown light exposure, canopy position, and signs and symptoms of EAB. Point quarter sampling was performed at each prism point, and any ash trees within 20 meters were assessed within each quadrant (Figure 2).

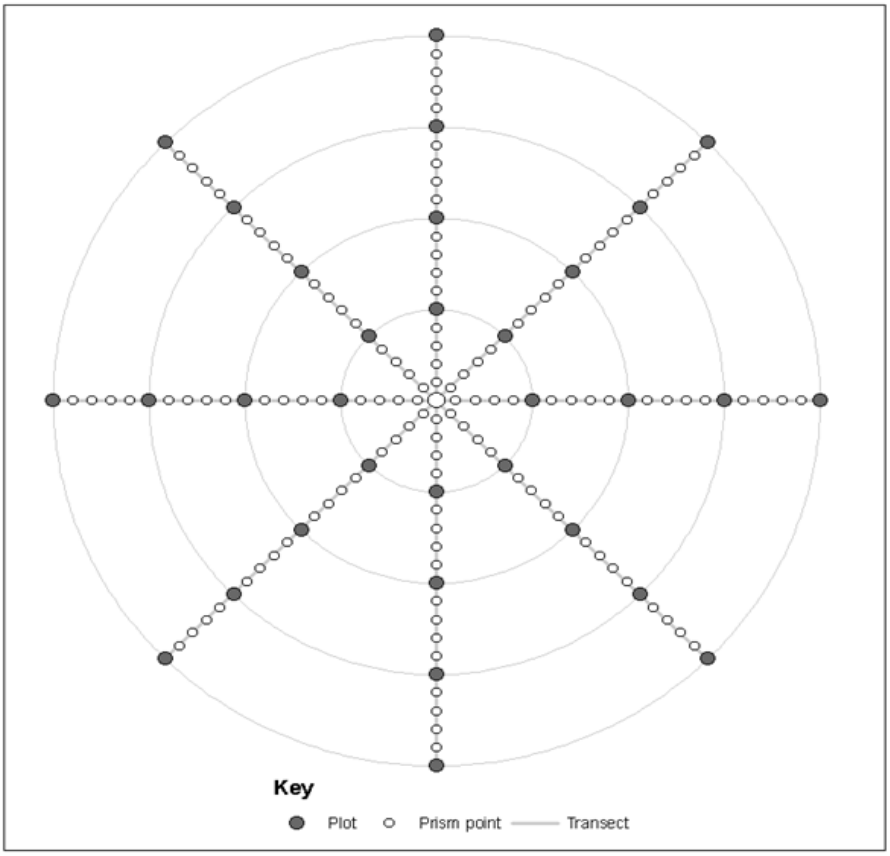

Figure 2. Ground survey sampling design

Ground survey located two additional infestations 0.8 km south and 1.2 km southwest of the original EAB find. A second population was located by ground survey in October of 2008 0.8 km west of the initial find. Transects were run from each of these additional populations, but no new infestations were located.

Destructive sampling and dendrochronology work will continue during the winter of 2009-2010. The rapid assessment of EAB infestations and ash resources using the radiating transects will be further evaluated in 2010.

MONITORING AND MODELING EAB EFFECTS IN FOREST ECOSYSTEMS: AN UPDATE ON OHIO MONITORING PLOTS AND ASH MORTALITY MODELING

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ABSTRACT

The effects of EAB on forest ecosystems are being studied through a collaborative research program involving USFS NRS-02 and Ohio State University. We are monitoring the decline and mortality of >4500 ash trees and saplings using a 1-5 rating system where 1 is a healthy canopy, 5 is a dead canopy, and 2-4 are stages of decline (Smith 2006), as well as changes in understory light availability, the responses of both native and invasive plant species, changes in species composition and forest structure, and effects on other organisms and ecosystem processes in over 250 monitoring plots in forests in Ohio and Michigan, representing a gradient of EAB infestation duration. The plots are located in forest stands representing different ages and habitat types to include all five ash tree species native to the region (*Fraxinus americana*, *F. pennsylvanica*, *F. nigra*, *F. profunda*, and *F. quadrangulata*). Yearly monitoring began in 2004 and is continuing.

A preliminary model of EAB-induced ash decline and mortality was presented at the EAB & ALB Research and Technology Development meeting in 2007 (Knight et al. 2008). This transition matrix model was based on >900 ash trees from 68 monitoring plots in 2005 and 2007, and tested with data (>500 trees) from 39 additional plots. The model predicted that a forest stand will progress from nearly all healthy trees to nearly all dead trees in a six year time period. The data collected since 2007 generally matches model predictions, although some ash stands died more rapidly than the model had predicted. We plan to revise the model using the additional yearly data that has been since collected, using a one year time step instead of a two year time step, and comparing other modeling techniques. The best model will be made available to managers.

We also plan to expand the model to ash saplings and seedlings, and to link the model to EAB population density, changes in understory light availability, and responses of forest ecosystems. Preliminary analysis suggests that light may increase gradually over a five year time period as ash trees die. However, not all stands experience this increase in light. Forest stands with abundant mid-story or understory trees, such as maple (*Acer* spp.) and elm (*Ulmus* spp.), may experience constant light levels despite ash mortality. As ash trees gradually die in these stands, the other species grow to fill in the canopy gaps.

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MATING FREQUENCY AND FECUNDITY IN THE EMERALD ASH BORER

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ABSTRACT

In theory, males maximize their fitness by frequent mating, while females mate only once provided they acquired sufficient sperm to fertilize all of their eggs. Nevertheless, multiple mating by female insects is the norm. Male and female emerald ash borers (EAB) mate multiple times. We examined the consequences of single versus multiple mating for female EAB longevity, fecundity and fertility. Newly emerged adult EAB were allowed to maturation feed for at least 7 days. We then set up three treatments. In two of the treatments, females that had been observed to mate were housed either alone (separate) or with their mate (together). In the third treatment, females were paired with a randomly chosen male (unobserved). Females were followed throughout their lifetime and laid eggs were counted and checked for viability. Weight and longevity of beetles did not differ among treatments. There was a significant positive correlation between fecundity and longevity in all treatments. Fertility was also positively correlated with longevity. The together treatment had the highest number of females laying eggs (27/31), followed by the unobserved treatment (19/31), and the separate treatment (17/31). The fecundity and fertility of females that did lay eggs did not differ among treatments. In the separate treatment, females that did not lay eggs were significantly lighter, and had a higher length/ weight ratio than females that did lay eggs. Females in the separate treatment mated one time, early in their adult lives, and their low length/weight ratio suggests non-laying females had not completed maturation feeding at the time of mating. The females in the other two treatments that did not lay eggs did not differ significantly in weight or length/weight ratio from females that did lay eggs. It is likely females that did not lay eggs in the unobserved treatment did not mate with their randomly-selected males. Our results suggest a single mating may be sufficient to ensure maximal fitness for females, but there is a high potential for failure of any one mating, and no apparent cost to longevity to multiple mating. Thus multiple mating is likely the best strategy for female EAB to maximize their fitness.

DEVELOPING AN EFFECTIVE AND EFFICIENT REARING METHOD FOR THE EMERALD ASH BORER

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis*, is a non-native insect from Asia that threatens ash trees in the urban and natural forests of North America. Research on this invasive insect and rearing parasitoids for release rely heavily on the ability to artificially rear the EAB. Methods exist to maintain EAB adults and obtain eggs, but result in inconsistent adult fecundity and longevity. An artificial diet that contains ash phloem exists but the phloem is time consuming to obtain and process.

We evaluated the effects of host plant, temperature, humidity, and oviposition substrates on adult survival and fecundity. Each treatment used 21-40 pairs of adults that emerged from infested wood collected in Michigan. The pairs were held at 25 °C, 65% relative humidity (RH) and 16 h:8 h (light: dark) cycle except in the temperature and humidity studies. To determine if group mating was an option we compared single pairs with 10:10, 15:5, and 20:5 (male: female) ratios. We also assessed temperature effects on time to egg hatch (60 eggs each at 20, 25, and 30 °C) and effect of pre-pupal chilling (22 per treatment at 5, 10, and 15 °C for 56 or 84 d) on pupation at different temperatures to determine if stockpiling insects was an option. We also evaluated larval development on eight artificial diets that did not contain host material (two containers and 100 larvae per treatment).

EAB adults did not survive or reproduce well when fed *Fraxinus griffithii* leaves, an evergreen ash of Asian origin, but did well when fed mature foliage of *F. uhdei*, an evergreen ash of North American origin. When adults were fed newly expanded *Fraxinus* foliage, fecundity, longevity, and the percentage of females that oviposited were all lower than when mature foliage of the same species was used.

The best oviposition substrate, of those tested, was a 2 x 20 cm bolt (either ash or PVC rod) wrapped with butcher paper around which a non-overlapping spiral of 1.3 cm wide strip of purple curling ribbon was secured. Using this substrate, 50% of the eggs were laid on the butcher paper under the curling ribbon which made removing and holding the eggs fairly easy. The eggs on butcher paper can also be cut apart and placed on artificial diet to allow the larvae that hatch to burrow in on their own.

Adult EAB longevity decreased as temperature increased from 20 to 30 °C. Only one female oviposited at 20 °C. Percentage females ovipositing and fecundity were higher at 25 °C than at either 20 °C or 30 °C. Percentage egg hatch was also highest at 25 °C. Female fecundity and survival were

lower at 55% and 75% RH than at 65% RH. There was little effect of percent RH on male survival. Egg hatch was lower at 55% RH than in the higher percent RH treatments.

Individuals mating when held in large groups resulted in higher fecundity per female but reduced female survival time by a couple of weeks compared to single pairs. The data suggest that 20 individuals per container (either 10 females and 10 males, or 15 females and 5 males) are optimal for egg production. Egg hatch from group matings was the highest (+80%) of any treatment we evaluated for adults. Egg hatch in general was good (averaging >60%) when the eggs were held in 24-well plates or petri dishes that were held in water boxes (to maintain high humidity).

The lower limit for egg hatch was between 14 and 16 °C (estimated by either linear or polynomial methods). The upper limit could not be accurately estimated without testing higher temperatures. We found eggs could be held at 20 ° C to slow hatching and at 15 °C to prevent hatch.

The seventh host-free artificial diet evaluated seemed to have the correct nutritional content and the proper moisture content to allow larvae to burrow in and survive. However, some larvae tunnel straight down and enter the plastic bottom of the dish partway, and then apparently cannot back out. About 90% of the larvae established on diet seven when presented in a 55 mm vented petri dish and held in a water box. The larvae grew as fast as would be expected in ash phloem. A petri dish with a tight fitting lid seems to hold too much moisture and reduces establishment. The best method for reintroducing the larvae into fresh diet after the first 5 weeks of rearing was to cut a chunk out of the center and place the larvae on the petri dish bottom and then cover it by reinserting the diet chunk. Diet for the larger larvae may need to be presented in a different form. We also experimented with group establishment in 90-mm petri dishes using eggs on butcher paper and produced similar results when we used single larvae infested in 55-mm petri dishes. Larvae in groups must be split up into singles by the third week to prevent cannibalism, but manipulating the smaller larvae can damage them. We have produced third generation adults using diet number seven.

We found we found that the longer pre-pupae were chilled the faster they will proceed to pupation once temperatures are warmer. The percentage of successful pupation was higher at 10 °C than for either 5 or 15 °C. We had some problems with desiccation, even using a water box, since these larvae were only held in well plates with a filter paper and no diet. There were still several problems to overcome in chilling and handling of the larger larvae.

Pupation and adult emergence progressed normally when the individuals were held in either vented 40-mm petri dishes or 6- to 12-well plates containing filter paper in the bottom for traction and to maintain moisture levels. The plates or dishes were held in a water box to maintain moisture. Adults were held for 8-10 days in this container then a leaf of ash foliage was added. Once adults begin feeding they are moved to quart containers and allowed to feed 10 days before mating.

These results provide good methods for maintaining adults and obtaining eggs that will hatch. Further improvement may be possible by using different containers or group rearing. More work is needed to develop a host-free artificial diet that can be used to rear larger numbers of larvae. The current best diet allows EAB larvae to complete development and the resulting adults survived and reproduced as well as those EAB that emerged from cut wood.

Regulatory, Management, and Outreach

FIREWOOD AS A POTENTIAL PATHWAY FOR EXOTICS IN THE SOUTHERN ROCKY MOUNTAINS

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ABSTRACT

Firewood can harbor and vector bark beetles, wood borers, canker, decay, and stain fungi. Regions of the western United States with the biggest draw for campers visiting National Parks are the interior west (4.1 million campers); west coast states (3.6 million campers); and Rocky Mountain states (2.5 million campers). Arizona, Colorado, Utah, Wyoming and New Mexico are ideal places to conduct research on firewood movement, as many visitors come to Rocky Mountain, Great Sand Dunes, Yellowstone, Grand Teton, Arches, Zion and the Grand Canyon National Parks.

We are investigating how much firewood is transported across state lines by campers coming to National Parks. We are also investigating the sources of retail firewood, the relative amount of retail firewood that is imported across state lines and the presence of live insects in the firewood in Colorado, New Mexico, Wyoming and Utah. Specifically our study objectives are to: 1) determine the introduction risk of EAB or other non-native pests into four western states (Colorado, Arizona, Utah, and Wyoming) through the transportation of firewood by out-of-state visitors to national parks; 2) determine the introduction risk of EAB and other non-native pests into four states with high urban populations in the southern Rocky Mountain region (Colorado, New Mexico, Utah, and Wyoming) through retail firewood sales.

Between March and July 2009, we conducted personal interviews of 1,656 campers at 30 campgrounds in thirteen National Parks from Grand Canyon NP in Arizona to Yellowstone NP in Wyoming. Seventy percent of these campers had firewood at their sites and 8-60% brought firewood from an out-of-state source to the park. Extrapolating these percentages with the number of overnight campers in 2008 from the National Park Service Public Use Statistics Office, we estimate that a potential of 1.2 million campers are bringing firewood from an out-of-state source to the thirteen national parks in one year.

We conducted phone surveys of various types of retail outlets (e.g. grocery stores, big box stores, convenience stores) to determine sources of firewood sales before purchasing over 300 bundles of firewood at various types of retail outlets in New Mexico, Colorado, Utah, and Wyoming. Based on manufacturers' labels (which may not be the source of the firewood), 45-67% of purchased firewood was from out-of-state. Most firewood bundles were conifer species (90%) with bark attached and 10% were hardwood species (with bark?). Sixty-five to 90% of the wood had evidence of pre-existing insect and/or fungal infestation (exit holes, galleries, stain, conks, etc). The firewood has been placed in rearing cages and emerging insects are collected monthly. The study will continue through the summer of 2010.

CITIZEN SCIENCE PROJECT LEADS TO EMERALD ASH BORER PARASITOID DISCOVERY

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ABSTRACT

An "EAB in the Classroom" curriculum is being developed based on a project with 7th and 8th grade advanced science students who studied EAB at their 15 ha school forest at Dempsey Middle School, Delaware, OH. EAB was discovered in an adjacent woodlot in 2005. Students established monitoring plots in 2006. In December 2007, one ash tree from each plot (selected as the ash tree closest to plot center in the 3-18 cm DBH size range, for a total of 35 trees) was felled and the trunk was cut into 1 m segments. In fall 2008, students used a 1-5 rating system (Smith 2006), where 1 is a healthy tree, 5 is a dead tree, and 2-4 are stages of decline, to rate the canopy condition of ash trees. In December 2008, four green ash (*Fraxinus pennsylvanica*) and 25 white ash (*F. americana*) trees, selected to represent each ash canopy condition and ranging from 8-25 cm DBH, were felled and cut into 1 m segments. In January 2008 and 2009, respectively, students collected data on woodpecker feeding holes, EAB exit holes, and exit holes of other species on the outside of the segments. After collecting external data, they removed the bark to collect data on EAB larvae, EAB prepupae, other insects, and percent cover of EAB galleries. In January 2008, all EAB and non-EAB insects were preserved. Later examination of these specimens revealed the presence of parasitoid larvae and cocoons. In January 2009, all parasitoids were collected and incubated for emergence, EAB prepupae were frozen for isotopic analysis, and non-EAB insects were preserved. Peeled logs, as well as one unpeeled log per tree, were incubated for insect emergence.

In 2008, data revealed a patchy infestation with some trees uninfested and other trees ranging from lightly infested with only a few EAB galleries to heavily infested with galleries covering >80% of the trunk surface area. EAB galleries were present at all heights in the trees in both years. In lightly infested trees in 2008 and 2009, galleries were only in portions of the trunk 4 – 8cm diameter and primarily in portions of the trunk 6 – 12 cm diameter, respectively. In large trees, these areas occurred higher on the trunk, while in small trees, these occurred lower on the trunk. In highly infested trees, EAB galleries occurred across a large range of trunk diameters, with the greatest gallery cover in the larger and lower portions of the trunk. The incidence of two-year development of EAB was negatively correlated with EAB gallery cover. EAB larval mortality due to woodpeckers and mortality due to competition were positively correlated with EAB gallery cover. Woodpeckers consumed up to 90% of EAB larvae in the trees with an average of approximately 50% of the larvae consumed in highly infested trees. Results from 2009 are reported by Flower et al. (this volume).

Fifty parasitoid cocoons and 12 parasitoid larvae in EAB galleries beside an EAB cadaver were collected and incubated and an additional nine parasitoid adults emerged from incubated logs (Kula et al. in press). A total of 2,567 EAB larvae and prepupae were recorded, giving a parasitism rate of 2.8%. Of the parasitoids that emerged, 29 were *Leluthia astigma*, which is native to the Nearctic region and had not been reported previously as a parasitoid of EAB. These were reared in the lab and parasitism of EAB was observed. A few *Metapelma spectabile* and unidentified species of ichneumonid wasps also emerged. The parasitoid species we observed differed from the four parasitoid species observed emerging from ash logs the previous year from the same site (C. Goebel, M. Bumgardner, and D. Herms, personal communication).

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COMPARING POTENTIAL MANAGEMENT OPTIONS TO SLOW THE SPREAD OF EAB POPULATIONS IN LOCALIZED SITES

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ABSTRACT

The spread of many invasive species, including the emerald ash borer, *Agrilus planipennis* Fairmaire, are characterized by stratified dispersal, whereby dispersal occurs by at least two separate mechanisms, a short distance and a long distance dispersal mechanism. This process leads to a spread pattern marked by the presence of satellite colonies ahead of the main invasion front. Following establishment, these satellite colonies typically grow, coalesce and ultimately greatly increase the speed of the invasion front. Consequently, any action to reduce their formation or growth can significantly decrease the spread of an invasive species. The small population sizes typical of newly formed isolated populations also make successful management more practical.

Using a simulation model, we evaluate two potential management options to slow the spread of *A. planipennis* in discrete outlier sites: 1) girdling ash trees to attract ovipositing female beetles and destroying the trees before larvae complete development, and 2) applying a highly effective systemic insecticide.

The underlying model used to examine the effect of the three management options on localized spread is a spatially explicit coupled lattice model of *A. planipennis* population growth and spread from small, isolated colonies. In brief, the model couples population processes relevant to the spread of *A. planipennis* onto matrices that record the quantity of available ash phloem and the number of *A. planipennis* larvae that develop in 1 or 2 years. Population processes linking these matrices include 1) emergence of adult beetles in a particular year, 2) dispersal of adult beetles, 3) population growth, and 4) loss of ash phloem through consumption by developing *A. planipennis* larvae, which were determined and parameterized using data collected from multiple field sites. Finally, onto this base model we incorporated the effect of the two management options.

Girdled trees were simulated in the model by removing the phloem area and the number of larvae expected to be in a girdled tree. As *A. planipennis* populations increase in an area, the proportion of stressed trees also increases. Consequently, the attractiveness of girdled trees relative to non-girdled trees is likely to diminish over time. Using observations from 16 sites, we fit a power function to describe

the relative attractiveness of girdled trees as population density increases. We used this relationship to estimate the proportion of larvae expected to be removed from the population per m^2 of girdled ash phloem present in each cell. For our insecticide applications, we assumed treatments completely protect trees and exhibit no repellency effects. Therefore, once insecticide treatments were applied to chosen cells, the larvae developing in the proportion of phloem contained in treated trees were assumed to be eliminated from the population prior to consuming a noticeable quantity of phloem. Thus, phloem area present in treated trees would be unaffected by larval feeding.

An important component of understanding the spread of an organism is the potential for directed movement to affect spread, which may affect the relative efficacy of management options. In addition to being more attractive compared to neighboring healthy trees, movement of beetles towards areas containing girdled trees has been observed (Siegert et al. unpublished results). Here, we simulated the attraction of *A. planipennis* to areas containing trees stressed by girdling or by damage induced by *A. planipennis* larvae by increasing the attraction of cells containing a girdled tree by a factor of 7. For cells without girdled trees, we increased the attraction to cells containing stressed trees as a linear function of the proportion of phloem consumed by beetles (100% when no phloem consumed, 700% when greater than 33 % of available phloem consumed).

Using this model, we performed simulations applying each of the management options outlined above, varying the radius of application from 0 to 1,700 m in 50 m increments (1 cell) and the proportion of phloem available to be treated in each cell from 0-90%. Simulations were conducted in homogeneous environments consisting of a matrix of 100×100 cells, where each cell was 50×50 m in size and contained 200 m2 of ash phloem. All infestations were initiated at the center of the environment with an initial population size of 100 adult beetles and assuming a 50: 50 sex ratio.

Our simulations indicated that the use of a highly effective systemic insecticide (e.g. emamectin benzoate) is likely to greatly reduce the local spread of *A. planipennis*. In particular, simulations indicated a fairly linear and consistently diminishing radial spread of *A. planipennis* with increasing proportion of trees available for treatment and radius of treatment applications. Up to a >90% reduction in spread was observed when 90% of phloem was treated in a 1,700 m radius from the origin of infestation. In addition, the use of insecticides proved highly effective when a dispersal bias towards areas containing stressed trees was or was not included. Simulations also indicated that the use of girdled trees is likely to reduce the radial spread of *A. planipennis*, albeit less than insecticide use. It is important to note that simulations also suggest that the design used to deploy girdled trees across an environment can have a considerable influence on the effectiveness of this management option. Because the relative attractiveness of girdled trees as an ovipositional substrate is maximized when *A. planipennis* density is low, placing girdled trees at the leading edge of the invasion wave would be ideal. However, delineating the leading edge is difficult and girdling trees too far ahead of the invasion wave could waste resources and exhaust the number of trees available for girdling prior to the arrival of *A. planipennis* individuals. More significantly, if *A. planipennis* ovipositing females are strongly attracted to areas containing girdled trees, establishing girdled trees too far ahead of the leading edge could potentially increase the radial spread of *A. planipennis*.

