United States Department of Agriculture

United States Forest Service

Animal and Plant Health Inspection Service

Forest Health Technology Enterprise Team
Morgantown, West Virginia

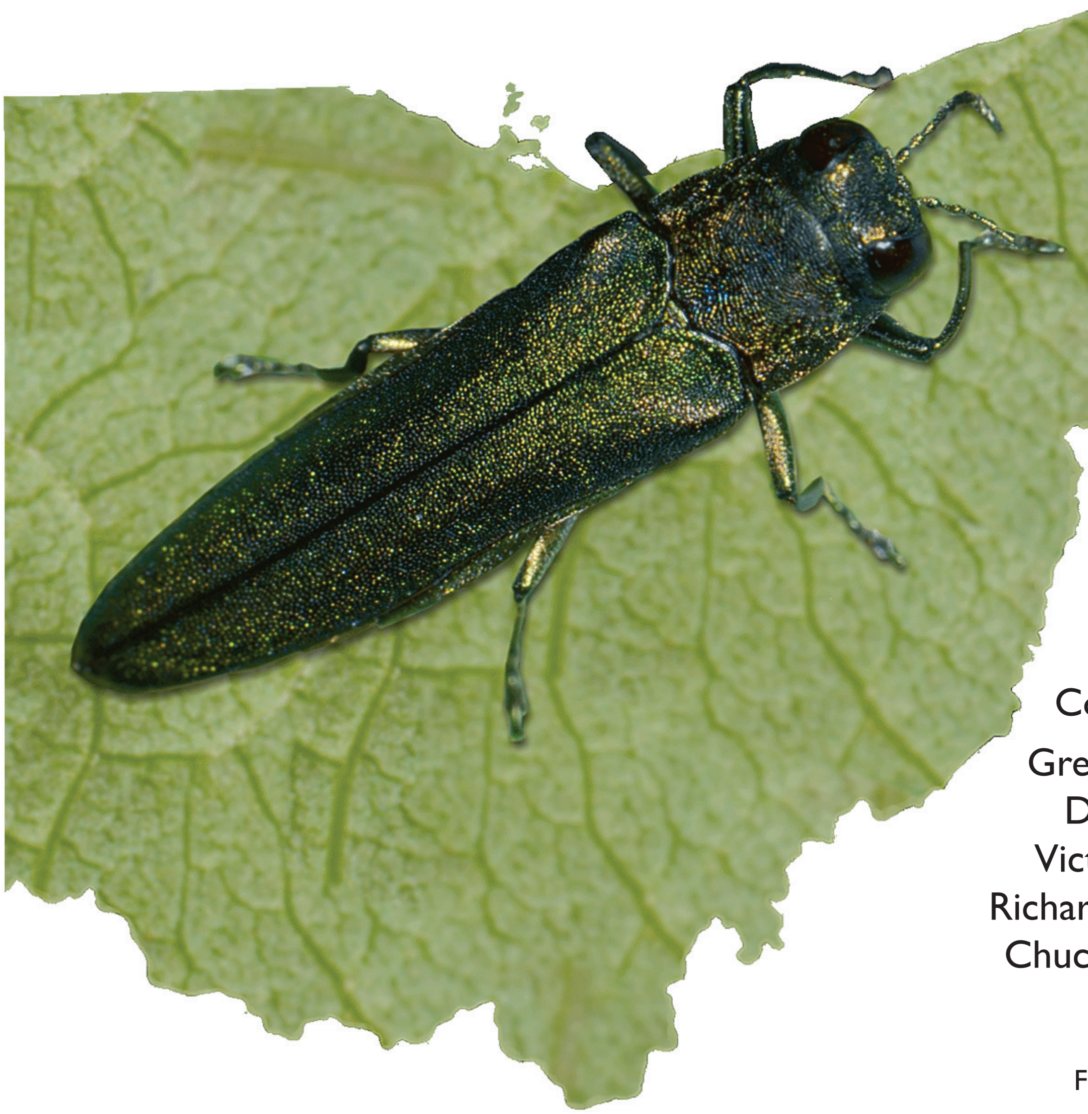
The Ohio State University

2011 Emerald Ash Borer National Research and Technology Development Meeting

October 12 & 13, 2011
Wooster, Ohio

Technology Transfer

Emerald Ash Borer



Compiled by

Gregory Parra
David Lance
Victor Mastro
Richard Reardon
Chuck Benedict

FHTET-2011-06

The abstracts were submitted in an electronic form, and were edited to achieve only a uniform format and typeface. Each contributor is responsible for the accuracy and content of his or her own paper. Statements of the contributors from outside of the U.S. Department of Agriculture may not necessarily reflect the policy of the Department. Some participants did not submit abstracts, and so their presentations are not represented here.