THE UPPER PENINSULA SLAM PROJECTS - AN INTEGRATED STRATEGY TO SLOW ASH MORTALITY IN EMERALD ASH BORER OUTLIER SITES

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ABSTRACT

SLAM is an integrated, multi-year strategy designed to suppress EAB population growth and delay the onset and progression of widespread ash mortality in isolated outlier sites. The components of a SLAM project include: 1) EAB surveys for distribution and density; 2) ash surveys for distribution and amount; 3) population suppression tools and tactics; 4) regulatory activities; 5) data management and evaluation; and 6) outreach and communications. Suppression tools and tactics include some combination of insecticide treatments, girdled ash trees (sinks), and ash utilization (phloem reduction).

A SLAM pilot project was initiated in Mackinac County, Michigan in fall 2007. Two relatively recent EAB introductions, one near the rural community of Moran and one in the city of St. Ignace, provided an opportunity to implement and evaluate a SLAM strategy. A grid survey for EAB has been conducted using girdled detection trees and baited panel traps in 2008 and 2009, documenting the local EAB infestation status. Detection trees have been cut and 100% peeled each year. Treatments were initiated in 2009 using insecticide injections and sink tree establishment and removal at both sites. Other treatment tactics and tools that have been or will be implemented include timber sales to reduce phloem and remove infested trees, and cut-and-leave projects to reduce phloem. The project includes an extensive outreach effort to communicate with local land owners and land managers. This SLAM pilot project is a cooperative effort between the Michigan Department's of Agriculture and Natural Resources; Michigan Tech University; Michigan State University; US Forest Service Northeastern Area, Northern Research Station, and Hiawatha National Forest; and USDA APHIS.

An ongoing evaluation effort will be initiated in an attempt to document success or failure at delaying the onset and progression of ash mortality at these sites. Further treatments and surveys are planned in 2010 and 2011.

Host Relations

HOST VIGOR AND EMERALD ASH BORER LARVAE ON GREEN AND WHITE ASH

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ABSTRACT

The emerald ash borer (*Agrilus planipennis* Fairmaire, Coleoptera: Buprestidae) is a devastating invasive pest of stressed and healthy ash trees in the United States. Previous research has shown that (1) emerald ash borer (EAB) adults are highly attracted to stressed trees and (2) most EAB larvae complete their development in one year on stressed trees (Cappaert et al. 2005; McCullough et al. 2009, 2009a; Tluczek 2009). Additionally, green ash (*Fraxinus pennsylvanica*) trees are often attacked sooner or succumb faster than white ash (*F. americana*) trees (Anulewicz et al. 2007).

We selected 20 green ash and 20 white ash trees (average DBH 5.5 ± 0.1 cm) planted in a randomized block design in a plantation at the Tree Research Center on the Michigan State University campus. One green and one white ash tree per block were randomly assigned to one of four treatments: girdling, fertilization, enhanced fertilization, or untreated control. Fertilized trees received a single granular application of Harrell's Pro-Blend with Micros 19-5-10 controlled release fertilizer at a rate of 40 g N per tree on 15 May 2008. In addition to the granular fertilizer, a trunk spray with the phosphite fungicide Agri-Fos® + Pentra-bark™ (a bark-penetrating surfactant), was applied to the trees assigned to the enhanced fertilization treatment on 15 May 2008. Agri-Fos has induced pathogen resistance by increasing defense mechanisms in some tree species (Garbelotto et al. 2007). To girdle trees, we removed a 20 cm wide band of outer bark and phloem 1 m aboveground on 28 May 2008. All trees were irrigated weekly during the study. A wild population of EAB colonized these trees throughout summer of 2008.

Foliage from all trees was collected on 16 Jul 2008 and analyzed for nitrogen, protein, and amino acid content. The Li-Cor LI-6400 system was used to measure photosynthesis rates on 30 Aug 2008. Foliage was analyzed weekly for chlorophyll content through the summer using the Minolta SPAD 502 Chlorophyll Meter. Total chlorophyll levels were significantly lower in foliage from girdled trees compared with other treatments and significantly lower in white ash trees compared with green ash trees through most of the summer. Foliage from girdled trees had the lowest levels of nitrogen, protein and amino acids, and these trees had lower photosynthetic rates compared with trees of other treatments. Fertilized green ash trees exhibited lower photosynthesis than the untreated or enhanced fertilization trees. Overall, neither fertilization nor enhanced fertilization consistently increased foliar nutrition variables compared to untreated trees.

Trees were felled and debarked in autumn 2008 to evaluate EAB larval density, mortality, and development rates between species and among treatments. Based on previous research, larvae overwintering as first, second or third instars were expected to require a second summer of feeding. Larvae that were overwintering as fourth instars or prepupae were expected to emerge as adults the following spring.

Within white ash trees, larval density was significantly higher on girdled trees than on trees of other treatments. Overall, very few larvae (average < 7 larvae per m2) were found on any of the non-girdled white ash trees. Within green ash, larval density and mortality was significantly higher on girdled trees than on untreated trees and trees treated with fertilizer + Agri-Fos, but larval density and mortality did not differ significantly between girdled trees and fertilized trees. An average of 321.3 ± 38.0 larvae per m2 were found on girdled green ash trees, and an average of 178.6 ± 28.9 larvae per m2 were found on girdled white ash trees. On fertilized trees and trees receiving enhanced fertilization, larval densities were higher on green ash trees than on white ash trees. Larval density did not differ between green ash and white ash on girdled trees or untreated trees.

Larval mortality was extremely high (nearly 40%) on girdled green ash trees. This mortality was caused by high intraspecific competition for phloem on these trees. Larvae developed significantly faster on girdled white ash trees than on white ash trees treated with enhanced fertilization. Variability in larval development on non-girdled white ash was high, however, because of the low larval densities on these trees. Most larvae on untreated or fertilized white ash trees would have required a second year for development, while nearly all larvae on the girdled white ash trees would have developed in 1 year. On the green ash trees, nearly 70% of the larvae on girdled and fertilized green ash and 40-55% of larvae on untreated green ash and green ash treated with enhanced fertilization would have developed in a single year.

Results suggest that white ash and green ash trees may respond differently to stress and vigor treatments. Fertilized green ash trees were colonized by EAB at higher rates than fertilized white ash, even with trees randomly arranged, illustrating the strong preference of EAB for green ash over white ash even when trees are healthy. More research is needed to determine how stress and vigor affect green and white ash physiology, EAB colonization and larval development. These discoveries could help to identify mechanisms of host tree resistance to EAB.

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THE FAST AND THE FURIOUS: INFLUENCE OF HOST VIGOR ON DEVELOPMENT AND MORTALITY OF EMERALD ASH BORER LARVAE

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ABSTRACT

When emerald ash borer, *Agrilus planipennis* Fairmaire, was first investigated after its discovery in 2002, it was thought to have a one-year life cycle. Adults emerged in the spring, laid eggs during the summer and larvae fed during the late summer and fall, overwintered as prepupae, then pupated in the spring and emerged as adults. However, in 2004, we determined that some emerald ash borer (EAB) larvae may require two years to develop. A two-year life cycle appears to be most common at recently infested sites where densities are relatively low and trees appear healthy. If prolonged development of EAB larvae is common, it would strongly influence EAB spread, population dynamics, and survey activities of program managers.

In 2006 and 2007, we examined the effects of host stress on the density, development, and mortality of EAB larvae. We also quantified larval mortality and determined the main sources of that mortality.

Methods. We established a randomized complete block design in a 15-year-old, well-stocked ash plantation with a low, nearly undetectable EAB population in 2006. In May of 2006 and again in 2007, we selected 90 healthy green ash trees (mean DBH of 12 cm). Each year, we randomly assigned 30 trees to one of three treatments: 1) girdling 2) exposure to methyl jasmonate (MeJa) which increases emission of stress-induced volatiles, or 3) untreated controls. Sticky bands (30 cm wide) coated with Tanglefoot were wrapped around the trunk of all 90 trees each year to capture adult EAB.

All trees were felled each winter and bucked into 1 m sections from the soil line to a height of 8 m. Each section was debarked and larvae were counted and identified to instar. Larval density was standardized by dividing the number of larvae by the area of bark peeled. Fourth instars and prepupae were recorded as 1-year larvae, while first, second, or third instars were classified as 2-year larvae because they would likely have fed for an additional year to complete development. Dead larvae were assigned to one of three categories: predation by woodpeckers, cannibalism, or death from unknown causes (most likely by a pathogen or desiccation).

Results. In 2006, an average of 0.9 ± 1.8 adult beetles per tree was trapped on sticky bands during the summer; nearly all of the 81 beetles were trapped on girdled trees. In 2007, the number of adults captured increased to a mean 4.7 ± 1.5 adult beetles per tree; again, nearly all of the 426 beetles

were trapped on girdled trees. Larval density significantly varied among treatments in both 2006 and 2007. Larval density on girdled trees was 70 larvae per m^2 in 2006 and 175 larvae per m^2 in 2007, which was six-fold higher than the larval densities found on control trees and trees treated with methyl jasmonate. A significantly higher proportion of larvae overwintered as fourth instars or prepupae on girdled trees than on control trees or trees treated with methyl jasmonate. After taking treatment into account, however, larval development did not significantly vary by larval density in either year. We also found that larvae feeding below the girdle developed significantly faster than larvae feeding above the girdle. Additionally, in both 2006 and 2007, larvae below the girdle had a wider horizontal feeding pattern than larvae above the girdle. The feeding pattern of larvae found above the girdle on girdled trees was similar to that of larvae feeding on control and methyl jasmonate-treated trees.

In 2006, 30.1% of larvae died, and in 2007, 35.7% of larvae died. Woodpecker predation was the highest cause of EAB mortality in both 2006 and 2007, accounting for 24% and 31% of the mortality, respectively. Mortality from unknown causes accounted for 6% and 4% of total morality, respectively. Cannibalism was minimal. No parasitism was observed in either 2006 or 2007.

COAST TO COAST ASH MORTALITY? POTENTIAL SUSCEPTIBILITY OF SELECTED WESTERN AND SOUTHERN ASH SPECIES

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ABSTRACT

Emerald ash borer (EAB) has become one of the most devastating invasive pests in North America. Tens of millions of ash (*Fraxinus*) trees in forests and millions of landscape ash trees in urban settings have already been killed by this Asian pest. Populations of EAB have been found in 13 states and two Canadian provinces so far. These infestations continue to expand and additional infestations will undoubtedly be discovered.

The range of ash spans the continent of North America and widespread ash devastation, similar to that in southern Michigan and northern Ohio, could potentially occur. EAB has successfully colonized every ash species it has encountered to date in eastern forests and urban areas, including green ash (*F. pennsylvanica*), white ash (*F. americana*), black ash (*F. nigra*), blue ash (*F. quadrangulata*), pumpkin ash (*F. profunda*) and a number of commercially available hybrids. There are at least nine other native ash species and several European species occurring in the US whose susceptibility to EAB is unknown. If one or more of these species proves to be resistant, then we may be able to identify these mechanisms of resistance and use them to enhance other vulnerable species. In addition, knowing the relative susceptibility of ash species to EAB could help municipal foresters, natural resource managers and regulatory officials plan for the arrival of EAB in their region. Our objectives were to 1) determine if adult EAB will feed on foliage of novel ash species and, if so, evaluate their longevity, and 2) determine whether female EAB will oviposit on novel ash species and, if so, evaluate larval survival and development.

For our first objective, we tested seven species including green ash, which occurs throughout most of the U.S. and Canada, Oregon ash (*F. latifolia*) whose range includes Oregon, Washington, California and British Columbia, and single-leaf ash (*F. anomala*) which occurs primarily in the southwestern U.S. We included two European species, European ash (*F. excelsior*) and flowering ash (*F. ornus*),

the Asian species, Manchurian ash (*F. mandshurica*) and European privet, (*Ligustrum vulgare*), a member of Oleaceae and widely distributed throughout the U.S. Seedlings were obtained from nurseries throughout the U.S. from January to April 2008, kept in cold storage until potted in June 2008, then maintained in a lathe shade house throughout the summer.

To assess leaf-feeding, leaf samples were taken from 15-21 plants of each species and pre-feeding leaf area was quantified using leaf-scanning software. One leaf and three similarly-aged, unfed EAB beetles were placed into a Petri dish and allowed to feed for 4 days. Leaf petioles were submerged in a water pic to prevent desiccation. After 4 days, leaves were re-scanned and the total leaf area consumed was determined. EAB fed significantly less on single-leaf ash and privet than on the other ash species. Seedlings were overwintered outside, then transplanted to an irrigated, pot-in-pot plantation in spring 2009. Leaf samples were taken from 8-12 plants of each species and bioassays were repeated using the same methods as in 2008, except that four EAB per plate were used and single-leaf ash, which did not overwinter well, was excluded. EAB fed significantly less on privet than on European, green, Oregon and Manchurian ash, while flowering ash was intermediate.

Beetle longevity on novel ash species was evaluated in 2008 by taking leaf samples from 12 plants of European, Manchurian and green ash, and privet. Three EAB were placed in a Petri dish with one leaf and allowed to feed for up to 14 days. Leaves were replaced and beetle mortality was recorded every 2-3 days. Nearly 100% of beetles died after 14 days on privet, significantly more than on European, Oregon, green and Manchurian ash. Oregon ash was intermediate, averaging 75% mortality. The study was repeated and expanded in 2009 to include single-leaf ash. There was significantly higher mortality on single-leaf ash and privet after 14 days than on the other species we tested.

In June 2009, foliage and logs were collected from western and southern ash species, including Carolina ash (*F. caroliniana*) from Florida, velvet ash (*F. velutina*) from Arizona and Utah, and single-leaf ash from Utah. Samples were collected from three trees of each species. Foliage was shipped overnight in coolers to MSU. Adult leaf-feeding bioassay methods were conducted as described in 2009. EAB fed significantly more on Carolina ash and velvet ash from AZ than on the other species.

To address Objective 2, logs were harvested from the above species, along with uninfested green ash from MI. Cut ends of each log were sealed and logs were shipped to MSU. Logs arrived within 3 days of cutting and were placed in cold storage. In July, one log of each species was attached to each of 15 ash trees at sites with high densities of EAB (n=15 logs/sp; 75 logs total). Logs were retrieved in mid-August. We examined each log for 5 min to count visible eggs. Logs were then debarked to determine larval density, stage and status. We found a total of 105 eggs on 20 logs. Nearly half the eggs (47%) were found on 6 green ash logs, 33% on 6 AZ velvet ash, 18% on 6 Carolina ash, and 2% on 2 UT velvet ash logs. No eggs were found on single-leaf ash logs. There were a total of 204 early instar EAB galleries on 24 logs. Overall, 66% of galleries were on 11 green ash logs, 25% were on 6 AZ velvet ash, 4% on 3 single-leaf ash, 3% on 3 UT velvet ash, and 2% on a single Carolina ash log. More than 95% of the larvae found on all species survived only to the first instar before dying because of log desiccation. Eight logs had eggs but no galleries, while 12 logs had larval galleries, but we could not find any eggs.

In summary, adult EAB fed to some degree on foliage from all species tested. Beetles consumed little single-leaf ash or privet foliage and few survived for 14 days on these species. Eggs and at least a few first instar galleries were observed on logs of all species tested. Unfortunately, logs desiccated before larvae could develop to later instars. Additional study is needed, preferably with live trees, to better assess larval development.

PRESERVATION OF ASH GENETIC RESOURCES BY LONG TERM SEED STORAGE: INTERAGENCY COOPERATION AND PROTOCOL DEVELOPMENT

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ABSTRACT

Breeding ash trees resistant to the EAB is one technique that is being explored to protect the ash resources. The occurrence of resistant individuals appears to be very low or zero in many areas. Therefore, it is necessary to somehow preserve a sufficient amount of adapted germplasm in advance of the borer. An interagency effort is coming together that has to date placed approximately 1000 trees into the National Plant Germplasm System that can be used to either reintroduce ash to devastated areas or breed resistant populations. Protocols for sampling populations of parent trees, seed collection, and seed handling are developed. The various approaches followed by different federal and state agencies are being woven into a comprehensive strategy.

A COMPARATIVE SYSTEM BIOLOGY APPROACH FOR UNVEILING THE RESISTANCE MECHANISMS OF ASH TREE SPECIES TO EMERALD ASH BORER

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ABSTRACT

The introduction of EAB into the United States threatens extirpation of native North American ash. Asian species of ash have co-evolved with the insect and have robust mechanisms of defense that are absent in indigenous ash population. Establishment of effective strategies for combating EAB to rescue American ash trees depends upon detailed understanding the resistance mechanisms present in Asian trees but absent in American ash trees. We propose a comparative top-down systems biology approach utilizing cutting-edge next generation sequencing technologies, and systemic data mining to unveil the resistance mechanisms to EAB in Asian ash species. Upon completion, this research will open up multiple avenues for augmentation of the resistance in American ash species via genetics engineering and conventional breeding.

We propose that a breakthrough can be achieved by performing a single experiment to generate both biological sequences and expression data for recognizing resistance mechanisms via state of the art statistical analysis and data mining. We will be able to extract the following information: A. Identification of biochemical pathways and control points responsible for resistance to EAB. B. Identification of genes conferring protein-derived resistance to EAB; C. Identification of gene-for-gene pairs (termed as R versus avirulence) in ash and EAB that determine the outcome of interaction; D. Identification of marker genes for early diagnostics and long-term breeding; E. generation of sequence and expression data resources for EAB research community, which is sought by ash research community.

We envision three major ways to utilize the knowledge generated in the previous phases: (1). Develop markers for easy detection of EAB infected trees. (2). Transfer resistance to native ash trees via genetics engineering. We will genetically modify the resistance-derived pathways, or individual genes encoding a protein that can interfere with EAB digestion or development process. (3). Knowledge gained in the resistance mechanisms will enable development of marker-assisted selection for acceleration of the back-crosses to American ash following the first round of inter-specific hybridization and identification of individuals carrying rare resistance alleles in native population.

The use of the top-down systemic approach can identify multiple focuses in one effort at low cost. Upon completion, we should reveal a few mechanisms that enable breakthroughs in detection, management and incorporation of these mechanisms into American ash trees. The information and genomics resources resulting from this project will lead to initialization of multiple projects targeting for complete eradication of EAB by focusing on the critical points or mechanisms. This is the most opportune time to initiate such a project because of the availability of high-throughput sequencing technologies and state of the art data mining methods.

USING STABLE ISOTOPES AS A TOOL TO INVESTIGATE THE IMPACTS OF EAB ON TREE PHYSIOLOGY AND EAB SPREAD

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ABSTRACT

As no economically or ecologically feasible option has been proposed to inhibit the propagation of EAB, all ash trees in the eastern United States remain at risk. In an effort to quantify the ramifications of ash mortality on standing carbon stocks in the eastern US we relied on Forest Inventory and Analysis estimates of standing forest biomass of ash wood based and adjusted this quantity based on bulk density and species specific carbon content. Forests in the Eastern US contain over 1.9 Pg C in standing aboveground *Fraxinus* trees, a quantity roughly equivalent to 1.5% of the world's annual gross primary production. Yet despite these potential consequences, the ecology, spread and impacts of EAB on ecosystems are not well understood.

Stable isotopes integrate chemical and physical processes at a variety of spatio-temporal scales and can be used to study the interactions between EAB and ash. Carbon naturally exists in the two stable isotopes ^{12}C and ^{13}C, the lighter isotope is naturally more abundant in the atmosphere and preferred in plant biochemical reactions such as photosynthesis. During times of stress, such as those induced by EAB or unfavorable climatic conditions, a plant incorporates more of the heavier isotope, and so will organisms that feed on that plant. Hence, the carbon isotopic composition ($\delta^{13}C$) of EAB may be used as a proxy for ash tree health.

This study used the carbon isotope ratio (^{12}C:^{13}C) of *Fraxinus* spp. leaf tissue to investigate relationships between tree-level stress and a user-friendly ash canopy condition rating method (AC) used by Ohio State University and USDA Forest Service researchers. AC is a visual assessment of canopy health graded on a 1 to 5 scale, where 1 represents a healthy canopy and 5 a dead canopy. Leaf tissue was collected from the upper canopy of 25 *F. americana* and 4 *F. pennsylvanica* in conjunction with a citizen science program described by Knight et al. (this volume). Percent EAB gallery cover, an assessment of larval activity and density, was estimated from gallery cover counts in six 25 cm^2 grids placed on each 1 m segment of each tree. EAB prepupae and emerged EAB adults were collected from each segment and analyzed for $\delta^{13}C$.

Results indicate a significant correlation between AC and leaf $\delta^{13}C$ isotopic enrichment. AC also exhibited a significant correlation with EAB gallery cover. There was also a significant positive correlation between the leaf carbon isotope ratio and EAB gallery cover. These results confirm that the mechanism by which EAB causes tree mortality is through the serpentine galleries formed

by larval feeding behavior which induces chronic water stress. Furthermore, these results validate the physiological basis of AC as an easy method to represent these processes.

An analysis of the $\delta^{13}C$ of both EAB prepupae and adults from these trees indicates a significant correlation between the $\delta^{13}C$ of EAB and that of leaf tissue, suggesting the $\delta^{13}C$ of adults can be used as integrators of their host tree and therefore AC rating. Utilizing a triple isotope (^{13}C, ^{15}N, and δD) approach to assess the isotopic composition of adult EAB from outlying or recently discovered populations may facilitate a better understanding of EAB dispersal patterns. This is based on the principle that the deuterium isotopic composition (δD, or ^{2}H) varies temporally and spatially according to altitude and atmospheric variables such as temperature and relative humidity. As a consequence of an EAB's diet, its tissue will reflect its local environment, and thereby an EAB adult trapped at a site will largely reflect the δD and ^{15}N composition from its parent tree or forest stand. When these measurements are combined with $\delta^{13}C$ then the spread of EAB can be quantitatively assigned to a region and an AC score within that region.

COMPARATIVE CONSTITUTIVE AND INDUCED PHLOEM CHEMISTRY OF NORTH AMERICAN, EUROPEAN, AND ASIAN ASH SPECIES THAT VARY IN RESISTANCE TO EMERALD ASH BORER

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ABSTRACT

Larvae of the emerald ash borer (EAB; *Agrilus planipennis*) feed on phloem of ash (*Fraxinus* spp.) trees. A common garden study conducted by Rebek et al. (2008) found that an Asian species, Manchurian ash (*F. mandshurica*), was resistant to EAB while North American white (*F. americana*), green (*F. pennsylvanica*), and a putative North American x Asian hybrid (*F. nigra* x *F. mandshurica*) were highly susceptible. A subsequent common garden study found that North American black ash (*F. nigra*) and Oregon ash (*F. latifolia*) were also highly susceptible to EAB, as were the European species *F. excelsior*, *F. ornus*, and *F. angustifolia*, which experienced high rates of EAB-induced mortality. However, North American blue ash (*F. quadrangulata*) was colonized at much lower rates comparable to Manchurian ash (Smitley and Herms, unpublished). Divergence in resistant and susceptible phenotypes between ash species may be due in part to differences in phytochemical profiles of their phloem, specifically phenolic chemistry. For example, Eyles et al. (2007) found significant qualitative differences in constitutive phloem phenolic chemistry between resistant Manchurian and susceptible green and white ash.

The objective of this study was to compare the constitutive phloem phenolic chemistry of North American (green, white, black, and blue), European (*F. excelsior*), and Manchurian ash using RP-HPLC-PDA and MSn. We found qualitative similarities in the constitutive phenolic chemistry of black, European, and Manchurian ash while individual compounds differed quantitatively. Green and white ash was similar in composition and quantity as reported previously by Eyles et al. (2007). The phenolic profile of blue ash comprised mainly hydroxycoumarins and showed no similarity to the other species included in this study. The similarities and differences in phenolic profiles among species corresponded to their phylogenetic relationships (Wallander 2008). An effective bioassay is now needed to investigate the effect of individual phenolic compounds on EAB larval performance.

The ability of methyl jasmonate (MeJA) (an endogenous plant stress hormone that regulates defense responses against herbivores) to induce ash phloem chemistry was also investigated by applying 1 M MeJA to the outer bark of Manchurian and white ash. MeJA significantly induced total phenolics in both ash species, suggesting that induced responses to EAB feeding, in addition to constitutive chemistry, may be important in this interaction. These studies lay the groundwork for investigating what role phenolics may play in ash resistance against EAB and whether phenolic chemistry may be

used to develop resistant ash through conventional breeding or as a means to identify resistance in native ash.

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GENOMICS OF EAB-ASH INTERACTIONS

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ABSTRACT

Emerald ash borer (EAB *Agrilus planipennis*), an invasive insect pest of ash trees (*Fraxinus* sp.) continues to have tremendous socio-economic and environmental impacts throughout North America (NA). Indeed it is thought that at the current rate of invasion, EAB has the potential to threaten the very existence of ash trees in NA. Despite this high impact status, to date no functional knowledge (ESTs, expressed sequence tags) exists for EAB and ash. Hence, there is an urgent need for learning the physiology-driven molecular strategies that enable EAB to thrive on its naïve host and to decipher the phloem transcriptome of ash species (susceptible NA and resistant Asian ash). We hypothesize that the successful establishment of EAB is primarily due to the transcriptomic adjustments in the digestive tract (midgut) of feeding larvae and that the phloem tissue produces resistant compounds in response to EAB attack. An initial sequencing pass using the newer-generation pyrosequencing 454 Titanium technology yielded ~35 Mb for the midgut of EAB and 65 Mb for phloem of ash. In total, 19,246 tentative unique genes (TUGs) were recovered from the EAB database, including consensus sequences from contigs and singletons. This dataset represents candidates involved in digestion, detoxification, antioxidant defense and structural components of the insect gut. On the other hand, 44,625 TUGs were recovered for ash, which revealed genes encoding for proteases (cysteine and aspartic), players of jasmonic acid pathway (e.g. lipoxygenases), pathogenesis related (PR) proteins and inhibitors, which are thought to play important roles in plant defense. These derived EST databases of EAB and ash represent a powerful tool for gene discovery and gene mining of candidate genes with putative function during the interactions.

Future studies are aimed at elucidating differential gene expression profiles (via microarrays or RNA-Seq): i) EAB infesting susceptible NA ash versus resistant Asian ash; ii) constitutive and induced responses in NA versus Asian ash; and iii) deciphering gene function via RNA interference (RNAi). The potential impacts of this project are – (i) thorough understanding of the molecular strategies deployed by EAB, (ii) unraveling the complement of transcripts expressed in ash phloem to aid in the identification of resistant factors; and (iii) provide fundamental knowledge about adaptations in invasive insect species. Such information will be of great importance in ash breeding programs and in studies aimed to better understand interactions between forest trees and their exotic and indigenous herbivores.

PROTEOMIC DIFFERENCES BETWEEN ASH SPECIES RESISTANT AND SUSCEPTIBLE TO THE EMERALD ASH BORER

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ABSTRACT

Emerald ash borer (EAB; *Agrilus planipennis*) larvae feed on phloem of ash (*Fraxinus* spp.) trees. All North American ash species that EAB has encountered are susceptible, while an Asian species, Manchurian ash (*F. mandshurica*) has been shown to be resistant. Recent phylogenetic analyses based on ITS and chloroplast gene sequences of all 43 *Fraxinus* species place North American black ash (*F. nigra*) and Manchurian ash in the same clade and section (*Fraxinus* section *Fraxinus*), yet black ash is highly susceptible to EAB. This stark contrast between phylogenetic similarity and phenotypic divergence provides an opportunity to investigate differences between the two species that may have a role in defense/resistance.

In order to identify proteins that may be responsible for resistance of Manchurian ash to EAB, we used Differential in Gel Electrophoresis (DIGE) to compare its constitutive phloem proteome with that of susceptible black ash. Differentially expressed proteins were sequenced using nano-LC-MS/MS and putative functions were assigned. Hierarchical clustering of 355 proteins with concentrations differing significantly (> 2.0-fold, $P < 0.05$) between phloem of black and Manchurian ash revealed the two species had almost opposite protein expression patterns, despite their very close phylogenetic relationship. Proteins in Manchurian ash that may be associated with resistance include enzymes involved in the synthesis of isoflavones and coumarins, oxidative enzymes, a PR-10 protein, and an aspartic protease. Future functional studies are needed to identify what roles these proteins may play, if any, in resistance of Manchurian ash to EAB. Discovery of resistance-related proteins could accelerate selection of resistant trees or aid in marker-based hybridization programs ultimately aimed at restoration of ash to natural and urban forests in North America.

GENETIC TRANSFORMATION OF GREEN ASH AND PLANT REGENERATION FOR DEVELOPING ASH RESISTANT TO THE EMERALD ASH BORER

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ABSTRACT

An adventitious shoot regeneration and rooting protocol was developed for green ash, *Fraxinus pennsylvanica*. The best regeneration medium for freshly isolated hypocotyls was Murashige and Skoog (MS) supplemented with 13.3 μM 6-benzylaminopurine (BA) plus 4.5 μM thidiazuron (TDZ). Seventy-six percent of hypocotyl segments produced adventitious shoots, with a mean number of adventitious shoots per explant of 2.7 + 0.5. The effect of in vitro-germinated seedling age on adventitious shoot regeneration from hypocotyl explants was also studied. Results showed that hypocotyl explants from freshly isolated embryos exhibited a higher organogenesis potential than 4–15-day-old explants. Adventitious shoots from hypocotyls were established as proliferating shoot cultures following transfer to MS basal medium with Gamborg B5 vitamins supplemented with 10 μM BA plus 10 μM TDZ. A high rooting percentage (73–90%) was achieved when in vitro shoots were rooted on woody plant medium (WPM) containing 4.9 μM indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) at 0, 2.9, 5.7, or 8.6 μM) with a combination of 10-day dark culture period followed by a 16-h photoperiod. The highest rooting (90%) of adventitious shoots or the number of roots per shoot (3.0 + 1.0) was obtained on WPM with 4.9 μM IBA plus 5.7 μM IAA. Rooted plants were successfully acclimatized to the greenhouse, and 100% survived after overwintering in cold storage. This regeneration system provided a foundation for genetic modification of green ash.

A genetic transformation protocol for green ash hypocotyl explants was developed. Green ash hypocotyls were transformed using *Agrobacterium tumefaciens* strain EHA105 harboring binary vector pq35GR containing the neomycin phosphotransferase (nptII) and β-glucuronidase (GUS) fusion gene. Pre-cultured hypocotyl explants were transformed in the presence of 100 μM acetosyringone using 90 s sonication plus 10 min vacuum-infiltration. Kanamycin at 20 mg l-1 was used for selecting transformed cells. Adventitious shoots regenerated on MS medium supplemented with 13.3 μM BA, 4.5 μM TDZ, 50 mg l-1 adenine sulfate, and 10% coconut water. GUS- and polymerase chain reaction (PCR)-positive shoots from the cut ends of hypocotyls were produced via an intermediate callus stage.

Presence of the GUS and nptII genes in GUS-positive shoots were confirmed by PCR and copy number of the nptII gene in PCR-positive shoots was determined by Southern blotting. Three transgenic plantlets were acclimatized to the greenhouse. Studies are underway using a construct containing the Cry8Da protein of *Bacillus thuringiensis* SDS-502 for genetic transformation of green ash for resistance to the emerald ash borer, *Agrilus planipennis*.

MAPPING ASH TREES ACROSS A BROAD SPATIAL EXTENT WITH MODERATE RESOLUTION TO SUPPORT EMERALD ASH BORER MANAGEMENT

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ABSTRACT

Rapid ecosystem invasion by the emerald ash borer (*Agrilus planipennis* Fairmaire) is forcing resource managers to make decisions regarding how best to manage the pest, but a detailed map of abundance of the host, ash trees of the genus *Fraxinus*, does not exist, frustrating fully informed management decisions. We have developed methods to map ash tree abundance across a broad spatial extent in Wisconsin using their unique phenology (late leaf-out, early leaf-fall) and the rich dataset of Landsat satellite imagery that can be used to characterize ash senescence with respect to other deciduous species. However, across environmental gradients, senescence can vary by days or even weeks such that ash brown-down occurs at different times even within the same (185x185 km) Landsat footprint. To address this issue, we used additional Moderate Resolution Imaging Spectroradiometer (MODIS) satellite-based products from NASA's North American Carbon Program (NACP) coupled with vegetation indices derived from the Landsat imagery to determine the phenological position of each Landsat pixel within a single idealized growing season. Pixels within Landsat images collected in different years were re-arranged in a phenologically-informed time series that described autumn senescence for all locations. This characterization of leaf-drop was then related to a field-collected dataset of forest composition, producing a spatially-generalizable model of moderate resolution (30m pixels) capable of predicting ash abundance across the state. Empirical models predicting ash abundance within two different Landsat footprints in Wisconsin indicate model fits for ash abundance of R^2=0.65 (north central WI) and 0.78 (eastern WI). Future work will focus on extension of the model to areas outside of the originally sampled properties, as well as a validation of the model results across landscapes in Wisconsin.

BALANCING MANAGEMENT COSTS AND SIZE OF THE URBAN FOREST WITH A WEB-BASED COST CALCULATOR

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ABSTRACT

Since its arrival in 2002, the development of management tools for the emerald ash borer has been an active area of research. Recent research on the effectiveness of a number of insecticide therapies has produced viable tools for protecting trees with a DBH of up to 25 inches. Despite the availability of these tools, city foresters are often faced with dwindling budgets and need to develop economically viable action plan. The web based emerald ash borer cost calculator was developed to meet this need. This calculator allows users to project the cost of EAB management programs that include any combination of treating, replacing or removing ash trees over a 25 year period. Arborists using this program are able to use local estimates of costs for replacing, removing or treating trees. Using a tree growth model developed for the city of Indianapolis, the calculator project the size of the forest over a 25 year period to give users a rough estimate of the benefits conveyed by each management strategy.

Using a street tree inventory from the city of Indianapolis to evaluate the default strategies presented in the calculator I determined that each strategy had distinct effect on forest size. Removing all ash trees without replacement was the least costly but left behind the smallest forest. Replacing ash trees was more costly and only temporarily depleted with forest size. After 25 years the replaced ash forest was over 90% of its original size. Treating all the ash trees had the lowest annual costs, highest 25 year costs, and saved the most trees. Strategies that mix ash tree replacement and treating ash trees produces forests that are intermediate in size between those produced by simply replacing or treating all ash trees. Protecting large trees (DBH > 12") saves more of the forest than protecting only the small trees. Over a 25 year period the cost of protecting large ash trees is substantially higher than the cost for protecting small trees.

There is an urgent need for developing new approaches to managing ash forests. Although insecticide use can buy time for the development of new options, the effectiveness of controls for larger trees (DBH>25") is largely unknown. Ash utilization options are needed to defray the cost of ash removal and replacement and to support the economic viability of tree replacement as a management option.

MODELING THE RISK OF SPREAD OF EMERALD ASH BORER AND COMPUTING ASH TREE HEALTH INDEX

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ABSTRACT

We describe a spatially explicit cell-based model used to calculate risk of spread in Ohio and Michigan, by combining the insect's flight and short-range dispersal (insect flight model) with human-facilitated, long-range dispersal (insect ride model). This hybrid model requires estimates of EAB abundance, ash abundance, major roads and traffic density, campground size and usage, distance from the core infested zone, wood products industry size and type of wood usage, and human population density. With the insect flight model, probability of movement is dependent on EAB abundance in the source cells, the quantity of ash in the target cells, and the distances between them. With the insect-ride model, we modify the value related to ash abundance based on human assisted factors like road networks, campgrounds (through a gravity model that takes traffic volumes and routes between EAB source areas and distances to campgrounds), wood products industry and population density. We attempt to show the advantage of our model compared to statistical approaches and to justify its practical value to field managers working with imperfect knowledge. Our model emphasizes the importance of road networks in distributing insects to new geographically dispersed sites. In Ohio, 52% of insect outbreaks fell within 1 km, 64% fell within 2 km and 81% fell within 4 km of a major highway.

We also describe an index of ash tree health based on canopy light transmission, epicormic shoots and D-shaped exit holes. Based on these measures, the quantitative index gives a classification of tree health (good, intermediate and poor) that can be used by home owners and property managers to more effectively manage their ash resources.

Chemical and Microbial Control

EVALUATION OF MULTI-YEAR APPLICATION OF NEONICOTINOID INSECTICIDES FOR EAB CONTROL

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ABSTRACT

The use of non-invasive, neonicotinoid trunk sprays for the control of emerald ash borer is appealing because they are fast, efficient, do not wound the tree or contaminate soil and require no special equipment for application. However, there are a number of questions surrounding the use of these products for emerald ash borer (EAB) control. How effective are such products at controlling both adult and larval life stages? How persistent are these products and can control be improved with multi-year applications? Do EAB experience an intoxication effect associated with imidacloprid? We designed a long-term study to address these questions.

In 2006, eight blocks, each consisting of four small, white ash trees (3-5" DBH) were selected at Seven Lakes State Park in Holly, MI (32 trees total). Trees were randomly assigned to one of four treatments: (1) trunk-injected imidacloprid [Imicide (10%) in Mauget capsules applied at a rate of 0.15 g a.i./ inch DBH], (2) trunk-sprayed imidacloprid plus Pentra-Bark [Macho 2F (21.4%) plus 3 oz Pentra-Bark per gal. spray applied at a rate of 1.7 g a.i./inch DBH], (3) trunk-sprayed dinotefuran [Safari 20 WP (20%) plus 3 oz Pentra-Bark per gal. spray applied at the 1.7 g a.i./inch DBH rate], and (4) untreated control. The Pentra-Bark (PB) is an agricultural surfactant that may improve the movement of the insecticide through the bark and into the xylem. Trees were treated in May of 2006, 2007, 2008, and 2009.

A composite sample of foliage was collected from four aspects of each tree and analyzed using ELISA to determine foliar residues in mid-June, mid-July and August. Adult leaf-feeding bioassays were also conducted in June and July, concurrently with foliage collections for residue sampling. Three 4-5 day old adult EAB were placed in a Petri dish with a single leaf sample collected from opposite aspects of each tree (2 leaves per tree) and allowed to feed for 4 days. Beetle mortality and intoxication were recorded at the end of 4 days. Canopy condition of each tree was assessed in late summer in all four years. In the fall of each year, cumulative EAB larval density was determined by surveying all stem sections greater than 5 cm in diameter for each of the smaller trees. On the largest trees, nearly half of the bark surface area was surveyed. The number of new EAB exit holes and woodpecker attacks

on larvae were counted then marked with a staple, enabling us to repeat sampling annually. Cumulative density of EAB emerging was determined each year.

When all four years of adult bioassay data were combined, results showed that all three insecticide treatments resulted in significantly higher adult EAB mortality than the control trees by Day 4. Dinotefuran+PB trees had significantly higher mortality (near 100%) than trees treated with imidacloprid applied as a trunk spray or via trunk injection, which averaged approx. 60% EAB mortality. Approximately 25% of beetles feeding on foliage from Imicide-Mauget treated trees appeared intoxicated after 4 days, significantly greater than in dinotefuran+PB or control trees. Imidacloprid+PB trees were intermediate (approx. 20% intoxicated). No trees exhibited any canopy decline or dieback in 2006, but dieback progressed thereafter in all treatments. By August 2009, each insecticide treatment had one dead tree, while three control trees were dead. Cumulative EAB larval density was highly variable within treatments, but density increased steadily over time in all treatments. All insecticide treatments reduced the density of EAB developing on the trees compared to controls. Larval density was 2 to 4 times greater in control trees than treated trees, but there was little difference among trees treated with different products. There was a positive linear relationship between larval density and canopy dieback (R^2=0.76).

As of fall 2009, most ash trees in the vicinity of our study area had been killed by EAB or are heavily infested. We plan to continue this study to determine if annual application of these neonicotinoid products can effectively protect trees until surrounding trees succumb and EAB populations collapse.

EVALUATION OF EMAMECTIN BENZOATE AND NEONICOTINOID INSECTICIDES: TWO-YEAR CONTROL OF EAB?

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ABSTRACT

We are continuing to evaluate the effectiveness and persistence of systemic insecticides for emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) control in landscape ash trees. Highly effective insecticides may also have a role in efforts to slow EAB population growth in localized outlier sites.

Our previous studies had shown that two neonicotinoid insecticides, imidacloprid and dinotefuran, could be applied as non-invasive trunk sprays. Projects were conducted in 2006-2007 with trunk sprays of Macho 2F (imidacloprid) or Safari 20% (dinotefuran) mixed with Pentra-Bark (a non-toxic, agricultural surfactant; Agrichem, Medina, OH). Results showed that both insecticides moved through the bark, into xylem and were subsequently translocated into foliage. The non-invasive trunk spray application method is efficient, requires no specialized equipment, does not introduce insecticide into the soil and does not require wounding the tree. It was not clear, however, whether the Pentra-Bark product was necessary, particularly for dinotefuran, which is much more soluble than imidacloprid. We also wanted to determine whether consecutive years of treatment with neonicotinoid insecticides would improve the efficacy of these products.

In addition, we were interested in evaluating the EAB control provided by emamectin benzoate, a compound used for a variety of pests in fish farming, veterinary medicine and agriculture. A formulation of emamectin benzoate, sold as TREE-äge, has recently received a special registration in several states for EAB control.

We initiated a two-year study beginning in May 2007 to compare the effectiveness of imidacloprid, dinotefuran and emamectin benzoate products for EAB control. We established a total of 25 blocks at three different sites spread across four counties in Michigan. Each block consisted of seven trees with average DBH per site ranging from approximately 7 to 14 inches.

The seven treatments represented in each block included (1) untreated control; (2) trunk injection with imidacloprid (Imicide; 10%, 3 ml Mauget capsules); (3) trunk injection with emamectin benzoate (4% solution applied with an ArborJet micro-injector); (4) a non-invasive trunk spray of

imidacloprid (Macho 2F + Pentra-Bark; (5) a non-invasive spray of Macho 2F without Pentra-Bark; (6) a non-invasive trunk spray of dinotefuron (Safari 20%) + Pentra-Bark; and (7) a non-invasive trunk spray of Safari without Pentra-Bark. Seven blocks of trees were felled and debarked in the 2007-2008 winter to quantify density of EAB larvae on each tree.

In May 2008, half of the remaining 18 blocks of trees were re-treated; the other nine blocks were left untreated. In both years, trunk sprays of imidacloprid were applied in early May, roughly 2 weeks before trunk injections of imidacloprid and emamectin benzoate were applied. Trunk sprays of dinotefuran were applied in late May.

We quantified insecticide residues in composite foliage samples collected from 8 locations in each tree in mid June, July and August 2008 using ELISA. For the trees treated in both 2007 and 2008, mean emamectin benzoate residues ranged from 5 to 8 ppm and dinotefuran residues ranged from roughly 2 to 3 ppm. Imidacloprid residues in trees treated with trunk sprays or with the Mauget capsules generally increased over the summer and were highly variable. Average imidacloprid residues in all months were < 2.5 ppm.

For the trees treated only in 2007, emamectin benzoate residues were detectable but averaged less than 1 ppm throughout the summer in 2008. Dinotefuran residues were highly variable but averaged approximately 2 ppm in June and July before sharply declining in August. Imidacloprid residues were detectable but generally averaged less than 1 ppm. Residues were consistently slightly higher on the trees treated with the 2007 trunk spray if the Pentra-Bark surfactant was added, but preliminary analyses indicated differences were not statistically significant.

Bioassays were conducted in mid June in 2008 to assess survival of EAB adults caged with leaves from each study tree. Two leaves were collected from opposite sides of each tree and three beetles were placed on each leaf for four days.

For trees treated in both 2007 and 2008, mortality of beetles caged with leaves from the emamectin benzoate trees was > 90% in all three bioassays For trees treated in both 2007 and 2008 with trunk sprays of dinotefuran or imidacloprid, or with the Mauget capsules, average beetle mortality ranged from 50-70%, while only 10-15% of the beetles on control trees died.