Cover photo and graphic by David Lance
Animal and Plant Health Inspection Service (APHIS)

The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture of any product or service to the exclusion of others that may be suitable.

References to pesticides appear in some technical papers represented by these abstracts. Publication of these statements does not constitute endorsement or recommendation of them by the conference sponsors, nor does it imply that uses discussed have been registered. Use of most pesticides is regulated by state and federal laws. Applicable regulations must be obtained from the appropriate regulatory agency prior to their use.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish and other wildlife if they are not handled and applied properly. Use all pesticides selectively and carefully. Follow recommended practices given on the label for use and disposal of pesticides and pesticide containers.

Federal Recycling Program
Printed on recycled paper

2011 Emerald Ash Borer National Research and Technology Development Meeting

October 12 & 13, 2011

Ohio Agricultural Research and Development Center
Wooster, Ohio

Sponsored by

The Ohio State University and
the United States Department of Agriculture
Animal and Plant Health Inspection Service

Compiled by

Gregory Parra[1], David Lance[2], Victor Mastro[2], Richard Reardon[3] and Chuck Benedict[4]

[1]USDA-APHIS PPQ, Raleigh, North Carolina
[2]USDA-APHIS PPQ, Otis ANGB, Massachussetts
[3]USDA-FS FHTET, Morgantown, West Virginia
[4]USDA-FS FHTET, Madison, Wisconsin

For additional copies of this or any of the previous five proceedings (FHTET 2010-01, FHTET 2008-07, FHTET 2007-04, FHTET-2004-02 and FHTET-2004-15), contact Gregory Parra, North Carolina, at (919) 855-7548 (gparra@ fs.fed.us) or Richard Reardon in Morgantown, West Virginia, at (304) 285-1566 (email: rreardon@fs.fed.us).

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. At this point, however, it is still too early to judge how effective these efforts are. There is simply no "control area" for comparison.

This volume contains abstracts from papers presented at a meeting that took place October 12-13, 2011, in Wooster, OH. The studies described herein were aimed at the broad goal of improving our ability to manage EAB populations. The focus of this compendium is work conducted in 2010 and 2011, although some of the abstracts describe work across a broader time frame. The abstracts cover a wide variety of subjects, starting with studies that add to our basic understanding of the insect and its physiology, interactions with its environment, and potential impacts on North America's natural and urban forests. At the other end of the spectrum, the studies describe highly applied research. There are ongoing efforts to improve methods and materials for using insecticides to control EAB populations. Products of regulatory treatment research have already improved our treatments for wood and wood products, and new methods such as the use of radio-frequency treatments are showing promise. Other groups looked at the potential of broader management strategies for slowing mortality of ash in an area or protecting landscape trees from EAB.

As part of its management strategy, the USDA-APHIS 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. These species had previously been evaluated for such factors as host specificity and ability to attack EAB and reproduce in the field. Operational releases of these insects began in 2010 as part of a 5-year implementation strategy. Ongoing research and development efforts described here includes assessment of releases of biological control agents, development of traps and other methods to improve our ability to monitor the effectiveness of those releases, foreign exploration for and evaluation of additional parasitoid species, assessing potential effects of natural enemies that are already established in North America, and improved rearing and parasite production methods. 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 evident, 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. Efforts described here include a renewed look at using multi-funnel traps for EAB, optimization of trap colors, and potential improvements in chemical lures, including an apparent female-produced pheromone.

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. Current studies are addressing molecular and allelochemical mechanisms of tree resistance to EAB as well as attempts to identify resistance in North American ash. Molecular and traditional plant-breeding methods are being investigated as means of developing resistant varieties.

Federal funding for emerald ash borer programs was substantially reduced starting in Fiscal Year 2012, placing the future of EAB Research and Development Meetings in doubt. However, the reduction in funding also places a premium on developing highly cost-effective methods of managing the pest, so research and development activities are expected to carry on, albeit at a reduced level. In 2011, APHIS Plant Protection and Quarantine issued a Request for Proposals and invested heavily in several multiple-year research projects in a number of areas. Foremost are projects focused on the discovery of host-resistance factors to EAB, and the utilization of these factors to develop resistant ash trees. The fruits of the research focused on managing EAB will benefit other programs challenged with managing other introduced and endemic pests in the future. These discoveries and developments will be critical to the maintenance of North American forests as a critical resource for the benefits that they provide to our economy and well-being.