For trees treated only in 2007, on average at least 90% of the beetles that fed on leaves from the emamectin benzoate died. Beetle mortality on trees treated only in 2007 with dinotefuron or either of the imidacloprid products did not differ significantly from the control trees. The addition of the Pentra-Bark surfactant did not appear to influence EAB mortality on trees treated with trunk sprays in 2007 only.

Larval density was assessed by felling and debarking areas on the trunk and canopy of trees during the 2008-2009 winter. Number and instar of larvae were recorded and standardized by m^2 of phloem exposed on each tree.

For trees treated in both 2007 and 2008, larval density on untreated control trees averaged approximately 90 larvae per m^2. Trees treated with emamectin benzoate in both 2007 and 2008 had less than 1 larva per m^2, equivalent to >99% control when compared to larval density on control trees. Larval densities on trees treated with trunk sprays of dinotefuran+ Pentra-Bark or imidacloprid+ Pentra-Bark in both 2007 and 2008 were 65-70% lower than on control trees, a difference which was significant. Larval density on trees treated with the Mauget capsules or trunk sprays without Pentra-Bark were intermediate.

For trees treated only in 2007, larval density on untreated control trees averaged approximately 58 larvae per m^2. The emamectin benzoate trees had less than 1 larva per m^2 or > 99% control. Larval density on trees treated only in 2007 with any of the neonicotinoid products, however, did not differ from untreated controls.

The nearly complete EAB control provided by the emamectin benzoate, even two years post-treatment, was striking. These results show that trees could be treated at two or perhaps even at three year intervals, which would dramatically reduce costs and logistical problems associated with annual treatments. The neonicotinoid insecticides, particularly the trunk sprays of dinotefuran+ Pentra-Bark and imidacloprid+ Pentra-Bark did reduce EAB larval density. The level of control was not nearly as high as that provided by the emamectin benzoate, however, and these products provided virtually no reduction in EAB larval density if they were not re-applied the second year.

MULTIYEAR EVALUATIONS OF SYSTEMIC INSECTICIDES FOR CONTROL OF EMERALD ASH BORER

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ABSTRACT

Research and experience has shown that insecticides provide a viable option for protecting high value ash trees (Herms et al. 2009). However, questions remain regarding their optimal use. In 2006, we initiated three studies in Toledo, Ohio to evaluate the effectiveness of systemic insecticides for protecting large caliper ash street trees from emerald ash borer in the face of extremely high pest pressure. Specific objectives were to: (1) evaluate efficacy of different rates of imidacloprid (Merit and Xytect) soil drenches made annually in spring or fall, (2) determine the number of years of control provided by different rates of a single emamectin benzoate (Tree-äge) trunk-injection, and (3) compare multi-year efficacy of emamectin benzoate (Tree-äge) and imidacloprid (Ima-jet) trunk injections applied once, consecutively, or in alternate years.

Objective 1: Efficacy of imidacloprid soil drenches applied in spring or fall

The following imidacloprid soil drench treatments were applied to a green ash (*Fraxinus pennsylvanica*) street tree planting ranging in size from 11-17 in DBH (mean = 14 in):

(1) untreated control (n=5)

(2) Merit 2F, 1.42 g ai/inch DBH applied spring 2006, 2007, 2008, and 2009 (n = 5)

(3) Merit 2F, 1.42 g ai/inch DBH, applied fall 2006, 2007, and 2008 (n = 5)

(4) Xytect WSP, 1.42 g ai/inch DBH, applied fall 2006, 2007, and 2008 (n = 4)

(5) Xytect WSP, 2.84 g ai/inch DBH, applied fall 2006, 2007, and 2008 (n = 4)

(6) Xytect WSP, 2.84 g ai/inch DBH, applied spring 2007, 2008, and 2009 (n = 3)

The experiment was designed as a randomized complete block with treatments applied as a basal soil drench with one gallon water. Dates of spring treatment dates were 18 May 2006, 13 June 2007, 23 May 2008, 10 May 2009; fall treatments were applied 4 Nov. 2006, 5 Nov. 2007, 21 Oct. 2008, and 19 Oct. 2009. Treatments were evaluated on 15 Aug. 2007, 26 Aug. 2008, and 11 June 2009 by visual estimation of percent thinning of the canopy following the 0-100% photographic scale of Smitley et al. 2008.

No trees showed visible symptoms of canopy decline in 2006 when treatments began. In 2007, one tree in each treatment showed some signs of canopy decline (Table 1). However, canopy decline in 2007 is probably not reflective of efficacy of the fall 2006 Merit or spring 2007 Xytect treatments, because canopy decline in 2007 reflects feeding injury by larvae that colonized trees in summer 2006

prior to initiation of these treatments. Decline of the untreated trees intensified substantially in 2008, and all untreated trees were dead or nearly so in June 2009, indicating severe pest pressure. All imidacloprid treatments significantly suppressed decline relative to the untreated control in 2008 and 2009. Trees treated with Xytect applied at the highest labeled rate (2.84 g ai/inch DBH) had very little canopy thinning than the other treatments in 2008 and in 2009 under extreme pest pressure. This observation is consistent with the hypothesis that decreased efficacy at lower rates may be due to dilution of imidacloprid within larger trees.

Table 1. Effects of imidacloprid soil drenches on percent canopy decline of green ash street trees from 2006 through 2009. Means within a column with different letters are significantly different (LSD test following significant ANOVA, $p < 0.05$).

	Percent Canopy Decline			
Treatment	2006	2007	2008	2009
Untreated Control	0	2	42a	90a
Merit 2F, 1.42 g ai/in, Fall	0	10	26ab	42b
Merit 2F, 1.42 g ai/in, Spring	0	4	16ab	16bc
Xytect WSP, 1.42 g ai/in, Fall	0	7	17ab	33b
Xytect WSP, 2.84 g ai/in, Fall	0	8	0b	10c
Xytect WSP, 2.84 g ai/in, Fall	0	10	3b	10c

Objective 2: Duration of efficacy of emamectin benzoate (Tree-äge) trunk-injections

The following rates of emamectin benzoate (Tree-äge) were applied as trunk injections using the Arborjet Viper Tree IV on 14 June 2006 to a green ash street tree planting ranging in size from 13-24 in DBH (mean = 21 in): 0.1, 0.2, 0.4, and 0.8 g ai/in DBH. The experiment was designed as a randomized complete block with five replicates per treatment. Treatments were evaluated on 15 August 2007, 26 August 2008, and 27 August 2009 by visual estimation of percent dieback of the canopy following the photographic scale of Smitley et al. (2008). Successful adult emergence from randomly sampled branches was also quantified as an index of treatment efficacy.

Initial signs of canopy dieback were evident on only two trees in 2007, the year after treatment (Table 2). In August 2008, two years after treatment, canopy decline was much more evident at the study site, becoming moderate to severe in the untreated control.

Table 2. Effects of emamectin benozoate trunk injections in 2006 on percent canopy decline of ash trees from 2007 - 2009. Means within a column with different letters are significantly different (LSD test following significant ANOVA, $p < 0.05$).

	Percent Canopy Decline			
Treatment	2006	2007	2008	2009
Untreated Control	0	8	53a	96a
Emamectin benzoate, 0.1 g ai/in	0	0	3b	25bc
Emamectin benzoate, 0.2 g ai/in	0	0	3b	55b
Emamectin benzoate, 0.4 g ai/in	0	0	10b	60b

All rates of emamectin benzoate continued to provide good protection in August 2008, as evidenced by excellent canopy condition, and greatly decreased exit hole density relative to the untreated control (data not shown), indicating two years of control from the single treatment.

The lower rates of emamectin benzoate began to show signs of decreased efficacy in the third year as evidenced by increased canopy decline in June 2009, which continued to increase through the growing season. However, even under extremely high pest pressure, the highest rate (0.8 g ai / inch DBH) provided three years of very effective control, as evidenced by extremely low canopy decline in June 2009 (which would reflect efficacy during 2006, 2007, and 2008). These results were corroborated by extremely low adult emergence in 2009 from the 2008 larval cohort. There were no signs of phytotoxicity in any of the treatments.

Objective 3: Multi-year efficacy of emamectin benzoate (Tree-äge) and imidacloprid (Ima-jet) trunk injections applied in a single, consecutive, or alternate years

The following emamectin benzoate (Tree-age) and imidacloprid (Ima-jet) trunk injection rates and treatment schedules were applied to a green ash street tree planting ranging in size from 13-25 in DBH (mean = 19 in):

(1) Untreated control (n=16)
(2) Tree-äge, 0.4 g a.i./inch DBH, applied in 2006 (n=8)
(3) Tree-äge, 0.4 g a.i./inch DBH, applied 2006 and 2008 (n=8)
(3) Ima-jet, 0.4 g a.i./inch DBH, applied in 2006 and 2007 (n=8)
(4) Ima-jet, 0.4 g a.i./inch DBH, applied in 2006 and 2008 (n=9)

Trunk injections were made using the Arborjet Viper Tree IV on 14 June 2006, 13 June 2007, and / or 24 June 2008, depending on treatment schedule. The experiment was designed as a randomized complete block. Treatments were evaluated on 15 August 2007, 26 August 2008, and 27 August 2009 by visual estimation of percent canopy decline following the photographic scale of Smitley et al. (2008). Successful adult emergence from randomly sampled branches was also quantified as an index of treatment efficacy.

No trees showed visual symptoms of decline in 2006. However, decline of untreated trees intensified rapidly from 2007 to 2009, by which time almost every untreated tree was dead or nearly so (Table 3). All treatments provided excellent protection for three years, through 2008. Only one of 16

trees treated with Tree-äge showed any sign of decline through 2008, and density of adult emergence holes was extremely low relative to untreated control trees (data not shown).

Table 3. Effects of emamectin benzoate (Tree-äge) and imidacloprid (Ima-jet) trunk injections on ash decline. Means followed by different letters are significantly different (LSD test following significant ANOVA, $p < 0.05$).

	Mean percent canopy decline			
Treatment	2006	2007	2008	2009
Untreated Control	0	6a	51a	90a
Tree-äge, 2oo6	0	0a	0b	13c
Tree-äge, 2006 & 2008	0	0a	3b	6c
Ima-Jet, 2006 & 2007	0	0a	0b	33b
Ima-Jet, 2006 & 2008	0	0a	9b	20bc

Emamectin benzoate continued to provide substantial protection in 2009, providing further evidence that a single emamectin benzoate injection can provide three full years of protection.

Trees treated with emamectin benzoate in 2006 and 2008 had minimal dieback in 2009, with only 1 of 8 trees showing light decline in 2008 and 3 of 8 trees showing light decline in 2009 (Table 3). Exit hole density was also extremely low in this treatment. These results confirm that emamectin benzoate can provide excellent protection of larger trees when applied every two years, even under high pest pressure, which is to be expected based on the results described above showing that a single application provided three years of control.

None of the trees treated with Ima-jet in 2006 and 2007 showed any signs of decline in 2008 (Table 3). However, trees in this treatment averaged 33% canopy decline in 2009, with 7 of 8 trees in this treatment showing some degree of decline, indicating that protection became compromised when treatment was skipped in 2008. Four of nine trees treated with Ima-jet in 2006 and 2008 exhibited noticeable decline in 2008 that intensified in 2009, when two additional trees also showed evidence of decline. This provides further evidence that skipping a year of treatment with Ima-Jet compromises control to some degree.

SUMMARY

Imidacloprid soil drenches applied in spring of fall provided excellent protection of large caliper ash trees when applied annually at the highest rate (2.8 g ai/in DBH). In the case of Xytect, this high rate has been registered for control of EAB. A single trunk injection of emamectin benzoate (Tree-äge) provided up to three years of control; however, control with imidacloprid (Ima-jet) injections became less consistent if a year was skipped between treatments. These soil and trunk applied systemic insecticides provide viable options for protecting large-caliper ash trees even under the most intense pest pressure.

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EVALUATION OF PREVENTATIVE NEONICOTINOID SOIL INJECTIONS FOR MANAGEMENT OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS* FAIRMAIRE)

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ABSTRACT

Practitioners require insecticide management options that are not only effective against emerald ash borer (EAB), but that are also cost effective, easy to apply and safer for humans and the environment. Systemic neonicotinoid insecticides are commonly applied by landscapers and tree care professionals to manage numerous insect pests on woody ornamentals. Insecticides containing the neonicotinoid active ingredients imidacloprid and diontefuran have been tested and are labeled for use on EAB. Imidacloprid and diontefuran can be applied as soil treatments using either basal soil drench or soil injection. In addition numerous imidacloprid products can be trunk injected and dinotefuran can be applied as a systemic bark spray for EAB. Soil injection application offers benefits over soil drench in that the insecticide is applied beneath mulch and turf directly in the root zone of the tree to maximize root absorption, minimize absorption by the mulch and turf and reduce the risk of run-off. When compared with invasive trunk injection procedures, soil injection treatments provide applicators with a method to consistently treat trees in a quick and predictable manner and can be done annually without wounding or injuring the tree.

To date, research trials using soil applied neonicotinoids have produced inconsistent efficacy data on EAB. Reasons for inconsistencies in results between trials may be attributed to the varied experimental designs used in each trial. Specifically, application protocol used, tree size, baseline EAB severity, and trial site characteristics may account for variability. Much of the EAB insecticide research produced to date has been on trees that were infested at the time of the initial application. Limited research exists comparing efficacy of preventive soil injection treatments on different diameter classes of ash. Recent research suggests that higher dosage rates of soil applied imidacloprid are required for ash trees 15 inches or greater. Little is known about the efficacy of the higher rate treatments of imidacloprid relative to other products labeled for EAB. Furthermore, little is known regarding combination treatments of soil applied dinotefuran and imidacloprid and their efficacy against EAB.

The objectives of this trial were as follows:

- Conduct multi-year field efficacy research in Illinois to determine if preventative soil injection applications of neonicotinoid-class insecticides can serve as suitable treatments prior to an initial infestation by EAB
- Determine if these treatments are effective in preventing the establishment of EAB larvae and adult feeding in medium (10-14 inch DBH) and large (≥ 15 inch DBH) ash trees
- Determine the efficacy of combination soil injection treatments of neonicotinoid insecticides
- Determine the efficacy of fall vs. spring soil injection treatments
- Evaluate the performance of annual soil injection neonicotinoid treatments with a single one time treatment of TREE-age over the duration of the trial.
- Evaluate the performance of soil injection treatments of imidacloprid when applied at the new maximum labeled dosage rate, which was approved by the EPA in March 2009.

To date, 2008 and 2009 insecticide applications have been applied and treatments have been annually assessed for canopy dieback and larval activity. Low levels of larval activity were identified in a small sub-set of trees within the plot confirming that EAB is present within our study location. Despite the presence of EAB larvae in some trees, it is still too early to draw conclusions on treatment efficacy for trees in both size classes as there were no significant differences in the change from 2008 to 2009 in visual dieback ratings for both size classes. As EAB pressure increases throughout the plot area, the possibility of treatment effects may become more apparent.

SECTORED FLOW AND RESERVOIRS: A SYNTHESIS OF 14C-IMIDACLOPRID TRUNK-INJECTION RESEARCH

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ABSTRACT

Imidacloprid applied as a trunk injection is often used as a means of control of emerald ash borer (*Agrilus planipennis*), but effectiveness can be variable. The objectives of this study were to determine the extent to which movement of imidacloprid is sectored within ash trees, concentration of imidacloprid in trees after over wintering, and how imidacloprid is distributed to leaves the year after injection. Highly sectored flow could result in uneven distribution of compound within trees and result in variable efficacy of insecticide.

Twenty-four trees (16 *Fraxinus americana*, 16 *F. pennsylvanica*) approximately 4.5 cm diameter were injected on June 28, 2006 with ^{14}C labeled imidacloprid (25 mCi ^{14}C-imidacloprid in 3.1 ml Imicide® (10% a.i) directly below a branch in the first whorl. Each branch of the first three whorls was labeled as 0º /180º or L90º / R90º depending on the location of the branch in relation to the injection point. Leaves from each of the branches in the first three whorls plus terminal leaders and fine roots were sampled through two growing seasons. Trunk cores were sampled during 2006. Tree stems were sampled 60 days after treatment (DAT) and during final destructive harvest. Litterfall was collected at the end of both growing seasons. At the conclusion of the experiment, ten trees were destructively harvested. Boles of trees were cut into sections at 10.0 cm, 1.0 m and 2.0 m above the center of the injection scar, and a 2.0 cm thick cross-section was removed. Cross sections were split into quarters using a hammer and chisel.

A 150.0 – 180.0 mg sub-sample of each individual tissue sample was processed in a biological tissue oxidizer (OX300 R. J. Harvey Instrument Corporation, Tappan, NY, USA). Stem samples were burned on a four minute cycle, leaf samples on a three minute cycle, and root and core samples on a two minute cycle. Resulting $^{14}CO2$ was trapped in scintillation cocktail and processed through a Packard Tri Carb liquid scintillation counter (LSC, Packard Bioscience, Currently PerkinElmer Life and Analytical Science, Waltham, Massachusetts, USA) on a one and one half minute cycle. Resulting counts per minute (CPM) were recorded for each sample and converted to imidacloprid equivalents after accounting for background activity and oxidizer and LSC efficiencies.

Imidacloprid equivalent concentration (IEC) in leaves varied with time, and orientation to the injection point. Results are consistent with the sectored flow hypothesis. Imidacloprid equivalent concentrations were higher in leaves of branches in the plane of the injection point (0º) as opposed to leaves of branches on the opposite side of the injection point (180º). Leaves from R90º branches had higher IEC than leaves from L90º branches. We conclude that ash trees have a zigzag xylem architecture pattern which could lead to variable IEC in the tree crown. During 2007, the year after injection, patterns of IEC in leaves were similar to the previous year; some evidence sectoriality was still present, and IEC in leaves from spring-injected trees was greatly reduced. Imidacloprid equivalent concentrations in leaves, stems, and litterfall were higher than IEC in roots, trunk cores, and stems, indicating that imidacloprid moves primarily through the xylem. Wood disks of the stem cross sections collected at 10 cm above the injection points had visible stains from the injected material. Samples taken from stained areas had IEC approximately 75 times higher than all other wood samples taken from the tree. Imidacloprid equivalent concentrations were the same for wood cross sections taken from 10.0 cm (no stain) and 1.0 m and samples taken from 1.0 m and 2.0 m for 0 injected trees.

These results support the sectored flow hypothesis which implies that variability of trunk-injected imidacloprid could be a result of xylem anatomy, timing of injection, and time after treatment. Moreover, results from sampling the stained wood tissue and IEC concentrations the year after injection suggest that imidacloprid pools may remain in the main truck and insecticide may continue to diffuse from this reservoir for several months after injection.

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

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ABSTRACT

We have tested *Beauveria bassiana* (commercial strain GHA in Laverlam's Botanigard®), alone or in combination with imidacloprid (in Bayer Advanced Tree and Shrub® formulation for homeowner use), against emerald ash borer (EAB), *Agrilus planipennis*, on green and white ash in a commercial tree nursery. During the course of this 4-year study, the EAB infestation increased rapidly. Overall, the prevalence of EAB-infested trees, based on presence of exit holes on the lower 2 m of each tree, rose from 1% of 360 trees sampled in 2006 to 73% of 207 trees sampled in 2009. In laboratory bioassays, adult beetles showed nearly complete mortality when exposed for 1 or 24 h to fungus-treated foliage collected 1 h after spray. Mycosis was significantly reduced when beetles were exposed to foliage collected 1 or 2 weeks after spray. Beetles exposed 24 h to imidacloprid-treated foliage had shorter life spans than controls and showed signs of intoxication. Beetles confined 4 h on fungus-treated bark up to 2 weeks after spray had significantly higher mortality than controls. Beetles collected on baited purple Coroplast traps showed signs of fungal infection indicating possible dispersal of the pathogen within beetle populations. Higher fungal persistence on treated bark suggests that such treatments may be useful for targeting adult populations prior to emergence or during oviposition.

CONTROL ACTIVITIES AND LONG-TERM MONITORING FOR THE WEST VIRGINIA EAB INFESTATION

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ABSTRACT

Project Background. An emerald ash borer population was discovered in late 2007 at a recreational area near Oak Hill in Fayette Co., West Virginia. A survey conducted within a ½-mile radius of the original trap tree identified over 300 ash trees, the majority of which showed no signs of EAB infestation. A control and monitoring study was initiated in 2008 due to the following favorable factors: limited ash resource over a large area; isolated and relatively light EAB population; infested ash trees were within a confined area. Ash trees were either cut down and disposed of (149 trees, mostly 3" DBH or less) or treated once by trunk injection in 2008 with emamectin benzoate (Tree-äge, Arborjet, Inc.) at varying dosages depending on tree diameter.

Baseline monitoring. Foliar samples from a sampling of the treated trees were collected and analyzed for pesticide residue in 2008 and 2009. An ELISA analysis being used to determine emamectin content in leaf tissue was been confirmed as accurate as compared with the LC/MS analytical method used by the manufacturer (Syngenta). Emamectin residue from the 2008 samples was present in significant quantities in all foliage samples, increasing by dosage applied but not differing between sample periods. The average residue found in the foliage ranged between 5 and 8 ppm. Residue levels for the 2009 samples have yet to be generated.

Sample trees were also assessed for D-shaped exit holes, woodpecker feeding and canopy crown ratings twice during each of the growing seasons of 2008 and 2009. In 2008, about thirty percent of the trees showed evidence of canopy dieback from the August observations. There were very few woodpecker attacks on these trees, and only 6 of the 31 trees had 2-7 putative EAB exit holes, evidence of a light infestation. Additional tree observations during June and August of 2009 evidenced that the trees remain healthy with stable canopy ratings, a 33 to 55% decline in observed EAB exit holes and 90 to 100% decrease in the number of woodpecker attacks.

Trapping. A ½ kilometer trapping grid was set up to monitor the general EAB population in the area. A more intensive trapping grid was targeted for those areas around the original infestation as well as for areas surrounding EAB infested trees that were newly identified in the early spring of 2009 (red dots, Fig. 1). All traps were EAB program panel traps, green in color and baited with Phoebe oil. Ninety-four trapping sites were established in 2008 along with 111 additional sites in 2009. Traps were checked once in July and again in August before being taken down for the season. Only 2 adult beetles were captured on two of the traps placed around the original infestation site in 2008 (purple

triangles, Fig. 1). In 2009 a total of 95 insects were captured on 27 traps, half of which captured only one insect each (red triangles, Fig. 1).

It is of interest to note that no insects were captured in either 2008 or in 2009 within the original ½ mile radius management area around the Ace Adventure Campground (red circle, Fig. 1). Study trees will be felled and examined in the fall of 2010 for a more complete assessment of treatment impact and EAB infestation levels.

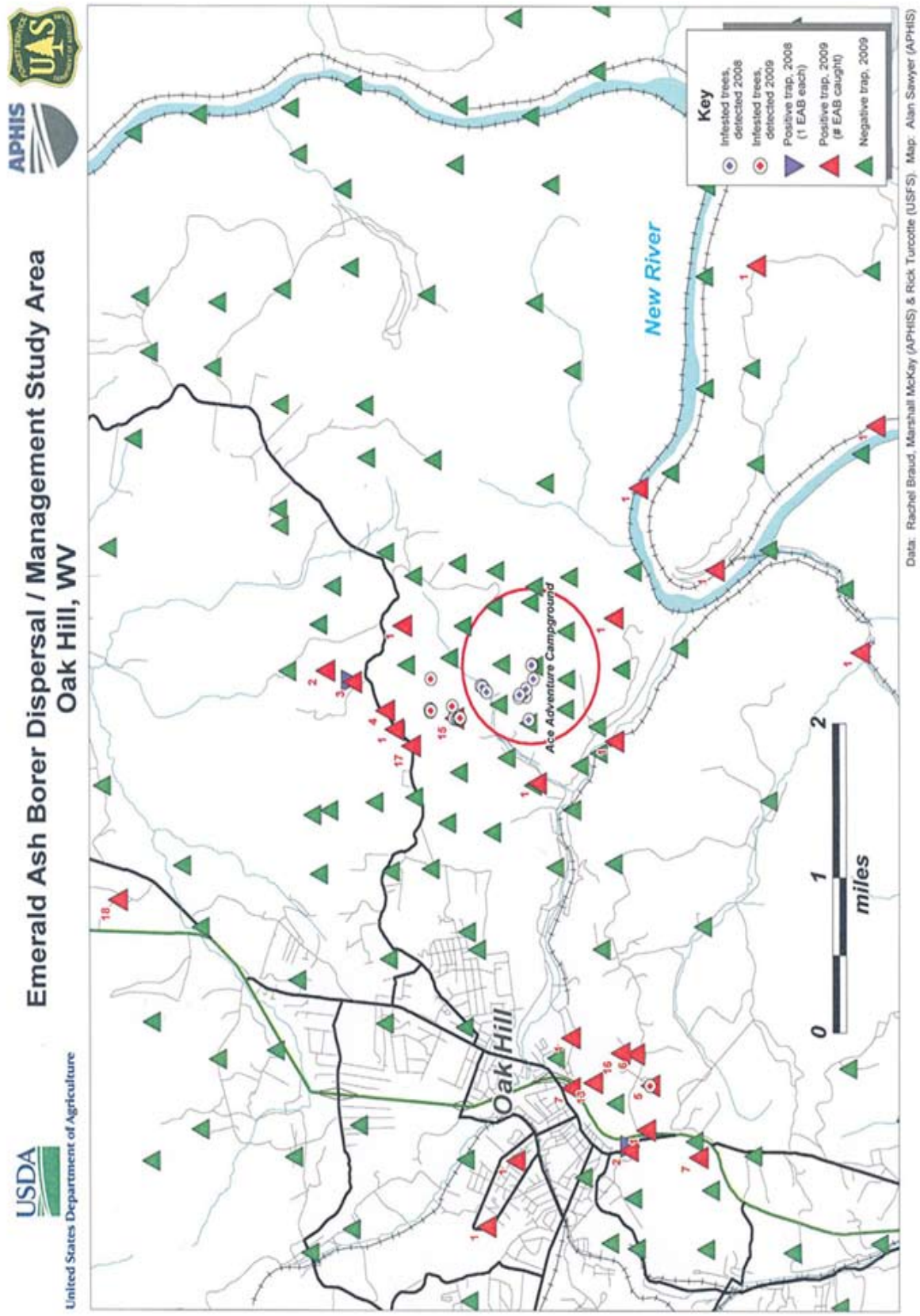

Figure 1. Trapping results and infested trees found near Ace Adventure Campground, 2009.

CONTROL OF EMERALD ASH BORER IN WOODLOTS WITH AERIAL APPLICATIONS OF SPINOSAD IN MICHIGAN, 2009-2010

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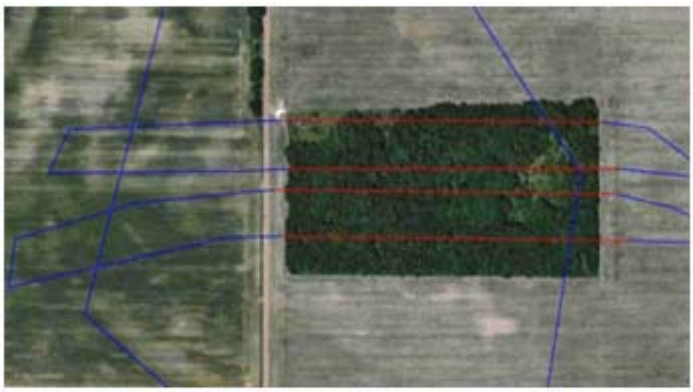
ABSTRACT

Suppression of emerald ash borer with aerial applications of spinosad, a biological insecticide, was tested using isolated woodlots in an intense farming area in Clinton County, Michigan. This area was recently infested with emerald ash borer, but woodpecker attacks and dieback were scarce in most of the test blocks. Isolated woodlots were selected for this study if they were surrounded by farm fields, contained enough ash trees to sample, and if some signs of EAB infestation were found. Ten suitable ash woodlots were chosen to be used as spray blocks and ten were picked to be unsprayed control plots.

Applications: Due to the cool spring and delayed emerald ash borer emergence, the applications were postponed 2 weeks. The first application was applied on 26 June between 5:50 and 7:10 am in excellent weather conditions. There was no breeze, the humidity was high and the skies were clear and sunny. The applications were made with Micronair AU5000 nozzles on an Air Tractor 502B set at 55 degree pitch. The aircraft was flown at 140 MPH and over the trees high enough to give a 125 foot swath. A second application was made on 2 July between 10 and 12 am. The weather conditions were overcast with high humidity until a slight clearing of the cloud cover about 9:30am. There was an occasional light drizzle during the application, but not enough to cause drip off the leaves.

Air Tractor 502B



Spray path over site # 3 (from PS)

Tarp Sampling: Two tarps were placed under 4 trees in each of six treated and six untreated woodlots between 18 June and 23 June. On 25 June, the tarps were swept to remove leaves and other debris. The contents of the tarps were emptied into plastic bags and taken back to the lab

to assay for emerald ash borer adults on 29 June. Tarp collections were again made between 6 and 7 July after which the tarps were removed from the woodlots. A total of 54 EAB adults (range 1 to 20) were collected from sprayed plots after the 1st application, but only 9 (range 0 to 4) were observed on tarps after the July application. Only two insects were observed on tarps placed within control plots for both applications.

Shotgun Sampling for Residue and Bioassay: Leaf samples from the upper canopy of 3 trees in each of the 6 sprayed and unsprayed sites listed in Table 1 were taken with a 12–gauge Remington 870 shotgun on 29 June (3 DAT) for residue analysis at the USDA/ APHIS facility at Otis. Three compound leaves from each sample were placed in a floral pick with water before they were fixed in an arena consisting of two quart sized food containers held together with a nylon stretch-band. Arenas were set-up on 30 June in a lab at Michigan State University. Approximately 8 adults were placed in each arena. EAB adult mortality was determined on 1 July (24 h exposure to leaf samples), 4 July (4 d exposure) and 6 July (6 d exposure).

Table 2. Foliage Bioassay. Data are mean percent EAB mortality for 24 adults placed on foliage samples collected from woodlots at 3 d after aerial application of spinosad

Site number	Treatment	n	Mortaity 24 h exposure (%)	1 d exposure (%)	4d exposure (%)	Mortality 24 h mean (%)	1 d mean (%)	4d mean (%)
1	Control	8	4.2	8.3	12.5	5.5	11.0	30.8
22	Control	8	4.0	12.0	60.0			
28	Control	8	0.0	0.0	25.0			
43	Control	8	16.7	16.7	33.3			
44	Control	8	0.0	0.0	25.0			
102	Control	8	8.3	29.2	29.2			
2	Sprayed	8	87.5	100.0	100.0	43.5	72.2	84.0
3	Sprayed	8	0.0	4.2	41.7			
33	Sprayed	8	40.0	100.0	100.0			
36	Sprayed	8	45.8	95.8	100.0			
53	Sprayed	8	29.2	62.5	66.7			
89	Sprayed	8	58.3	70.8	95.8			

Canopy thinning and Emergence Hole Sampling: Between 27 July and 11 September, 2009 a team of 3-4 people visited each site to identify and tag 30 ash trees in each plot, measuring their DBH, surveying them for emergence holes, and providing a canopy thinning rating. Ash trees were identified first by their bark and then verified by their canopies of opposite-branching compound leaves. Flat, numbered aluminum tags were nailed to the trunk about 6 feet from the ground with aluminum nails, facing the direction of the following team members. Percent dieback ratings were produced by taking into consideration both the size of the individual leaves of the canopy and the fullness of the canopy as a whole. The bottom 20 feet on the tag side of each tree trunk was videotaped. The taping was done with a tripod mounted digital video camera set up 6 paces from the tree to be sampled. Video was focused on the inner 75% of the tree's visible trunk surface and was taken in increments as

it moved up the tree, pausing at the top of every frame for the focus to adjust for a clear view of the bark. The same trees sampled in 2009 will be revisited in 2010.

The videos taken in the woodlots were viewed at later dates on a classroom projection screen by teams of two in order to count the number of EAB emergence holes. One site was done per sitting, taking approximately one hour to view 30 trees. Emergence holes were identified by the presence of a definitively D-shaped hole. Both team members reviewed and agreed upon each emergence hole before it was entered in the final data. The location on the trunk of the emergence holes was recorded in order to assist the samplers in 2010 in distinguishing old from new holes. The sampling done in 2009 is for the purpose of establishing the background population of emerald ash borer in the different woodlots.

Table 3. Mean percent canopy thinning and emergence holes at the 20 study sites (30 trees per site, a total of 600 trees). At 15 sites emergence holes were also counted from the ground to a height of 5 feet

Site	Treatment	Percent canopy thinning (n = 30)	Emergence holes to 20' on video (n = 30)	Emergence holes visual to 5' (n = 30)	Mean % canopy thinning (n = 10 sites)	Mean emergence holes (n = 10 sites)
1	Control	56.8	8	-	38.3	12.0
22	Control	62.3	13	-		
44	Control	30.0	16	7		
102	Control	14.1	9	5		
41	Control	26.6	16	7		
43	Control	31.8	9	5		
25	Control	51.1	5	2		
47	Control	39.2	6	2		
28	Control	35.2	21	4		
111	Control	36.1	17	0		
89	Sprayed	25.8	5	-	40.9	7.6
2	Sprayed	53.9	16	-		
3	Sprayed	55.7	11	-		
23	Sprayed	30.0	6	2		
29	Sprayed	56.8	14	5		
108	Sprayed	32.9	10	1		
33	Sprayed	39.7	9	0		
36	Sprayed	43.7	0	4		
100	Sprayed	28.9	1	0		
53	Sprayed	41.2	4	0		

Biological Control

BIOLOGICAL CONTROL OF EMERALD ASH BORERS: THE ROLE OF INDIGENOUS NORTH AMERICAN PARASITOIDS

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ABSTRACT

Background. Because the emerald ash borer is an introduced pest, most of the biological control research emphasis has been placed on the classical approach. In the classical approach, exotic (not native) pest species are controlled by the introduction and establishment of exotic biological control agents from the pest's region of origin. Generally, with exotic invasives, little attention is paid to native natural enemies, because very few attack an adventive pest, and those that do are usually generalists that use other hosts or prey in the environment. Nevertheless, it is not wise to ignore native natural enemies, because (1) not all exotic natural enemies become established, (2) successful biological control does not necessarily result from successful establishment of an exotic natural enemy, and (3) sometimes there is substantial switching to the invasive herbivore. Cornell and Hawkins (1993) conducted a meta-analysis of the parasitoids of 87 non-indigenous insect herbivores, and found that an average of four native parasitoids was acquired in introduced regions, with over 25% of hosts acquiring 10 parasitoid species. Moreover, there are over 160 species of Agrilus in the North American fauna (Arnett 1985), so the likelihood of one or more indigenous natural enemies switching over to *Agrilus planipennis* seems substantial.

Several earlier studies have been conducted in Michigan to see if native or indigenous natural enemies were attacking emerald ash borer and might have potential as control agents of the pest. An early study, conducted by Bauer et al. (2005) resulted in the discovery of six indigenous ectoparasitoids of immature EAB: *Atanycolus hicoriae*, *A. simplex*, *Spathius floridanus*, (Braconidae), *Dolichomitus* sp. (Ichneumonidae), *Phasgonophora sulcata* (Chalcididae), and one non-native ectoparasitoid, *Balcha indica* (Eupelmidae). Larval parasitism by these six species combined, however, accounted for <0.5% EAB mortality; however, Cappaert and McCullough (2008) later found a species of *Atanycolus* exerting substantial levels of parasitism on EAB, at a heavily-infested state park north of the Detroit area. The purpose of our study was to complement the previous studies in the Great Lakes Region by surveying EAB infestations in the Middle Atlantic States (Pennsylvania and Maryland) to see (1) what parasitoids were attacking the pest there and (2) whether any of them might have potential as biological control agents for EAB.

Methods. Surveys were conducted in three physiographic regions: (1) unglaciated Allegheny plateau, in the vicinity of Cranberry (Butler Co.), PA; (2) ridge and valley, in the vicinity of Granville (Mifflin Co.), PA; and (3) piedmont, in the vicinity of Cheltenham (Prince Georges Co.), MD. In all cases, the predominant forest type was oak-yellow poplar. We sampled ash trees exhibiting symptoms of EAB (e.g., exit holes, epicormic growth, bark splits) in several habitats (woodlots, hedgerows, yards, and parking lots). We examined boles from the ground to a height of 2 m, peeling or removing bark to obtain immature stages of EAB. Woodpecker predation was estimated on the basis of EAB exit holes, living larvae collected and apparently successful attacks. Exposed immature EAB and parasitoids were

then collected with flexible aluminum forceps, placed in cells of plastic culture plates lined with wet filter paper or plastic tubes with ventilated caps, respectively, and returned to the USDA ARS Beneficial Insects Introduction Research quarantine facility (Newark, DE) to rear the parasitoids to the adult stage. In addition, we attempted to rear F_2 progeny in quarantine to verify the host-parasitoid relationship and to assess the affinity of the parasitoid for EAB. This was done by implanting immature stages of EAB into ash sticks freshly-cut from green ash trees using methods modified from Liu and Bauer (2007).

Results and Discussion. At least 10 species of hymenopterous parasitoids were recovered from hosts collected in Pennsylvania and Maryland: two eupelmids, *Balcha indica* Mani & Kaul and *Eupelmus pini* Taylor; three braconids, *Atanycolus nigropyga* Shenefelt, *Atanycolus* sp., *Spathius laflammei* (Provancher); and four ichneumonids, *Dolichomitus vitticrus* Townes, *Dolichomitus* sp., *Orthizema* sp., and *Cubocephalus* sp.

Balcha indica, an adventive species from southern Asia (Gibson 2005), was recovered in all three regions, and was the dominant parasitoid recovered in our studies. It can attack larvae, prepupae and pupae. We successfully started a culture of this eupelmid in quarantine, and a study of its bionomics is in progress. It might have potential for use in an augmentative biocontrol program directed against EAB for the following reasons: (1) females can live 10 weeks or more, so they could be stockpiled for release; (2) reproduction is thelytokous, so mating and mate finding are not necessary, an advantage when foraging at low host densities; and (3) females are present from late summer to the following spring, which presents a large window for attacking hosts.

Eupelmis pini, an indigenous species, was recovered only in western Pennsylvania, but has also been reported from EAB in Michigan by Liu et al. (2004). It has previously been recovered from white pine weevil, *Pissodes strobi* (Taylor 1929). Though *Eupelmus* has both endo- and ectoparasitic species, *E. pini* developed ectoparasitically on EAB in our laboratory assays, and like *B. indica*, reproduced thelytokously.

Atanycolus nigropyga was reared from large larvae of EAB collected in western and central Pennsylvania. In addition, an undetermined *Atanycolus* sp. was reared from EAB collected in Maryland. The genus *Atanycolus* is large, having 36 species in N. America (Arnett 1985), and attacks primarily cerambycids and buprestids. At least four different species have been recovered from EAB in the Great Lakes Region (Cappaert, personal communication). Females of *Atanycolus* spp. first paralyze a suitable host and then lay a single egg on it or nearby. Upon hatching, the *Atanycolus* larva develops externally on the host, and upon completion of feeding, spins a cocoon at the end of the host gallery.

Spathius laflammei was reared from larvae of EAB collected in western and central Pennsylvania. Most *Spathius* spp. attack subcortical Coleoptera. The only other host record we found for this gregarious ectoparasitoid was the weevil *Magdalis olyra* Herbst. Another indigenous species of *Spathius*, *simillimus* Ashmead, was recovered from EAB in Michigan by Bauer et al. (2005). Lab assays with *S. laflammei* were positive, and a culture was started.

Two species of *Dolichomitus*, *vitticrus* Townes and an undetermined species, were reared from EAB collected in western Pennsylvania and Maryland, respectively. Species in this genus have been reported from a wide range of xylophagous insects including cerambycids, buprestids, curculionids, bark beetles and even sessiids. Females of *Dolichomitus* lay a single egg on the host larva, and development of the parasitoid is external. The mature larva spins a distinctive, semi-transparent cocoon, in which it overwinters. Bioassays in which we exposed lab-reared EAB larvae to females of *Dolichomitus* spp. did not yield any parasitized hosts.

Two cryptine ichneumonids, *Cubocephalus* sp. and *Othizema* sp., were recovered in low numbers from EAB in western Pennsylvania. Species in both genera have been reported from a wide-range of wood boring and concealed Coleoptera and Lepidoptera. Our lab assays with EAB on these ichneumonids were negative. Because many members of the subfamily Cryptinae use echo-location to detect their hosts (Laurenne et al. 2009), the artificial galleries in which we implanted EAB might not have provided the proper stimulus to elicit oviposition by females tested.

Based on the results in western Pennsylvania (n = 1,041), parasitism by all species combined was low, about 4%. Woodpeckers and fungal pathogens were major sources of mortality, killing about 59% and 12% of the EAB we found. Thus, total apparent mortality was substantial, ≈75%. Because of the low parasitism, prospects for successful natural control of EAB appear slim, so the classical approach appears to be the most promising biological control avenue to pursue at this time. Nevertheless, it is possible that indigenous parasitoids might become better adapted to this new pest with the passage of time. As evaluation of interagency classical biological research in progress continues, parasitism by indigenous species needs to be monitored to see whether its incidence increases over time.

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LIFE CYCLE OF *ATANYCOLUS* SP. NR. HICORIAE, A NEWLY DESCRIBED NATIVE PARASITOID OF EAB

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ABSTRACT

Since the discovery of emerald ash borer in 2002, researchers have worked to identify native natural enemies that might help to mitigate the damage caused by *Agrilus planipennis*. While a number of native parasitoids have been found to attack *A. planipennis*, they collectively killed less than 3% of the total *A. planipennis* population. In the fall of 2007, we observed *Atanycolus* parasitism of *A. planipennis* at one site in Oakland County, Michigan. The parasitoids were initially identified as a variant of *A. hicoriae*, but subsequent molecular and morphological differences became apparent as additional specimens were collected. The parasitoid warranted the status as a new species; soon to be described as *Atanycolus cappaerti* Marsh, as described by P. Marsh et al. in an upcoming issue of the Great Lakes Entomologist. Preliminary studies in 2007 indicated that this new species parasitized emerald ash borer at higher rates, averaging at least 20% within the sites. Understanding the biology of *A. cappaerti*, and its interactions with *A. planipennis* is necessary to evaluate the influence this parasite may have on *A. planipennis* dynamics and its potential for augmentative biocontrol.

In 2008, we began to assess the geographic distribution, seasonal abundance, adult emergence and longevity, larval development rates, host range, and density dependence of *A. cappaerti* as compared to *A. planipennis*. Geographic distribution of *A. cappaerti* was determined by evaluating records provided by MSU and Forest Service scientists who either reared *A. planipennis* from ash logs or dissected logs infested with *A. planipennis*. Initial results show that *A. cappaerti* has only been recorded from two Michigan counties. Given that this species was only recently distinguished from other native *Atanycolus* spp., this was not unexpected.

Seasonal abundance of *A. cappaerti* life stages in relation to *A. planipennis* development was determined by periodically dissecting trees in ash stands in two locations: Seven Lakes State Park in Oakland County and Commons Park Nature Preserve in Genesee County, Michigan. Trees were felled and debarked at three week intervals through the summer and sample. The number of *A. cappaerti* larvae and cocoons were recorded. Parasitism rates averaged 62% at the Seven Lakes site and 16% at the Commons Park site. However, in 2009, this rate dropped to 1% at the Seven Lakes Park site and 0% at the Commons Park site. The cause of the precipitous drop in parasitism in 2009 is likely a result of the overall demise of ash at both sites and possibly weather conditions unfavorable for the parasitoid.

Periodic observation showed that *A. cappaerti* overwintered under the bark as pupae. The following May, adult *A. cappaerti* emerged two weeks before *A. planipennis* adults emerged. This cohort of wasps parasitized *A. planipennis* larvae that had over-wintered from the previous year. In June and July, a second generation of parasitoids emerged. These wasps parasitized the current year *A. plani-*

pennis larvae, and then over-wintered as pupae. Larvae of *A. cappaerti* were able to develop from egg to cocoon in two weeks. Density of *A. cappaerti* was inversely related with *A. planipennis*. This may reflect parasitization of *A. planipennis* larvae that overwintered as early instars, a pattern which is most commonly observed in trees that have not yet been heavily infested.