Table of Contents

*Abstracts appear in the order in which they were presented at the meeting.

*Posters appear in alphabetical order according to title.

Abstracts*

Host Interactions

*Abstracts appear in the order in which they were presented at the meeting.

AN OVERVIEW OF EMERALD ASH BORER HOST RESISTANCE RESEARCH SINCE 2003

D.A. Herms[1], P. Bonello,[2] D. Cipollini,[3] J.L. Koch,[4] K.S. Knight[4] O. Mittapalli,[1] and D.R. Smitley[5]

[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691
herms.2@osu.edu

[2]Department of Plant Pathology, The Ohio State University, Columbus, OH 43210

[3]Department of Biological Sciences, Wright State University, Dayton, OH 45435

[4]USDA Forest Service, Northern Research Station, Delaware, OH 43015

[5]Department of Entomology, Michigan State University, East Lansing, MI 48824

ABSTRACT

Emerald ash borer (EAB, *Agrilus planipennis*) has killed millions of ash trees (*Fraxinus* spp.) in managed and natural landscapes of North America since its accidental introduction from Asia. The most widely distributed ash species endemic to eastern North American are highly susceptible, including green (*F. pennsylvanica*), white (*F. americana*), and black (*F. nigra*) ash (Smith 2006). Ash mortality now exceeds 99% in forests of southeast Michigan within 50 km of the epicenter of the invasion (Herms et al. 2009), and as EAB continues to spread, it threatens the existence of ash in North America on a continental scale, with devastating economic and environmental impacts. The development of EAB resistant ash trees will be critical for long-term reforestation and preservation of ash in natural and urban forests.

We have initiated a collaborative research program with the goal of developing EAB-resistant ash trees. Objectives include identification, selection, and breeding of EAB-resistant germplasm; elucidation of mechanisms of resistance; and use of functional genomics to establish the molecular foundation for identification of resistance mechanisms and genes, as well as genetic markers to track desirable traits through the breeding and selection process.

In Asia, EAB does not devastate its endemic hosts, but does severely impact North American ash species planted there (Wei et al. 2004). This suggests that Asian ashes are inherently resistant by virtue of their coevolutionary history with EAB. Consistent with this hypothesis, a common garden study established in 2003 at the Michigan State University Tollgate Education Center confirmed that *F. mandshurica* 'Mancana' is much more resistant to EAB than North American green and white ash cultivars (Rebek et al. 2008), and have identified secondary metabolites and defense proteins in phloem tissue that may provide the mechanistic basis for this resistance (Eyles et al. 2007; Cipollini et al. 2011; Whitehill et al. 2011). Progress has been made in development of an artificial diet that can be used to evaluate the biological activity of candidate resistance compounds in bioassays.

A second common garden experiment established in 2004, also at the Michigan State University Tollgate Education Center, confirmed that resistance of *F. mandschurica* to EAB extends to sexually propagated individuals. Blue ash (*F. quadrangulata*) was found to more resistant to EAB than other North American taxa, while the following taxa were found to be highly susceptible (mortality ≥ 70%): *F. excelsior* 'Aueafolia', and *F. latifolia*. *F. ornus*, and *F. oxycarpa* 'Raywood'.

Sequencing of the ash transcriptome and proteomic analysis comparing this resistant Asian ash species to susceptible North American species have identified several candidate genes of interest (Mittapalli et al. 2010; Bai et al. 2011; Rajarapu et al. 2011; Whitehill et al., 2011). Work is underway to validate the roles of these candidate genes in resistance to EAB and to develop genetic markers to facilitate targeted breeding.

Progress has been made in overcoming barriers to hybridization of Asian and North American ash species (Koch et al. 2010a). Extensive surveys of ash stands in Michigan and Ohio have revealed a very small proportion of ash that are still healthy where EAB-ash induced mortality exceeds 99% (Knight et al. 2010; Koch et al., 2010b). These "lingering ash" may be naturally resistant and thus provide a potential source of rare resistance genes in native ash populations.