Longevity of adult *A. cappaerti* was determined by collecting adults reared from logs from infested trees in Seven Lakes and Commons Park. Adults were kept in clear plastic cups with ambient light and temperature. Adult males were found to live for a mean 15 days and females were found to live for a mean 32 days. The female: male ratio was found to be 6:1.

A host range study was performed at the Seven Lakes Park site to determine if *A. cappaerti* would parasitize the native two-lined chestnut borer, *Agrilus bilineatus* (Weber) in white oak (*Quercus alba* L.). In August, infested *Q. alba* logs paired with infested green ash, *Fraxinus pennsylvanica*, logs were affixed vertically to an infested *F. pennsylvanica* tree. Logs were removed and debarked in September. In addition, quaking aspen, *Populus tremuloides* Michx, trees infested with the native poplar borer, *Agrilus liragus* Barter and Brown, were felled and sampled. *A. cappaerti* parasitized an average of 18% of the *A. bilineatus*, 12% of the *A. liragus*, and 43% of the *A. planipennis* larvae.

Additional studies of *A. cappaerti* are underway to determine its geographical range and host range. Further research is needed to thoroughly assess parasitism rates in relation to *A. planipennis* density. If methods for rearing *A. cappaerti* in the lab can be developed, *A. cappaerti* could be considered for augmentative control of *A. planipennis* in areas with low density *A. planipennis* populations. The original core of the *A. planipennis* infestation in southeast Michigan or newly established outlier sites might be appropriate for such releases.

EXPLORATION FOR EAB AND ITS NATURAL ENEMIES IN SOUTH KOREA AND THE RUSSIAN FAR EAST 2004-2009

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ABSTRACT

As EAB spreads throughout North America, it is increasingly important that the biological control program establish a diverse guild of natural enemies representing many microclimates and habitats. Until 2004, most foreign exploration for natural enemies of EAB was carried out in China. To expand the range of species and regions, we have explored in South Korea from 2004 to the present and in the Russian Far East since 2007. In both regions, EAB populations are present on the two native ash species, *Fraxinus rhynchophylla* (Korean ash) and *F. mandshurica* (Manchurian ash). However, EAB is rarely encountered in native habitats, probably because of host resistance in the Asian ashes and the activity of natural enemies. Thus, our first step in foreign exploration for biological control agents was to locate EAB populations.

We investigated many riparian habitats in South Korea containing native ashes during 2004 and 2005 and sought EAB populations through the commonly used techniques of girdling trees and placing sticky bands near the girdles, as well as hanging purple and white plastic cross-vane sticky traps. Despite our considerable efforts over two seasons, we did not trap any EAB using these methods. In the late summer of 2005, we finally discovered a small population of EAB near the city of Daejeon, in central Korea. One hundred and ten Korean ash trees were planted along a country road and were under obvious water stress and attack by EAB. In May 2006, we girdled branches on several trees to stress them, increase attack by EAB, and hopefully, attract natural enemies. We peeled the branches in April 2008 and very rapidly found three natural enemy species: a *Spathius* species (Hymenoptera: Braconidae), a *Tetrastichus* species (Hymenoptera: Eulophidae) that was tentatively identified as *T. telon* Graham (J. LaSalle, pers. comm.), and *Teneroides maculicollis* Lewis (Coleoptera: Cleridae). The *Spathius* species is a gregarious ectoparasitoid, the *Tetrastichus* sp. is a gregarious endoparasitoid, and *T. maculicollis* appears to act as a solitary parasitoid, consuming the host and pupating inside the EAB pupal cell. Similar searches were carried out in 2009, but fewer parasitoids were found. We found a second EAB population in 2007 in Yangsuri (approximately 50 km east of Seoul) where Korean ash trees were damaged during construction of a fire road in the Dongguk University forest. We collected a *Tetrastichus* species and *T. maculicollis* at this site in 2009. The larval parasitoids collected at both Korean sites in 2008

and 2009 were transported to the Otis quarantine lab, but could not be colonized, primarily because of mite outbreaks in the laboratory.

All explorations in the Russian Far East were made in and around the city of Vladivostok. This is a good area in the region for investigating parasitoids; areas about 700 km farther north, around the city of Khabarovsk and on Sakhalin Island, have appropriate tree species, but fewer EAB and parasitoids. The most common ash species used as street trees in Vladivostok are *F. mandshurica* and *F. pennsylvanica* (green ash), a North American species that is susceptible to EAB. Trees of *F. pennsylvanica* infested by EAB were identified in 2007 and 2008. Trees were felled in two locations in late 2008, and billets were placed inside cloth bags in April 2009. Parasitoid adults were collected as they emerged from the billets, and cocoons or adults were gathered later by peeling bark. Two parasitoids were discovered: a *Spathius* species and a *Tetrastichus* species. Neither of these species nor their Korean counterparts have been positively identified. However, the Korean and Russian *Spathius* species are very similar in appearance (i.e., possibly the same species), and both are distinctly different from the Chinese species, *Spathius agrili* Yang. Several hundred Russian parasitoids were hand carried to the Otis Lab in May 2009, and the *Tetrastichus* species is currently being reared. After identification, host range testing, and obtaining of release permits, these four parasitoids – whether species or biotypes – from two regions of the Far East should prove to be valuable additions to our arsenal against EAB.

ADVANCES IN REARING EMERALD ASH BORER AND ITS PARASITOIDS

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ABSTRACT

Three parasitoids of the emerald ash borer (EAB), *Agrilus planipennis*, have been approved for release in the United States, and in order to produce large numbers for release, optimization of rearing methods for EAB and its parasitoids was necessary.

Advances have been made in rearing both EAB adults and larvae. Our method of holding EAB adults in groups of 10 and providing coffee filters as an oviposition substrate eliminated the need for sexing individuals, ensuring mating, and the necessity of using ash twigs as an oviposition substrate. We also developed an artificial diet based on ground ash bark and phloem. Rearing is more successful in the winter when humidity is low because reducing diet moisture and keeping it dry seems to be critical. We are testing the efficacy of silica gel packs as a means to maintain low humidity inside the plastic bags where the diet cups are stored. Further research is planned to develop a totally artificial diet (eliminating the need for ground ash bark and phloem) and to rear the larvae through to the adult stage for continuous production.

The egg parasitoid, *Oobius agrili*, is provided 0- to 7-day old EAB eggs that were laid on ash twigs or coffee filters. The key advance in rearing *O. agrili* is our ability to induce diapause in progeny by rearing the adults under short day conditions (8:16, L: D photoperiod). The parasitized eggs can then be stored in the refrigerator (4ºC) for at least 10 months. This allows year-round rearing and stockpiling for summer release.

To oviposit in the laboratory, *Spathius agrili*, a larval ectoparasitoid, requires EAB larvae that are concealed and feeding. To create these conditions we drill a chamber in a small ash twig, insert a large EAB larva, and then wrap the twig with floral tape. The parasitoid females oviposit on EAB larvae through the floral tape. Experiments revealed that *S. agrili* females lay more eggs when reared in the presence of EAB adults feeding on ash foliage than when reared in chambers with no ash or EAB. Improvements in *S. agrili* rearing methodology resulted in increased longevity (24 to 50 days), increased percentage of females ovipositing (51% to 97%), and increased eggs per female (10.8 eggs to 42.2 eggs). Cold storage of *S. agrili* pupae is possible at 10ºC for three months, but by six months the adults failed to emerge. Research to develop methods of storing *S. agrili* for up to nine months is continuing.

Rearing improvements for *Tetrastichus planipennisi*, a larval endoparasitoid, included abandoning arenas consisting of plastic bags or boxes containing small diameter (~1 cm) ash sticks. We now use arenas made from ventilated snap-top plastic cups, each containing larger diameter (~5 cm) sticks. After peeling down flaps of bark and cutting insertion channels, a late-instar field-collected larvae is inserted into each channel; the bark flap is then sealed back over the channel with a narrow strip of Parafilm®. These changes increased the reliability of *T. planipennisi* rearing and substantially increased the number of progeny produced per parasitized host.

These improvements in rearing methods resulted in 2.5-, 3.4-, and 29.3-fold increases in numbers of *O. agrili*, *S. agrili*, and *T. planipennisi* released, respectively, in 2009 compared to 2008. Our ability to release larger numbers of parasitoids will greatly enhance our ability to study their establishment and impact on EAB populations and ash decline or recovery at field sites. These methods were provided to the EAB Biocontrol Laboratory in Brighton, MI for use in the EAB Biocontrol Program.

A FIRST-YEAR REPORT ON OPERATIONS AT THE BRIGHTON, MI EAB BIOLOGICAL CONTROL FACILITY

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ABSTRACT

In the first year of fully operational status at the Brighton, MI Emerald Ash Borer Biological Control Facility (BCF), several major accomplishments were achieved. We have established successful colonies of all three parasitoid species to be produced at the BCF: *Oobius agrili*, *Spathius agrili*, and *Tetrastichus planipennisi*. The BCF produced and released over 12,000 *Spathius agrili* (3,000 males and over 9,000 females) and 6,000 *Tetrastichus planipennisi* (just over 1,800 males and 4,100 females) EAB parasitoids between June and October 2009. *Oobius* parasitoids have begun to be stockpiled in diapause for release next summer as well. In addition to the above released totals, the BCF has contributed several thousand additional insects (that were not released to the field) to provide material for laboratory research projects with cooperators.

All major equipment purchases originally made for the facility at its inception have been completed, and additional needs (as well as modifications to the original equipment) are being assessed and addressed as necessary. Staffing will continue to increase over the course of the current fiscal year to address needs such as directly rearing the insects and harvesting infested ash material containing parasitoid hosts.

The BCF has begun to conduct an extensive research cooperation with ARS, FS, and APHIS personnel to elucidate improved methodology for rearing large numbers of parasitoids – including, but not limited to, diet development, storage of parasitoid larvae and pupae, induction of diapause in the parasitoids, and increased efficiency in the entire EAB rearing process, to support an ever-growing population of parasitoids. These projects will be carried out primarily at the BCF, with additional experimental replication occurring in cooperating facilities around the US.

UPDATE ON EMERALD ASH BORER BIOLOGICAL CONTROL RESEARCH IN THE U.S.

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ABSTRACT

Emerald ash borer (EAB) (*Agrilus planipennis*), an invasive buprestid native to northeast Asia, has killed tens of millions of ash (*Fraxinus*) trees in infested areas of eastern North America. EAB apparently arrived in infested solid-wood packaging materials from China in the early 1990's near Detroit Michigan, but was not identified as the cause of ash mortality until 2002.

The search for EAB natural enemies started in 2003 at field sites in southeast Michigan (Bauer et al. 2004). In the laboratory, five hymenopterans were later confirmed to successfully parasitize EAB larvae, however, no egg parasitoids were found (Bauer et al. 2008). Four native larval parasitoid species, *Atanycolus hicorae*, *A. simplex*, *Spathius simillimus* (Braconidae), *Phasgonophora sulcata* (Chalcididae), and one exotic species, *Balcha indica* (Eupelmidae) were confirmed to parasitize late-instar EAB larvae (Bauer et al. 2008). However, less than 1% of EAB larvae in Michigan were parasitized. In a separate study of EAB natural enemies in western Pennsylvania, two species of eupelmid, *B. indica* and *Eupelmus pini*; two braconids, *Atanycolus* nigropyga and *Spathius laflammeii*; and one ichneumonid, *Dolichomitus vitticrus*, were confirmed to parasitize EAB larvae (Duan et al. in press). These species resulted in ~3.7% parasitism of EAB larvae. More recently, EAB parasitoids in Ohio documented parasitism by another native braconid species, *Leluthia astigma*, with parasitism reaching ~2.8% (Kula et al. in press). Except for the two eupelmid species (*B. indica* and *E. pini*), these wasps are reported in the literature as parasitoids of native *Agrilus* spp. Moreover, all are ectoparasitoids of late-instar larvae, except for *P. sulcata*, which is an endoparasitoid. Although the diversity of EAB parasitoids is relatively high and variable from site to site and from year to year, reports of prevalence remain substantially lower than for EAB in northeast China (Liu et al. 2007) and for native Agrilus spp. in North America. One exception, however, was the discovery in 2007-2008, of EAB-larval parasitism ranging from 9 to 71% by *Atanycolus* sp. at

two sites in southeast Michigan (Cappaert and McCullough, in press). This confirms that some native parasitoids have the potential to switch from native hosts to EAB under certain circumstances.

EAB biocontrol research in China yielded the following potentially useful parasitoid species: *Tetrastichus planipennisi* (Eulophidae), *Spathius agrili* (Braconidae), and *Oobius agrili* (Encyrtidae) (Liu et al. 2003, Zhang et al. 2005). *T. planipennisi* is a gregarious larval endoparasitoid of 2nd-, 3rd-, and 4th-instar EAB larvae, completes up to four generations per year, overwinters in mature larvae host galleries, and produces an average of 68 adults per host. In ash trees north of Beijing, *T. planipennisi* is the most abundant and widespread parasitoid of EAB. *O. agrili* is a parthenogenic, solitary egg parasitoid that completes about two generations per year and diapauses as a mature larva inside EAB eggs; its known distribution in China is limited to a single site in Jilin province. At that site, the distribution of *T. planipennisi* and *O. agrili* overlap, and in 2005, parasitism suppressed EAB densities by ~75% on infested green ash trees (*F. pennsylvanica*) (Liu et al. 2007). *S. agrili*, a gregarious larval ectoparasitoid of 3rd- and 4th-instar EAB larvae, produces an average of 8 adults per host, and is found more commonly in ash trees southeast of Beijing.

By 2007, research findings on parasitoid biology, laboratory rearing, host specificity, and risk assessment were completed and submitted to APHIS, along with requests for permits to release of the three EAB parasitoids from China in Michigan. An Environmental Assessment was compiled and posted on the Federal Register for public comment by APHIS. After review by researchers, land managers, and the public, APHIS issued a "Finding of No Significant Impact" (Federal Register 2007) and granted release permits for the three parasitoid species in Michigan in late July 2007.

The objective of the 2007 parasitoid releases was to assess reproduction and overwintering of these parasitoid species in central Lower Michigan. Parasitoid release sites with high ash densities were selected and approved on state and township lands. The ash trees showed symptoms of building EAB populations (some canopy decline and wood-pecker feeding on mid to upper trunks), but had low larval densities in the lower trunks based on sampling using bark windows. From FS laboratory colonies, *O. agrili* (~700 females) were released July and August and *T. planipennisi* (~600 mated females + some males) were released July through September at two different sites in Ingham County. Reared by the APHIS Otis CPHST laboratory, *S. agrili* (~100 mated females + some males), were released in September at one site each in Gratiot, Oakland, and Saginaw counties. The parasitoids were released as adults on GPS-marked epicenter trees at each site. In winter 2008, four release trees were felled and cut into logs for parasitoid recovery. For the two larval parasitoids, every other log section was peeled, and immature EAB and parasitoids were reared in the laboratory for parasitoid emergence. The other log section was placed in a cardboard-rearing tube for emergence of adult EAB and parasitoids. For *O. agrili*, release-tree logs were either 1) inspected for EAB eggs, which were then removed and held for parasitoid emergence in Petri dishes, or 2) placed in cardboard tubes for emergence. We successfully recovered *O. agrili* from one of the Ingham Co. sites and *S. agrili* from the Oakland Co. site, confirming field reproduction and overwintering in Michigan.

In 2008, we selected EAB-infested sites in Michigan, Indiana, and Ohio where the three parasitoid species were released for long-term monitoring of parasitoid establishment and efficacy; three of the Michigan parasitoid-release sites are being monitored with cohort life tables and other methods. Due to successful research on *O. agrili* diapause induction, ~2100 were available for release during EAB's egg laying period; these were released in June at Indiana and Ohio sites and July through August at Michigan sites. However, fewer than anticipated larval parasitoids were available for release because of

unexpected rearing problems at the two laboratories, thus ~600 mated *T. planipennisi* females (+ some males) were released from June through October at Michigan sites, and ~300 mated *S. agrili* females (+ some males) were released in August at Michigan and Ohio sites. For recovery of larval parasitoids in 2008, trees were sampled and peeled during either the fall or winter after release; egg parasitoid recovery was done by sampling EAB eggs from ash bark, and rearing them in the laboratory. At the three life table parasitoid-release sites, trees were randomly selected and dissected in July 2009, resulting in the recovery of *O. agrili* and *T. planipennisi*. We also recovered *T. planipennisi* ~800 m away from the release epicenters at two of our release sites by dissection of heavily infested ash trees.

In 2009, we increased the number of parasitoids released per site due to better rearing methods. In addition, the construction and staffing of the new APHIS EAB Biocontrol Laboratory in Brighton, MI was completed in January. After technology transfer of the rearing methods, transfer of parasitoid stock cultures, periodic training, and trouble shooting, the personnel at the EAB Biocontrol Laboratory successfully reared ~7,000 *S. agrili* females (+some males) and ~1,000 *T. planipennisi* females (+some males) for field release this summer. The combined total from the three laboratories resulted in release of ~8,000 mated *S. agrili* females (+some males), ~20,000 mated *T. planipennisi* females (+some males), and ~5,200 *O. agrili* at research sites in Michigan, Ohio, Indiana, Illinois, and Maryland.

At the release sites, we are monitoring for parasitoid reproduction, overwintering, establishment, spread, and prevalence. This is done by peeling infested trees; emergence traps stapled to tree trunks; sentinels logs with EAB eggs or EAB larvae; and by caging the larval parasitoids on tree trunks with EAB larval insertions. We are also collecting data to evaluate the impact or efficacy of biocontrol by comparing changes in ash condition and EAB densities in parasitoid-release vs. control (non-release) sites. Using hand-held computers with high resolution GPS, parasitoid-release and recovery data is being recorded for each tree. For 50 ash trees (<25-cm DBH) in Michigan and Ohio release and control sites, the following data were collected in 2008 and 2009: species, DBH, crown class, epicormic shoots, woodpecker feeding, and EAB exit holes.

In 2008, we started cohort life table studies to determine stage-specific mortality of EAB by each parasitoid species. This three-year study is being replicated at three parasitoid-release and control sites in Ingham Co., Michigan. For the last two years, parasitoids were released on ash trees with EAB egg and larval cohorts. In 2008 and 2009, *O. agrili* was recovered at low prevalence (<1% parasitism) from eggs sampled at the three release sites; none were detected at control sites. To assess establishment of the larval parasitoids at our life table sites by 2009, trees were randomly selected, peeled, and larvae were reared to determine parasitoid prevalence. In fall 2009, *T. planipennisi* was recovered from ~10% of EAB at the three release sites. At two of the sites, EAB larvae parasitized by *T. planipennisi* were found about 800 m from their initial release points, suggesting good dispersal potential for this species. In 2009, we also started laboratory and field research to better understand potential interactions and niche partitioning between the two larval parasitoids, *T. planipennisi* and *S. agrili* (Ulyshen et al. in press; Gould, personal communication).

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LIFE TABLE ANALYSIS TO ASSESS THE IMPACT OF INTRODUCED PARASITOIDS ON THE EMERALD ASH BORER

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ABSTRACT

In 2008, at three forested locations in Ingham Co., Michigan, we began a cohort life table analysis of the emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), to quantify the impacts of three parasitic hymenopteran species [two larval parasitoids, *Tetrastichus planipennisi* (Eulophidae) and *Spathius agrili* (Braconidae), and one egg parasitoid, *Oobius agrili* (Encyrtidae)] introduced from China as biological control agents. At each location, we established a parasitoid-release site and a non-release (i.e., "control") site. In both 2008 and 2009, we established EAB egg and larval cohorts at each of the six sites. The egg cohorts were created on the trunks of ash trees by placing individual eggs under flakes of bark and by attaching small egg-covered logs that had been created in the laboratory. The larval cohorts were created by caging ovipositing EAB onto the trunks of trees in the field. In both years, after establishing the cohorts, all three parasitoid species were released in the three releases sites.

In both years, 30 - 70% of the EAB eggs in the cohorts were removed by unknown predators and, at the three release sites, we documented low rates (i.e., < 1%) of egg parasitism by *O. agrili* None of the EAB larval cohorts established in 2008 was parasitized by *T. planipennisi* or *S. agrili*, but one of those established in 2009 was parasitized by *T. planipennisi*. Furthermore, ~10% of EAB larvae dissected from randomly selected trees in July 2009 at the release sites were parasitized by *T. planipennisi*. Parasitism by *Spathius agrili* was not documented in either year at any of the sites. The cohorts of EAB eggs and larvae established in the summer of 2009 will be recovered in the summer of 2010 to further assess parasitoid establishment and impact on EAB populations.

INTERACTIONS BETWEEN *SPATHIUS AGRILI* (HYMENOPTERA: BRACONIDAE) AND *TETRASTICHUS PLANIPENNISI* (HYMENOPTERA: EULOPHIDAE), LARVAL PARASITOIDS OF THE EMERALD ASH BORER

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ABSTRACT

Three hymenopteran parasitoid species native to China are being released in the United States as biological control agents for the emerald ash borer (EAB) (Bauer et al. 2008). *Spathius agrili* Yang (Braconidae), an ectoparasitoid, and *Tetrastichus planipennisi* Yang (Eulophidae), an endoparasitoid, both attack EAB larvae, raising concerns that interspecific competition following field releases may compromise establishment of one or both species. The main objectives of this study were to 1) compare parasitism rates of the two species when presented either separately or together with EAB larvae, 2) determine whether hosts previously parasitized by one species are acceptable to the other and 3) compare the host-locating efficiencies of the two species.

Experiment 1. Interactions between *T. planipennisi* and *S. agrili* were first assessed by comparing the following treatments 1) EAB larvae presented to *T. planipennisi* only, 2) EAB larvae presented to *S. agrili* only and 3) EAB larvae presented to *T. planipennisi* and *S. agrili* together. This experiment was carried out both in the laboratory and in the field using cup enclosures (each containing 5 EAB larvae inserted either individually into small sticks or together into large sticks) and cage enclosures (each surrounding a section of trunk containing 10 inserted EAB larvae), respectively. While *S. agrili* and *T. planipennisi* exhibited similar parasitism rates (i.e., essentially parasitizing all healthy and available larvae) when presented alone with EAB larvae for 12 d or more, *S. agrili* out-competed *T. planipennisi* when the two species were presented with EAB larvae together. This was particularly evident in the laboratory where there was no evidence of parasitism by *T. planipennisi* when *S. agrili* was present. In the field, however, *T. planipennisi* parasitized larvae in the presence of *S. agrili* on three occasions (although two of those larvae were also parasitized by *S. agrili*), possibly because the caged enclosures in the field were larger than the cup enclosures in the laboratory and because the ratio of *T. planipennisi* to *S. agrili* was higher in the field (3.33) than in the laboratory (2.5).

Experiment 2. Each species of wasp was presented with EAB larvae previously parasitized by the other species in the laboratory. *Spathius agrili* parasitized larvae previously parasitized by *T. planipennisi* but not the reverse. This is not surprising considering that larvae parasitized by *T. planipennisi*, a koinobiont, remain active for about a week (J. Duan, unpublished data) while those parasitized by *S. agrili*, an idiobiont, are paralyzed by the female before ovipositioning. It is likely that both species locate

their hosts by detecting feeding vibrations. If so, larvae parasitized (i.e., paralyzed) by *S. agrili* would be undetectable to *T. planipennisi*. While none of the *S. agrili* progeny on larvae previously parasitized by *T. planipennisi* completed development, *T. planipennisi* progeny were apparently unaffected by the presence of *S. agrili*. However, it remains unclear which species would "win" if the two species were to parasitize the same larva simultaneously. Because *S. agrili* eggs deposited on larvae previously parasitized by *T. planipennisi* are essentially wasted, it is in the interest of *S. agrili* to avoid such larvae. Our results suggest that while *S. agrili* will occasionally accept larvae previously parasitized by *T. planipennisi*, the species prefers healthy larvae.

Experiment 3. *S. agrili* and *T. planipennisi* were presented with EAB larvae for 27 hours to assess the short-term parasitism rates of each. In that time, *S. agrili* parasitized 32% of the larvae on average whereas *T. planipennisi* parasitized none.

Efforts are underway in Michigan and surrounding states to establish populations of *S. agrili* and *T. planipennisi* in the field. To facilitate successful establishment, we recommend releasing the two species separately in space or time to limit the antagonistic interactions observed in this study. This seems particularly prudent given that parasitoid establishment rates are generally higher in single-species introductions than in multiple-species introductions (Mills 2006). However, the implications of our results are somewhat limited by the fact that nothing is known about parasitoid ratios under natural conditions. Furthermore, it is difficult to predict how the two species will interact in nature based on a study in which the parasitoids were confined to small enclosures with limited resources. Long-term monitoring efforts will be needed to determine if *S. agrili* and *T. planipennisi* populations can coexist in North America forests. However, it is encouraging to note the distributions of these species overlap at some sites in China (Liu et al. 2003).

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SEMIOCHEMICALS FOR *SPATHIUS AGRILI*: A PRELIMINARY LOOK

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ABSTRACT

Long-rage attractants for *Spathius agrili* (Hymenoptera: Braconidae), an important larval ectoparasitoid of the emerald ash borer (*Agrilus planipennis* EAB), might be useful tools in the EAB biocontrol program. Attractants could be used in monitoring the survival of newly released *S. agrili* population, population density measurements, and the monitoring the dispersal of existing populations. We compared the volatiles emissions of virgin male and female *S. agrili* and detected at least 10 male- and two female-specific compounds. A tentative identification of the sex-specific compounds indicated that the compounds are relatively simple to obtain or to synthesize, making a practical tool more promising. Preliminary GC coupled antennal electrophysiology (GC-EAD) showed that some of the compounds were antennally active, indicating that GC-EAD might be a valuable tools for this insect to detect behavioral significant semiochemicals. Single virgin males emitted relatively large amounts of the male-specific compounds; these emissions stopped when volatile collections were made from groups of males. The highest amounts of male-specific compounds were emitted from the abdomen. The female-specific compounds could only be detected in the emissions of virgin females; none of the compounds were detected with mated females. Preliminary wind tunnel behavioral studies showed that male *S. agrili* are attracted at long-range to the collected emissions of single male *S. agrili*. All preliminary results so far points to a pheromone system in *S. agrili* that might be adaptable to a monitoring system.

Survey

USE OF UNWOUNDED ASH TREES FOR THE DETECTION OF EMERALD ASH BORER ADULTS: EAB LANDING BEHAVIOR

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ABSTRACT

Incorporation of multiple trapping techniques and sites within a survey program is essential to adequately identify the range of emerald ash borer (EAB) infestation. Within natural forests, EAB lands on stick band traps wrapped around girdled ash trees at a rate similar to unwounded ash trees. The objective of this study was to identify characteristics of ash trees that allow for prediction of EAB landing behavior. This predictive model may be used to select unwounded ash trees for EAB detection survey where cost and policy may be prohibitive to the use of plastic, panel traps or girdled trap trees.

Two sites (Burt Lake and Harrisville State Parks) in the northern Lower Peninsula of Michigan were selected in 2008. Two sites (Farnsworth and Providence Metroparks) in northwestern Ohio and two sites in central Lower Peninsula of Michigan (Deford and Shiawassee State Game Areas) were selected in 2009. For the 2008 sites, all ash trees (N=377) within the campgrounds at these parks were wrapped with a 0.5 m wide sticky, plastic band centered at breast height (1.37 m). For the 2009 sites, 20 ash trees were selected within each site in Ohio and 40 ash trees were selected within each site in Michigan. Each tree was wrapped with the sticky, plastic band. Traps were inspected every 2 weeks and all adult EAB were collected. Using DBH, the surface area of the plastic band and density of EAB (EAB/m^2) were calculated. Canopy light exposure (CLE) and vigor ratings were assessed for each tree. The categorical data CLE and vigor were used to create a predictive model for EAB/m^2. A model was created using the 2008 sites in Michigan (2008 model) and a similar model was created using the 2009 sites in Ohio (2009 model). The 2009 sites in Michigan (Deford and Shiawassee State Game Areas) were used to test the predictive abilities of the two models.

The 2008 model predicted the EAB/m^2 at Deford well, with the predicted values not differing from the observed values in a paired t-test. However, the 2008 model under-predicted EAB/m^2 at Shiawassee, resulting in a significant paired t-test. In contrast, the 2009 model over-predicted EAB/m^2 at Deford, but was successful at predicting EAB/m^2 at Shiawassee. The differences in the models demonstrate the differences between the populations at the sites used to create the 2008 model and those used to create the 2009 model. For example, a tree with a CLE of 4 and a vigor rating of 3 resulted in drastically different predicted EAB/m^2 values using the two different models (2008 = 5.39, 2009 = 303.14). When used to predict the EAB/m^2 values at sites with comparable EAB population size, each model performed well, predicting values that did not differ from the observed. Plotted as three-dimensional surfaces, the two models exhibited similar visual anomalies, which, although producing vastly different EAB/m^2 values, illustrate the overall similarities in EAB landing behavior. One such anomaly was a visual ridge created by trees with a CLE of 4. The predicted EAB/m^2 was greater for a tree with a CLE of 4 than those rated as 3 or 5. EAB activity level increases with increased direct

sunlight. A greater CLE equates to less shading by neighboring canopies. However, trees with CLE of 5 had lower predicted EAB/m^2 values, which may be due to a spatial disconnect between these trees and the forest. For trees used in the 2008 model, those with a CLE of 5, because they were open grown, were significantly farther from another ash tree than trees with other CLE ratings.

EAB population size variability makes it difficult to effectively model the actual captures of EAB/m^2. This is clearly demonstrated by the fact that the two models did not effectively predict both Deford and Shiawassee; only one model was effective at each of the sites. While the models cannot effectively predict the actual capture abilities of a single tree, they did produce visually similar models that can be used to effectively select a tree that has the greatest probability of capturing EAB. A tree with a CLE of 4 and a vigor rating of 4 will most likely be effective at detecting EAB. Some natural resource managers and private land owners decline the request to girdle ash trees for trapping and detection of EAB. Since unwounded ash trees are just as effective in adult captures and have larval densities similar to girdled ash trees, unwounded trees may be a less expensive alternative to other trapping techniques and a simpler alternative to girdled trap trees.

A MULTICRITERIA RISK MODEL TO GUIDE NON-DESTRUCTIVE SURVEYS OF EMERALD ASH BORER

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ABSTRACT

Detection surveys have been an important tool for discovering populations of emerald ash borer (*Agrilus planipennis*, EAB) in previously uninvaded areas. Many of these populations have been established undetected for many years acting as satellites from which EAB can spread naturally. It is likely that other unidentified satellite populations of EAB are present elsewhere. Using known locations of EAB, a multicriteria risk model was developed for the Upper Peninsula of Michigan. This model uses geographic information system data to determine the areas at highest risk for invasion. The model incorporates land cover, proximity to roads, and proximity to campgrounds as factors affecting spread of the insect. The preliminary model correctly predicted 79.8% of known invasion sites to be at high risk. Using the predicted risks a ground survey identifying signs and symptoms of infestation was conducted in summer 2009 focusing on high risk areas. This method of detection allows for an increased number of survey sites over a large area and is non-destructive in that it does not use girdled trap trees or hanging traps.

ASPECTS OF THE PHEROMONE CHEMISTRY OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA: BUPRESTIDAE)

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ABSTRACT

Evidence has suggested that mate location in emerald ash borer (EAB) is mediated by long range response to ash volatiles (Crook et al. 2008), short range response to visual cues (Francese et al. 2005) and contact pheromones (Silk et al. 2009, Lelito et al. 2009) rather than long-range pheromones (Rodriguez-Soana et al. 2006, Lelito et al. 2007, 2008). More recently, Bartelt et al. (2007) discovered an electrophysiologically-active EAB female-produced macrocyclic lactone, (3Z)-dodecen-12-olide (3Z-lactone), which is a putative EAB long-range pheromone. However, no behavioral activity of this compound has yet been reported. We hypothesized that response of EAB to this lactone might be synergized by host volatiles, similar to the critical synergistic attraction to pheromone/host odor combinations reported for *Tetropium fuscum* (Coleoptera: Cerambycidae) (Silk et al. 2007). Here, we report the testing of this hypothesis with the objective of developing an improved tool for early detection of EAB infestations.

GC/MS analysis of extracts from female volatiles of EAB confirmed the presence of 3Z-lactone with retention time and EI-full scan mass spectra against authentic synthetic material. We synthesized both the 3Z-lactone (Boden et al. 1993) and the stereoisomer, (3E)-dodecen-12-olide, the 3E-lactone (Blakemore et al. 1998). The 3E-lactone, if present, was below the detection limit and could not be confirmed by GC/MS or GC/EAD. GC-EAD responses to female EAB volatiles and to synthetic 3Z-lactone and to the 3E-lactone showed that the majority of antennal responses were, surprisingly, to the small (3E)-component of the synthetic lactone, however, there were also a small number of replicates where the antennae responded to the large *(3Z)-component* of the synthetic. Antennae response to female EAB volatiles did not show any consistent responses but some responses at the retention window of 3Z-lactone in the extracts were detected.

We tested the attraction of EAB to traps baited with synthetic 3Z-lactone, 3E-lactone, ash host volatiles (simulated by Phoebe Oil), and combinations of 3Z-lactone, 3E-lactone both separately and together with host volatiles on purple prism traps in field trapping bioassays in Ontario, Canada. Purple prism traps baited with Phoebe oil (a natural source of specific sesquiterpenes) caught significantly

more EAB of both sexes than unbaited traps, and the admixture of the 3Z-lactone or the 3E-lactone with Phoebe oil did not increase the trap catch of EAB.

In preliminary olfactometer assays, however, the highly EAD-active 3E-lactone (but not the 3Z-lactone) induced significant upwind orientation of male EAB but not females. Phoebe oil appeared to be unattractive alone and also appeared to inhibit responses to the lactones in admixture. We conclude that neither the 3Z- nor the 3E-lactones synergize Phoebe oil–baited purple traps but EAD and olfactometer data suggest that the 3E-lactone (paper in preparation) exhibits pheromonal activity and this macrocyclic lactone motif may be important to *A. plannipennis* chemical ecology not evidenced from trap capture data alone. Further research is warranted.

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ATTRACTANTS FOR THE EMERALD ASH BORER: A RESEARCH UPDATE

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ABSTRACT

We investigated the stereo chemistry of three ash (*Fraxinus* spp.) bark sesquiterpenes ((-)-α- cubebene, (+)-7-epi-sesquithujene, (-)-eremophilene) that are antennally active for the emerald ash borer (*Agrilus planipennis*, EAB) and confirmed that the stereochemistry of these ash sesquiterpenes are identical to those found in the essential oils of Phoebe and Manuka. The three sesquiterpenes were identified in volatile collection from white ash (*F. americana*), green ash (*F. pennsylvanica*), black ash (*F. nigra*), and tentatively from Manchurian ash (*F. mandshurica*). Electrophysiological dose-response studies of purified ash sesquiterpenes showed that female EAB antennae are more sensitive to (-)-eremophilene than male antennae. The responses of male and female antennae to either (-)-α- cubebene or (+)-7-epi-sesquithujene did not show any differences. Phoebe oil distillates containing relative large amounts of the oxygenated sesquiterpene eremoligenol can be chemically converted to an oil containing 25% (-)-eremophilene and this oil has been formulated as field baits, releasing 10 mg/day of (-)-eremophilene. These baits were tested during the 2009 EAB field seasons (see abstract by Crook et al.).

ATTEMPTS TO ENHANCE THE EFFICACY OF *AGRILUS PLANNIPENNIS* TRAPS AND LURES

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ABSTRACT

During 2008 and 2009 several field tests were done in an attempt to improve lures and traps for emerald ash borer (EAB) surveys.

Lure test- Manuka oil (oil distillate of *Leptospermum scoparium*) and Phoebe oil (oil distillate of *Phoebe porosa*) contain antennally active 'ash' sesquiterpenes that are attractive to *A. planipennis* when used with purple traps (Crook et al. 2008). Purple traps baited with Phoebe oil or Manuka oil have been shown to have 70-88 % detection rates (i.e., at least one adult caught) compared to 47 % for girdled trap tree treatments (Marshall et al. 2009). During 2009, we attempted to improve trap catch by field testing several enhanced distillates of Manuka and Phoebe oil, on purple traps hung at 6 m along the edges of EAB-infested ash stands. Treatments were Manuka oil 50 mg; Manuka distillate 50 mg (antennally active compounds increased x2, 'non ash-like' compounds removed); co-lure 50 mg (New Zealand Manuka oil distillate); 80/20 Manuka /Phoebe oil 50 mg; Phoebe oil 50 mg; Phoebe distillate (antennally active compounds increased x2, 'non ash-like' compounds removed); Phoebe oil 50 mg plus eremophilene 10 mg.

Phoebe oil baited traps tended to catch more beetles than Manuka oil and co-lure treatments. This supports the previous findings of Crook et al. (2008) who suggested that the increased catch on Phoebe lure baited traps was due to the oil containing 7-epi-sesquithujene, which Manuka oil lacked. Both the Phoebe oil distillate and the Phoebe oil enhanced with eremophilene caught significantly more beetles than Manuka oil or the Manuka distillate. None of the newer Phoebe oil treatments did significantly better than the 80/20 Manuka/Phoebe lure (current program lure). The Phoebe oil distillate (which uses x2 the amount of Phoebe oil) and the eremophilene plus Phoebe oil did not have good enough trap catch to justify the expense of using them for survey use.

The 80/20 Manuka/Phoebe oil lure is currently the most economically viable 'sesquiterpene-based' lure for *A. planipennis*.

Trap test 1- In 2008 two types of iridescent prism traps were field tested ('chromaflair' metallic flake pigment that enables color shifting from green – purple). Small and large flaked paint treatments were tested against a new plastic green trap (Crook et al. 2009) at 6 m height along infested ash stands. All traps (n = 6) were baited with 50mg per day Phoebe-oil lures. The green traps caught significantly more beetles that iridescent trap treatments. Green plastic trap catch averaged 170 beetles per trap.

Trap test 2- Based on results of electro-retinogram studies, we wanted to see if Green prism trap catch could be enhanced by using U.V. (350 nm) or Red (645 nm) lamps (situated inside them). All green traps had fifteen 10-cm-diameter holes punctured per side (including empty controls). U.V. lamp/green traps, red lamp/green traps and green control traps (no lamps) were glued and hung in ash trees at 10 m height (n=5). Traps were left all day taken down at 11 pm. Significantly more beetles were caught on traps with no lamps (controls) suggesting that both lamp types had a repellent effect on *A. planipennis*.

Trap test 3- Based on the coloration and highly iridescent nature of adult *A. planipennis* bodies, we wanted to see if images or iridescent paint were attractive in field tests. High resolution images of *A. planipennis* bodies (enlarged and 'to scale' versions), elytra, abdomen, thorax were printed, laminated and glued onto mini prism traps (20 x 25 cm) along with several traps painted with chromoflair iridescent paints that shift color depending on viewing angle (green to purple; red to gold). A green plastic mini prism was used as a control. A printed light green trap was also made (wavelength closely matching green plastic). This was used as a background color for two other photo traps (an enlarged beetle, and line of three females-to scale). Mini-prism treatments were hung in the canopy of ash trees using a bucket truck and monitored weekly through the flight season. Overall, treatments that had light green coloration on them (as well as the control plastic green) caught significantly more insects than hi-res-image traps or iridescent-painted traps. These findings support recent observations by Crook et al. (2009) in that a green trap (approx 540 nm, 66% reflectance) is highly attractive to *A. planipennis* when placed in the upper canopy of ash trees.

Trap test 4- in 2009 we ran preliminary field tests in Canada and the USA to see if a 'knockdown trap' was possible option for *A. planipennis* survey programs. A modified intercept purple trap (flat sided, not cross-vaned) was used for three treatments. One was glued with a spray application of 'ASTRO' insecticide where as another was sprayed with insecticide only (non-glued). The third treatment was a non-glued trap impregnated with a contact pheromone component (9-methyl-pentacosane), which was then sprayed with insecticide. These three treatments were compared against a standard purple prism glued trap. All traps were baited with 80/20 Manuka/Phoebe oil lures. Preliminary counts from USA tests showed that non-glued traps caught less than half the amount of beetles as glued treatments. The contact-pheromone, non-glued, insecticide-treated trap caught half as many beetles as a non-glued trap treated with insecticide alone. A better trap design and color may lead to a more efficient 'knockdown' trap in the future. This would eliminate the use of glue in survey traps thus making them reusable.

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DETECTION OF A LOW DENSITY EMERALD ASH BORER INFESTATION: TRAP DESIGN, PLACEMENT, AND ASH DISTRIBUTION

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ABSTRACT

Effective and efficient methods to detect recently established infestations of emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) would enable managers to rapidly implement strategies to slow EAB population growth and spread. Identifying new EAB infestations remains difficult, however, because ash trees with low densities exhibit virtually no external symptoms. Many scientists are engaged in efforts to improve the design of traps used to capture adult EAB and the lures used to attract the beetles.

Most field evaluations of new EAB traps or lures, however, have been conducted in areas where EAB populations are at moderate to high densities. Researchers obviously need to capture beetles to assess differences among lures or traps; data consisting of only zeros cannot be analyzed. But in sites where EAB densities are building, many ash trees are likely to exhibit decline symptoms and emit stress-related volatiles, which may affect either the visual response of beetles to the traps or the olfactory response of beetles to the lures being evaluated. Ideally, traps and lures developed for EAB detection should be tested in sites where EAB is present but at such low levels that few, if any trees are symptomatic or stressed by larval feeding.

We initiated a study in 2007 to evaluate the effectiveness of four EAB trap designs in a 40 acre site in Jasper Township in central Michigan. The site includes recent clear cut areas that are mostly open, forested areas where ash is relatively abundant in the overstory, and forested areas with little ash. A systematic grid consisting of 64 cells, each 50 x 50 m (0.625 acres) was overlaid on the site. All ash trees > 1 inch in diameter were inventoried by size class in winter 2007-2008 and the total area of ash phloem was calculated for each grid cell. In addition, 18 ash trees distributed systematically through the site were felled and at least four areas on the stem and main branches were debarked to estimate EAB larval density. A total of two live EAB prepupae were found on a single tree along with a few old galleries and woodpecker attacks, indicating that although EAB had been present in the site for at least 2-3 years, density remained very low.

In May 2008, we divided the site into 16 blocks, each consisting of 4 grid cells (2.5 acres). One type of trap was randomly assigned to each grid cell in each block. Traps evaluated included (1) a girdled ash tree with a sticky band; (2) the EAB trap used by APHIS in 2008 which consisted of a purple prism trap suspended in the mid to upper canopy of an ash tree and baited with a Manuka oil lure; (3) a double-decker trap with two purple panels baited with the Manuka oil lure, a leaf blend lure

and an ethanol lure and (4) a double decker trap with two green panels baited with Manuka oil, leaf blend and ethanol lures. There were four blocks that had only the three traps because ash trees were not available for girdling. The other 12 blocks had one of each trap type, including a girdled tree. Traps were set out in mid June and checked at 2 week intervals.

Because the EAB density was so low in this site, heavily infested ash logs were transported to the site and placed in the center of the 40 acres in early June. Old exit holes were marked on the logs. At the end of the summer, we counted the number of new exit holes and determined that slightly more than 400 adult EAB emerged during the summer.

We captured a total of 87 EAB beetles from 1 July through 19 Sept 2008. Captures peaked in July; 40% of the beetles were caught between 9 and 30 July. The purple double-decker traps captured 65% of all beetles and 81% of the purple double-decker traps captured at least one beetle. The green double-decker traps and canopy traps captured 18% and 5% of the beetles, respectively. Roughly half of the double-decker green traps captured a beetle while only four of the canopy traps had a beetle. Sticky bands on 5 of the 12 girdled trees had adults. If we assume that all beetles captured emerged from the logs at the central release point, our trapping efficiency was roughly 21%. We used logistic regression to determine if the number of beetles captured on individual traps was related to ash phloem area in the grid cell, the distance from the trap to the release point, the interaction of the two factors, exposure of the trap to sun or the type of trap. Only trap type was significant factor in the regression analysis and only the purple double-decker traps captured significantly more EAB than the other traps.