REFERENCES

Bai, X., L. Rivera-Vega, P. Mamidala, P. Bonello, D.A. Herms, O. Mittapalli. 2011. Transcriptomic signatures of ash (*Fraxinus* spp.) phloem. *PLoS ONE*, 6(1):1-12

Cipollini, D., Q. Wang, J.G.A. Whitehill, J.R. Powell, P. Bonello, and D.A. Herms. 2011. Distinguishing defense characteristics in the phloem of ash species resistant and susceptible to emerald ash borer. *J. Chem. Ecol.* 37:450-459.

Herms, D.A., Gandhi, K.J.K., A. Smith, J. Cardina, K.S. Knight, C.P. Herms, R.P. Long, and D. McCullough. 2009. Ecological impacts of emerald ash borer in forests of southeast Michigan. *In:* K. McManus and K.W. Gottschalk, eds. *Proceedings. 20th USDA Interagency Research Forum on Invasive Species 2009. Jan. 13-16, 2009, Annapolis, MD.* pp. 36-37. USDA FS GTR NRC-P-51, Newtown Square, PA: USDA, Forest Service, Northern Research Station. 114 pp

Knight, K.K., D.A. Herms, J. Cardina, R. Long, J. Rebbeck, K.J.K. Gandhi, A. Smith, W.S. Klooster, C.P Herms. 2010. Emerald ash borer aftermath forests: the dynamics of ash mortality and the responses of other plant species. *In:* C.H. Michler and M.D. Ginzel, eds. *Proceedings of Symposium on Ash in North America*, p. 11. Gen. Tech. Rep. NRS-P-72. Newtown Square, PA: USDA Forest Service, Northern Research Station. 64 p.

Koch, J.L, M.E. Mason, D.W. Carey , K.S. Knight, T. Poland, and D.A. Herms. 2010a. Overcoming obstacles to interspecies hybridization of ash. *In:* C.H. Michler and M.D. Ginzel, eds. *Proceedings of Symposium on Ash in North America*, p. 41-44. Gen. Tech. Rep. NRS-P-72. Newtown Square, PA: USDA Forest Service, Northern Research Station. 64 p.

Koch, J.L, M.E. Mason, D.W. Carey , K.S. Knight, T. Poland, and D.A. Herms. 2010b. Survey for tolerance to emerald ash borer within North American ash species. *In:* C.H. Michler and M.D. Ginzel, eds. *Proceedings of Symposium on Ash in North America*, p. 60. Gen. Tech. Rep. NRS-P-72. Newtown Square, PA: USDA Forest Service, Northern Research Station. 64 p.

Mittapalli, O., X. Bai, P. Mamidala, S.P. Rajarapu, P. Bonello, and D.A. Herms. 2010. Tissue-specific transcriptomics of the exotic invasive insect pest emerald ash borer (*Agrilus planipennis*). *PLoS ONE* 5(10)1-12.

Rajarapu, S.P., P. Mamidala, D.A. Herms, P. Bonello, and O. Mittapalli. 2011. Antioxidant genes of the emerald ash borer (*Agrilus planipennis*): gene characterization and expression profiles. *J. Insect Physiol.* 57:819-824.

Rebek, EJ, Herms DA and Smitley DR. 2008. Interspecific variation in resistance to emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (*Fraxinus* spp.). *Environ. Entomol.* 37:242-246.

Smith, A. 2006. Effects of community structure on forest susceptibility and response to the emerald ash borer invasion of the Huron River Watershed in southeastern Michigan. M.S. Thesis, The Ohio State University, Columbus.

Wei, X., D. Reardon, Y. Wu, and J.-H. Sun. 2004. Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in China: a review and distribution survey. *Acta Entomol. Sinica* 47:679-685.

Whitehill, J.G.A., S. Popova-Butler, K.B. Green-Church, J.L. Koch, D.A. Herms, and P. Bonello. 2011. Interspecific proteomic comparisons reveal ash phloem genes potentially involved in constitutive resistance to emerald ash borer. *PLoS One* 6(9): e24863 doi: 10.13171/journal.pone.0024863.