During the 2008-09 winter, the 12 girdled trees were felled and alternate sections (39 inches long) were debarked to determine EAB larval density. In addition, 29 non-girdled ash trees distributed systematically through the site were also felled and at least four sections on each tree were debarked. While only 5 of the 12 girdled trees captured adult EAB on sticky bands, all 12 girdled trees had EAB larvae. Seven of the 29 non-girdled trees had zero larvae. Larval density on girdled trees was approximately 5-fold higher than on non-girdled trees, which averaged roughly 2.5 larvae per m^2. These results reinforce the importance of debarking girdled trees to determine if larvae are present; relying on adult EAB captures on sticky bands is not indicative of infestation.

RESPONSE OF MALE AND FEMALE EMERALD ASH BORERS, *AGRILUS PLANIPENNIS*, TO GREEN LEAF VOLATILE AND SESQUITERPENE LURES

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ABSTRACT

We conducted several trapping experiments in 2008 and 2009 on the emerald ash borer (EAB) in green ash plantations in southern Ontario, Canada, to compare the attractiveness of the green leaf volatile (GLV) lure (Z)-3-hexenol (Z3-6:OH) (7.6 - 80 mg/d) against standard Manuka oil (50 mg/d) and Phoebe oil (50 mg/d) pouch lures placed in green prism traps in the ash canopy and purple traps on ash trees at the 1.5 m level. GLVs are typical emissions from ash foliage on which EAB adults feed, whereas sesquiterpenes are typical emissions from ash bark where females oviposit. As fatty acid derivatives and terpenoids, respectively, they represent two different classes of stress volatiles. Total catch by the Z3-6: OH lure was superior to catches by the sesquiterpene lures when placed in light green traps in the ash canopy and was equal or better than Manuka oil in purple traps at 1.5 m. Manuka oil and Phoebe oil baited traps tended to catch both male and female beetles with a slight preference for females, whereas catches with Z3-6: OH baited traps showed a strong male bias consistent with previously obtained antennal responses in GC-EAD bioassays. The differential effect of various lures on the sexes deserves further investigation. Adding other currently available GLV lures (i.e., C6 alcohols, aldehydes or acetates) in various combinations with Z3-6: OH, failed to improve catches. We conclude that light green prism traps baited with (Z)-3-hexenol should be considered for area-wide detection surveys for EAB. In an attempt to extend the range of possible GLV blends that can be tested against EAB, we evaluated a newly developed autonomous pump, Pher-Emit (Med-e-Cell, San Diego, CA) that allows greater control over blend composition and blend release rates.

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EVALUATION OF TRAPS AND ATTRACTANTS FOR EAB: EFFECTS OF TRAP COLOR, PLACEMENT, HOST VOLATILES, RELEASE RATE AND RELEASE DEVICES ON MALE AND FEMALE RESPONSES

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ABSTRACT

Efforts continue toward developing improved detection tools for emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire). Many factors have been found to influence attraction of EAB to artificial traps including trap color (Francese et al. 2005), design (McCullough et al. 2008) and placement (Francese et al. 2008), lure composition (Crook et al. 2008; de Groot et al. 2008; Grant et al. 2009), and release rate (Crook et al. 2008a; Grant et al. 2009) of attractive volatiles. We conducted several trapping experiments to evaluate these factors on male and female EAB responses by comparing different trap designs, lure combinations, and release devices.

I. Trap Type Experiments

An experiment was conducted in 2008 to compared three trap types, color, and baits. The trap types included: 1) prism traps hung in the lower or mid-canopy of ash trees; 2) double-decker traps that consisted of a 3-m tall PVC pipe with a prism trap attached to the top and a second prism trap attached 60 cm below the top prism; and 3) purple billboard traps that consisted of a large (120 cm × 120 cm) flat panel of corrugated plastic fastened to a 180 cm-tall PVC pipe. The colors tested were purple and light green. The baits included a four-component leaf blend (LB) ((*Z*)-3-hexenol, (*E*)-2-hexenol, (*E*)-2-hexenal, and hexanal released from bubble caps at 3.8, 3.8, 13, and 13 mg/day, respectively), ethanol (800 mg/day, ultra-high release devices), and Manuka oil (50 mg/day, pouches). Manuka oil is a distillate of the New Zealand tea tree and contains 4 of the 6 antenally-active sesquiterpenes present in ash bark (Crook et al. 2008). The experiment consisted of 20 blocks of 9 treatments laid out in randomized complete blocks at nine field sites in southeast Michigan. Treatments included: 1) unbaited purple double decker trap; 2) purple double decker trap baited with the LB, ethanol, and Manuka oil; 3) unbaited green double decker trap; 4) green double decker trap baited with the LB, ethanol, and Manuka oil; 5) unbaited purple canopy trap; 6) purple canopy trap baited with Manuka oil (i.e., the standard trap used by the USDA APHIS detection program in 2008); 7) unbaited green canopy trap; 8) green canopy trap baited with Manuka oil; and 9) billboard trap baited with the LB, Manuka oil, and ethanol. We captured a total of 4218 EAB. After adjusting for trap surface area, there were significant effects for trap design, color, and bait. Overall, 1) double decker and billboard traps captured significantly

more EAB than canopy traps, 2) purple traps captured significantly more EAB than green traps, and 3) baited traps captured significantly more EAB than unbaited traps.

II. GLV Lures vs. Sesquiterpenes Lures in Prism Traps

We conducted several experiments comparing green leaf volatile (GLV) lures with Manuka oil or Phoebe oil lures (each 50 mg/d pouches) in green or purple prism traps. Phoebe oil is a distillate of the Brazilian Walnut tree and contains 5 of the 6 antennally-active sesquiterpenes present in ash bark (Crook et al. 2008). The first experiment conducted in 2008 compared purple prism traps at 1.5 m on rebar poles and baited with the LB (described above) alone or with Manuka oil, and to (*Z*)-3-hexenol alone or with Manuka oil. The second lure experiment was conducted in 2009, and compared purple prism traps hung on rebar poles baited with the LB alone or combined with Manuka oil or Phoebe oil, to (*Z*)-3-hexenol alone or combined with Phoebe oil. Both experiments had 10 replicates of each treatment. In both years, the LB was no more attractive than the single GLV, (*Z*)-3-hexenol, and adding Manuka oil or Phoebe oil to GLVs did not enhance attraction compared to the green leaf volatiles alone.

A third experiment, conducted in 2009, compared green prism traps placed in the lower canopy (approx. 3 m high) of ash trees and purple prism traps hung 1.5 m above ground on rebar poles. Traps were baited with 1) Phoebe oil (50 mg/day pouches) 2) the LB (described above); 3) (*Z*)-3-hexenol (2 bubble cap each at 3.8 mg/day); 4) (*Z*)-3-hexenol and (E)-2-hexenol (1 bubble cap each at 3.8 mg/day); or 5) unbaited control. Each treatment and trap color was replicated 10 times. There was no significant difference in the number of females captured on green traps in the canopy baited with Phoebe oil, the LB or the two green leaf alcohol lures. However, significantly more males were captured on green traps in the canopy baited with (*Z*)-3-hexenol compared to unbaited traps. Traps baited with Phoebe oil or the other GLVs were intermediate. There was a strong male bias in sex ratio to traps baited with (*Z*)-3-hexenol. For purple traps at 1.5 m above ground, both males and females were significantly more attracted to traps baited with Phoebe oil than traps baited with GLVs.

III. Active Lure Dispensers vs Passive Lure Dispensers

We conducted several experiments in 2009 to evaluate active release devices (pumps and high volume sprayers) to compare GLVs and Manuka oil (including fractionated Manuka oil) or Phoebe oil lures. The first experiment tested purple double decker traps baited with the LB or (Z)-3-hexenol combined with a sesquiterpene fraction of Manuka oil, Manuka oil, or a mixture (80:20) of Manuka oil and Phoebe oil. The sesquiterpene fraction of Manuka oil was released from Pher-Emit pump dispensers (Med-e-Cell, San Diego CA) at 17 mg/day, whereas Manuka oil and the mixture of Manuka and Phoebe oil were released from pouches at 50 mg/day. The experiment had 12 replicates. Treatment combinations that contained the LB were no more attractive than the respective combinations with (*Z*)-3-hexenol. On the other hand, combinations that included Phoebe oil were more attractive than the respective combinations with the sesquiterpene fraction of Manuka oil, and combinations with Manuka oil were intermediate.

The second experiment was conducted with purple prism traps hung on rebar poles, and compared Manuka oil (50 mg/day pouches) to Manuka oil released from Pher-Emit pumps at 50 mg/day, the volatile monoterpene fraction of Manuka oil released from Pher-Emit pumps at 35 mg/day, the less volatile sesquiterpene fraction of Manuka oil released from Pher-Emit pumps at 17 mg/day, octane solvent released from Pher-Emit pumps or unbaited controls. Significantly more females than

males were attracted to Manuka oil and its fractions but there were no significant differences among treatments for either sex.

We conducted a third experiment to evaluate different release rates and release devices for Manuka oil and the LB. We compared the standard passive release devices (bubble caps or pouches) to active dispensers (pumps or sprayers) at higher release rates. The experiment included 12 replicates of four treatments in purple double decker traps: 1) the LB released from bubble caps as described above and Manuka oil (50 mg/day pouches); 2) the LB and Manuka oil released from Pher-Emit pumps at 34 mg/day (combined release rate for the LB) and 50 mg/day (Manuka oil); 3) the LB and Manuka oil released from Pher-Emit pumps at 340 and 250 mg/day, respectively; and 4) the LB released from bubble caps and Manuka oil released from sprayers at 4200 mg/day. Sprayers consisted of battery-powered automatic air-freshener sprayers that were modified to actively spray at 20-minute intervals. The sprayers were connected to solar cells so that they were activated only during daylight hours (approximately 7 AM – 8 PM). The active dispensers for the LB and Manuka oil and higher release rates did not enhance attraction of EAB.

Conclusions:

Overall, large, highly apparent trap designs (double decker and billboard) captured more EAB than the smaller prism traps hung in the canopy of ash trees. Both purple and light green traps were attractive to EAB. Color preferences were not consistent between experiments and may be influenced by trap placement, lure, and the responding sex of EAB. For the LB, a simplified lure consisting of only (*Z*)-3-hexenol was as attractive as the four-component blend, and was especially attractive to males. For the sesquiterpenes lures, Phoebe oil, which contains five antennally-active sesquiterpenes, was more attractive than simplified Manuka oil fractions, and Manuka oil, which contains four antennally-active sesquiterpenes, was intermediate. Higher release rates using active release dispensers did not enhance attraction of EAB to GLVs or Manuka oil compared to the standard passive dispensers.

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IMPROVING EAB SURVEY METHODOLOGY

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ABSTRACT

The objective of this ongoing research is to develop a survey tool that exploits cues that beetles use to locate hosts or other beetles. Such a tool could enhance efforts to contain and control North American populations of the pest. Field assays were performed to determine the optimal color, height and adhesive material for *Agrilus planipennis* traps.

In color assay studies, we evaluated several greens of varying wavelengths and reflectance. Traps of corrugated plastic in standard purple and green colors were used as bases for comparison. Among traps with maximum reflectance at varying green wavelengths (500 – 570 nm), those ranging in wavelength from 525 to 540 nm caught significantly more adult *A. planipennis* than traps of other wavelengths. In the 530-540 nm range of the electromagnetic spectrum, there was no significant difference among traps in the 22 to 67 % reflectance range, but traps painted with a peak reflectance of 49 % caught more beetles than standard purple or green plastic traps. Male to female ratio was highest on green traps.

The current height recommendation for survey traps is 6 m (20 ft). Trap placement within the canopy has been shown to play a major role in capturing adult *A. planipennis*, but little is known of the effect of color at the current program height. Purple and green traps were placed at three heights in relation to the tree (1.5 m, 6 m, and 13 m). More EAB were caught on green traps in the mid-canopy (13m) than on purple traps at any of the three heights or green traps in the lower canopy and near the ground. While not significantly different, green traps caught almost twice as many EAB in the lower canopy (6m) compared to purple traps placed in the mid- and lower canopy. Where possible, placing prism traps of either color near the ground should be avoided as there was at least a six-fold difference in the number of EAB caught between traps in the canopy and those placed near the ground. Male to female ratio was highest on green traps regardless of height.

Currently traps for emerald ash borer are coated in Tangletrap insect trap coating and then stuck together face to face prior to transport. This material is extremely messy and the method of delivery inconvenient. We compared three hot-melt adhesives with the current trap coating on purple prism traps in a field assay. One adhesive was removed from the assay early due to its inability to catch any insects. There were no significant differences among any of the remaining glues, so the new hot-melt adhesives may serve as a viable alternative to the current glue used in survey traps

MULTI-STATE COMPARISON OF DETECTION TOOLS AT LOW EMERALD ASH BORER DENSITIES

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ABSTRACT

Emerald ash borer (EAB) detection tool development has been a major focus of research efforts in recent years as the search for an effective detection survey methodology continues. The objectives of this study were to 1) compare the effectiveness of EAB detection trapping tools at low density sites in Indiana, Michigan, Missouri, New York, Ohio, Pennsylvania, Virginia, and Wisconsin, USA, 2) identify the most effective of available trapping techniques for capturing EAB at low density, and 3) develop monitoring and trapping recommendations for managers in locations with and without confirmed EAB populations.

Five different trap types were compared within 79 sites during 2009. Trap types included a girdled trap tree with a 50 cm wide Tanglefoot coated plastic wrap centered at breast height, green prism trap hung at 13 m, purple prism trap hung at 1.5 m, purple prism trap hung at 6 m, and a double-decker purple prism trap in an opening 30 m from an ash tree. All prism traps had 80:20 Manuka oil: Phoebe oil lure. All single prism traps were hung from the canopy of an ash tree. Traps were established on 50 m spacing. Each site had one replicate of each trap with the exceptions of girdled trap tree (65/79 sites) and the double-decker trap (49/79 sites). Traps were established during April and May 2009, checked every 2 weeks, and all adults were collected. During mid-summer, vigor rating, canopy dieback, uncompacted live crown ratio, crown light exposure, and crown class/position were assessed.

Green prism traps hung at 13 m within ash canopies captured the greatest number of EAB adults. However, this was not significantly different from the purple prism traps hung at 6 m with the same lure. There were no significant relationships between canopy light exposure (the number of sides of the canopy receiving full sunlight) and the number of EAB adults captured on the different trap types. There was a significant positive relationship between tree vigor and percent dieback with EAB adults captured for the green prism traps. These relationships are probably due to increases in vigor rating is actually a decrease in actual health and canopy density of the tree. Since the green traps are hung at 13 m in the upper portions of the canopy, trees with higher vigor ratings (i.e., poor health and less canopy) and greater percent dieback have greater amounts of sunlight penetrating the canopy and reaching the traps. EAB has greater activity levels with greater direct sunlight and may be more active in these canopies with lower vigor ratings and increased dieback, resulting in increased capture rates on the green traps. Increased light resulting in increased capture rates may also explain the positive relationship between the purple traps at 6 m (i.e., the base of the canopy) and EAB captures.

The detection of EAB adults was not independent of the different trap type used. The purple prism trap at 6 m and the double-decker trap had the highest rates of detection (81.0 and 95.2 percent, respectively). The green prism traps that resulted in the greatest number of individuals captured, only had 66.7 percent of traps actually detecting EAB. Since the purple prism traps at 6 m did not differ from the green traps in number of adults captured and were not greatly (except for percent dieback) influenced in capture rates by the ash trees they were associated with, the high rates of detection of EAB suggest these are the best traps for use in a detection survey program. While the double-decker traps had 95 percent detection rates, these traps have limitations in their placement. It is recommended that they be placed in open areas at least 30 m from an ash tree. In some parks or other managed areas, the need for an opening may be limiting factor, as it was in establishing this study. Also, the double-decker includes a 10 ft PVC pipe as a pole for trap attachment. This is an added cost, as is the two purple prism traps compared to a single trap used in the other purple prism traps in this study.

High capture rates by the green prism traps at first glance suggest that it may be an effective trap for EAB detection. However, these traps had lower detection rates and were greatly influenced by the individual ash tree they were associated with. Since the purple prism traps were not significantly different from the green traps in capture rates of EAB adults and not greatly influenced by the tree they were associated with, the high detection rates suggest that the purple prism traps were more effective than the green prism traps. Also, in situations that allow for their use, the double-decker traps may be effective as well. It is important to note that whichever trap is incorporated into a survey program, none of them have a 100 percent detection ability. The traps in this study were placed at sites where EAB had previously been detected and populations were known to exist. Even at sites where it is known to exist, none of the traps used in this study detected EAB at every site. Limited effectiveness in detection suggests that multiple trap types, surveys for signs and symptoms, and peeling of ash trees are necessary to provide an adequate detection survey protocol resulting in a definitive presence/absence determination of EAB populations.

BIOSURVEILLANCE WITH *CERCERIS FUMIPENNIS* (HYMENOPTERA: CRABRONIDAE) – THE 2009 COMPARISON TRIALS

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ABSTRACT

During the summer of 2009 three EAB monitoring tools were tested in Southern Ontario (Purple Prism Traps [PPT], Wrap Trees [WT] and Mobile Wasp Colonies [MWC]). The Wrap Tree is a variant of the trap tree.

An extremely cool/wet spring and summer severely reduced the local availability of *C. fumipennis* nests required to build and maintain MWCs. It also reduced the number of suitable weather days during which to simultaneously compare all three tools. Of the 24 prospective comparison sites (12 in the city of Hamilton and 12 in the Turkey Point Region of Norfolk County), trials were successfully completed at a quarter of them (n = 6), all in Norfolk County. For each monitoring tool the following five variables, pertaining to efficiency and effectiveness, were quantified.

a) Financial costs associated with setup, maintenance and disposal of PPTs, WTs and MWCs.
b) Person-hours associated with setup, maintenance and disposal of PPTs, WTs and MWCs.
c) Rate of detection, (ratio of EAB positive sites at which the PPT, WT and MWC successfully detected EAB).
d) EAB capture rates associated with the PPTs, WTs and MWCs, (number of EAB collected by a tool, divided by the number of days the in use at the site).
e) By catch (non-EAB Buprestidae) capture rates associated with the PPTs, WTs and MWCs, (number of non-EAB Buprestidae collected by a tool, divided by the number of days the in use at the site).

Summary of Results:

Table 1.1 Tally of values for Late Season EAB monitoring (July 11th to August 15th)

	Cost to monitor a site	Person hours to monitor a site	EAB detection rate	EAB capture rate	By catch capture rate
PPT	$5	1 hr 9 min	4 of 5	0.08 (SD = 0.06, n = 4)	0.03 (SD = 0.09, n = 26)
WT	$9	1 hr 9 min	2 of 5	0.083 (n = 2)	0.02 SD = 0.02, n = 26)
MWC	$38	15 hrs	5 of 5	6 (SD = 8.60, n = 5)	7.19 SD = 5.87, n = 5)

The monitoring tools were Purple Prism Traps (PPTs), Wrap Trees (WTs) and Mobile Wasp Colonies (MWCs) at 6 sites of 'questionable EAB infestation'. Sites of 'questionable EAB infestation' were sites near a known infestation but no EAB had been detected at the sites during spring 2009 visual surveys. Of the 6 sites, 5 of these sites were ultimately positive for EAB during the summer of 2009.

While a small sample size, this comparison still sheds light on the following three tools. The MWC (when available) are a more sensitive (detection rate) and a more productive (capture rate) EAB monitoring tool than either the PPT or WT. The "cons" at the moment are that the MWCs are more costly and time consuming. Currently *C. fumipennis* may be best implemented as a niche EAB detection tool. Perhaps all EAB detection tools should be classified this way as each tool has various limitations in addition to their benefits and managers require a quiver of EAB survey detection tools.

If there is sufficient interest in using the most effective tool (sensitive and productive) for the job, rather than just the least costly (financially and temporally) then new effort should focus on wasp husbandry (i.e., refinement of lab reared mobile colonies, produce a degree day model for the species, etc). The refinement of wasp husbandry will reduce costs and labor associated with the MWCs while enhancing the availability of *C. fumipennis* for use in MWCs.

Cerceris fumipennis has demonstrated its ability to naturally perform an extraordinary job collecting buprestids. The route ahead should involve taking advantage of a wasp service that is already being provided. Continuing to utilize and refine the use of *C. fumipennis* is not merely valuable for our immediate EAB concerns but it will also no doubt assist with monitoring for other pest Buprestidae and ultimately fuel the development of other forms of biosurveillance.

CREATING A STATE-WIDE BIOSURVEILLANCE PROGRAM FOR EMERALD ASH BORER

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ABSTRACT

Early detection of invasive insects is an important component of a management plan. For emerald ash borer, the solitary ground-dwelling wasp, *Cerceris fumipennis* (Hymenoptera: Crabronidae), can aid in early detection. This non-stinging wasp is native to the United States east of the Rocky Mountains, and provisions its nest with adult buprestid beetles. *C. fumipennis* is also an efficient predator of EAB when present (Careless et al. 2009). Using an insect in this way to detect a pest species is an example of biosurveillance.

Over 60 wasp colonies were located throughout central and southern Maine during the summers of 2008 and 2009. If colonies were of sufficient size (at least 30 nests) and within approximately 400 m of ash trees, they were used for biosurveillance. During the summer of 2009, suitable colonies were paired with local volunteers who were given supplies and trained in how to monitor colonies and take prey from returning wasps. The goal was to have volunteers collect 50 beetles from each site.

Eighteen individuals or groups of volunteers were involved in this project. Volunteers included city arborists and parks staff, Maine Entomological Society members, Girl Scout troops, families and other interested individuals.

Full or partial biosurveillance was carried out by volunteers or Maine Forest Service staff at 24 sites. Although not all beetles have yet been sent in, it is expected that approximately 500 buprestids have been collected in this program during 2009. Forty-five buprestids have so far been identified, and include 6 genera and 15 species, including *Spectralia gracilipes*, a new buprestid genus and species for Maine.

In comparison, during the summer of 2009, 26 purple prism sticky traps were hung in the canopy of ash trees at 13 locations. However, no buprestids were collected using this method.

Further information about Maine's biosurveillance program can be found at: www.main.gov/cerceris

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