DECIPHERING THE DEFENSE STRATEGIES IN THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS* FAIRMAIRE

Swapna Priya Rajarapu,[1] Xiaodong Bai,[1] Praveen Mamidala[1]
Pierluigi Bonello,[2] Daniel A. Herms,[1] and Omprakash Mittapalli[1]

[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691
rajarapu.1@osu.edu

[2]Department of Plant Pathology, The Ohio State University, Columbus, OH, 43210

ABSTRACT

Phytophagous insects exploit their host resources by employing defense mechanisms including avoidance, excretion, target modification, sequestration and metabolic resistance. Among these metabolic resistance is the most effective and prominent mechanisms in dealing with the deleterious compounds encountered during host interactions. Metabolic resistance includes the overproduction of several detoxification (cytochrome P450s, glutathione-S-transferase and carboxylesterases) and antioxidant enzymes (catalase, CAT; superoxide dismutase, SOD; glutathione peroxidase, GPX.) Together these enzymes contribute for successful adaptation/ invasiveness of insects in any introduced habitats. Understanding these mechanisms in a successfully adapted invasive pest like the Emerald Ash Borer, (*Agrilus planipennis* Fairmaire) might shed light on the crux of survival strategies used by this beetle. Hence, we undertook tissue specific (midgut and fatbody) transcriptomic study to unveil the putative detoxification process in *A. planipennis*.

Our 454 pyrosequencing efforts of *A. planipennis* larval tissues (midgut and fat body) feeding on the green ash (*Fraxinus pennsylvanica*) resulted in 62, 834 expressed sequence tags (ESTs). Among these we found a high number (75) of ESTs encoding for cytochrome P450 in fat body and midgut (45) tissues. Also, we predicted 22 ESTs encoding for antioxidant genes in midgut and 12 in fat body. Further, a tissue level profiling of these detoxification and antioxidant genes in the larvae (3rd and 4th instars) feeding on green ash revealed interesting patterns. The P450 genes belonging to different families demonstrated a tissue specific distribution wherein high mRNA levels of CYP6A and CYP6B were found in the Malpighian tubules and midgut respectively. Intriguingly, CYP9 showed high expression in the midgut and integument. These tissue specific expression profiles better explains the putative role of P450s in biotransformation/sequestration of toxins encountered through diet and contact. Profiling of antioxidant genes displayed high expression of CAT in the midgut. On the other hand, peak mRNA transcript levels of SOD were observed in Malpighian tubules. These genes might be involved in encountering the direct defenses from the plant in the form of reactive oxygen species or dealing with the oxidative stress caused due to feeding on diet with high amounts of toxins. A functional analysis of these genes via RNAi will shed more light on adaptation strategies employed by *A. planipennis* for its successful colonization in novel ecological niches.

MECHANISMS UNDERLYING VARIATION IN RESISTANCE OF ASH SPECIES TO EMERALD ASH BORER: EFFECTS OF EXPERIMENTAL GIRDLING ON LARVAL PERFORMANCE AND DEFENSIVE CHEMISTRY OF ASH

Vanessa L. Muilenburg, P. Larry Phelan, and Daniel A. Herms

Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691
muilenburg.1@osu.edu

ABSTRACT

Emerald ash borer (EAB) (*Agrilus planipennis*) larvae feed on phloem and outer xylem of ash (*Fraxinus* spp.) stems, producing galleries that can girdle and kill trees. There is substantial interspecific variation in ash resistance to EAB. North American species, such as green (*F. pennsylvanica*) and white ash (*F. americana*), are much more susceptible than Asian species such as Manchurian ash (*F. mandshurica*) that share a coevolutionary history with EAB. To elucidate mechanisms underlying this variation in resistance, we experimentally girdled white, green, and Manhurian ash trees, which is predicted to substantially weaken resistance in the portion of the trunk below the girdle relative the portion above the girdle. We quantified performance of EAB larvae originating from experimentally inoculated eggs and natural oviposition, as well as potential constitutive and induced resistance mechanisms above and below the girdle, and on corresponding locations on non-girdled tees.

We found that green ash has higher densities of EAB galleries than white and Manchurian ash. With each of the three species, girdled trees were more susceptible to EAB colonization than ungirdled trees, as indicated by higher density of galleries. Within a tree, there were more galleries below than above the girdle, but there were no differences in number of larvae. This may indicate that larvae below the girdle produced longer galleries due to faster development rates than larvae feeding above the girdle. Ongoing work will evaluate the contribution of constitutive and induced phenolics to resistance by analyzing the relationship between larval performance/density and phenolic profiles.

GENE DISCOVERY IN ASH (*FRAXINUS* SPP.)

Loren Rivera-Vega,[1] Xiaodong Bai,[2] Praveen Mamidala,[1] Pierluigi Bonello[3] Daniel Herms,[1] Jennifer L. Koch,[4] Mary E. Mason[1] and Omprakash Mittapalli[1]

[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691
rivera-vega.2@osu.edu

[2]Center for RNA Molecular Biology, Case Western Reserve University, Cleveland, OH 44106

[3]Department of Plant Pathology, The Ohio State University, Columbus, OH 43210

[4]US Forest Service, Northern Research Station, Delaware OH 43015

ABSTRACT

Ash trees (*Fraxinus* spp.) are widely spread throughout eastern North America and represent an important tree species in urban landscape and natural settings. Since the accidental introduction of the invasive insect pest emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) millions of ash trees have been killed by this devastating pest. However, in Asia, EAB's natural habitat, the damage to native ash is isolated and only observed in trees under stressed conditions, indicating some level of resistance presumably due to a shared co-evolutionary history. Despite efforts to contain EAB, it continues to spread through the eastern United States and parts of Canada, threatening the survival of the entire ash genus. Despite the high impact status of EAB, there is little information available about the molecular genetics of any North American or Asian *Fraxinus* species. In order to reduce this lack of knowledge, a functional genomics approach was taken.

Two next generation sequencing platforms were used to obtain four databases containing ash expressed sequence tags (ESTs). In 2009, 454 pyrosequencing was used to create a pooled database containing transcripts from blue (*F. quadrangulata*), black (*F. nigra*), green (*F. pennsylvanica*), white (*F. americana*) and Manchurian ash (*F. mandshurica*). This database yielded 58,673 high quality sequences. Given that this was a pooled database is was difficult to make comparisons among the species. In 2010, three independent databases for black, green and Manchurian ash were created using Illumina GAII platform each yielding 35,676, 41,885, and 37,516 high quality sequences, respectively. A series of bioinformatic analyses were done in order to identify potential metabolic pathways associated to these sequences. In general, more than 130 metabolic pathways were associated to each database. Also, more than 3,000 protein domains were identified. Molecular markers, both single nucleotide polymorphisms (SNPs) and microsatellites were predicted. There are currently more than 40,000 molecular markers identified and in need of validation.

The availability of these databases opens the door for many research studies. Gene characterizations, identification of genes potentially involved in resistance, plant breeding programs and population genetics studies can benefit from these databases.

CHARACTERIZING PHLOEM BROWNING IN ASH: A POSSIBLE DEFENSE MECHANISM TO EMERALD ASH BORER

Sourav Chakraborty,[1] Amy Hill,[1] Don F. Cipollini,[2] Justin G.A. Whitehill[1] Daniel A. Herms,[3] and Pierluigi Bonello[1]

[1]Department of Plant Pathology, The Ohio State University
2021 Coffey Road, Columbus, OH 43210
chakraborty.27@osu.edu

[2]Department of Biological Sciences, Wright State University
3640 Colonel Glenn Highway, Dayton, OH 45435

[3]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691

ABSTRACT

Emerald ash borer (EAB, *Agrilus planipennis* Fairmare; Coleoptera : Buprestidae) threatens to decimate the ash trees of North America. The most widely distributed North American species are highly susceptible to EAB. However, Manchurian ash, an Asiatic species, is resistant, possibly due to its co-evolutionary history with the beetle. In recent years, our lab has investigated biochemical resistance traits of ash to EAB. We are particularly interested in understanding mechanisms responsible for insterspecific variation in resistance of ash to EAB. Resistance mechanisms of Manchurian ash could include defensive proteins and secondary metabolites. In addition, tissue browning, which is associated with the oxidation of phenolic compounds, may play an important role. The mechanism of tissue browning is attributed to the activity of phenol oxidase enzymes. Both peroxidases (POD) and polyphenol oxidases (PPO) convert soluble phenolics to corresponding quinones. Reactive quinones then undergo polymerization resulting in tissue browning. Cipollini et al. (2011) observed that phloem of Manchurian ash browns faster than that of white ash (*Fraxinus americana* L.) in response to wounding and in buffer extracts. Molecular events involved in the browning reaction are not fully understood. Mechanical wounding initiates tissue browning *in planta*. Phenolic compounds present in ash phloem tissue will react to aerial oxygen as well as to host PODs and PPOs, resulting in the synthesis of toxic quinones in ways that may be similar to host responses to larval feeding. We have initiated studies to identify and quantify quinones formed during the browning reaction in Manchurian and black ash (*Fraxinus nigra* Marsh.), a close phylogenetic relative of Manchurian ash that is very susceptible to EAB, and to determine how the reaction is affected by drought stress.

REFERENCES

Cipollini, D., Q. Wang, J.G.A. Whitehill, J.R. Powell, P. Bonello, and D.A. Herms. 2011. Distinguishing defense characteristics in the phloem of ash species resistant and susceptible to emerald ash borer. *J. Chem Ecol.* 37:450-459.

SURVIVAL ANALYSIS OF TWENTY TAXA OF *FRAXINUS* SPP. UNDER ATTACK BY EMERALD ASH BORER (EAB)

Sakthi K. Subburayalu[1] and T. Davis Sydnor[2]

[1]School of Environment and Natural Resources, The Ohio State University,
2021 Coffey Road, Columbus, OH 43210-1085
subburayalu.1@osu.edu

[2]USDA Forest Service (volunteer)

ABSTRACT

Ten replicates each in twenty taxa representing nine species and one interspecific cross in the genus *Fraxinus* were evaluated for time to the first positive strike by EAB. Trees were planted in 2004 and 2005 in a 30 m wide median strip in a Toledo, OH boulevard. Trees had two growing seasons to establish prior to the first EAB strike seen in 2007. Pressure from EAB was very high from 2006 to 2010 in Toledo, OH with most trees in the neighborhood collapsing in 2008. Trees were maintained by city crews and not irrigated. Trees were never treated to control EAB.

Kaplan Meier Survival Analysis was used to calculate the probable time of the first strike as a means of assessing resistance of the twenty taxa to EAB attack. Data was right censored meaning that trees dying or removed from something not related to EAB such as two plants stolen shortly after planting in 2004 were removed from the analysis.

White, green, and black ash selections showed signs of attack beginning in 2007. Two European ash species began to decline after 5 years while *F. excelsior* Golden Desert began to decline after six years. A single individual of Northern Treasure Ash (*F. manshurica x nigra*) suffered a strike after six years. Blue ash (*F. quadrangulata*) and Manchurian ash (*F. mandshurica*) have not been attacked to date by EAB. We feel that blue, Northern Treasure, and Manchurian ashes may well offer useful levels of resistance for landscape use especially when EAB pressure is moderate to low.

ASH MORTALITY IN OHIO FORESTS INFESTED BY EMERALD ASH BORER: SURVIVAL ANALYSIS SHOWS THAT AREAS WITH HIGHER ASH DENSITY DIE SLOWER

Kathleen S. Knight, John P. Brown, and Robert P. Long

[1]USDA Forest Service Northern Research Station
ksknight@fs.fed.us

ABSTRACT

We examined factors that affect mortality of ash trees in 31 forest stands infested by emerald ash borer (EAB) in Ohio, USA. Our data show that ash populations in forested sites can progress from healthy to almost complete mortality of mature trees within five to seven years. Survival analysis showed more rapid mortality in stands with lower densities of ash trees. This is probably due to both the biology of emerald ash borer and ash. EAB can efficiently locate ash trees even at low densities in mixed stands. We hypothesize that this leads to concentration of EAB on few trees in low ash density areas, which causes rapid decline of these trees. In high ash density areas, the EAB's are spread out among many trees, so each tree may have lower attack rates early in the infestation, allowing the trees to decline more slowly in these sites. This contradicts the generally accepted paradigm that greater host density increases relative pest abundance and host mortality. Therefore, the management strategy of reducing ash tree density is unlikely to protect the remaining trees from EAB and may actually hasten the mortality of those trees. However, reductions in ash density via diversification may be desirable for other silvicultural, conservation, and management objectives in preparation for EAB. Reductions in ash density may also be appropriate in combination with other management strategies, such as lethal trap trees, to slow the spread of EAB. Survival analysis also showed that mortality was more rapid for trees shaded by other trees and for trees initially exhibiting dieback, and also showed some differences among habitat or ash species. In management scenarios where hazard tree removal must be spread over several years due to budget constraints, focusing initial tree removal on stressed trees is recommended.

IT'S GOOD TO BE BLUE: EVIDENCE THAT *FRAXINUS QUADRANGULATA* MAY PERSIST IN NORTH AMERICAN FORESTS

S.R. Tanis[1] and D.G. McCullough[1, 2]

[1]Department of Forestry, Michigan State University, East Lansing, MI 48824
tanissar@msu.edu

[2]Department of Entomology, Michigan State University, East Lansing, MI 48824

ABSTRACT

Previous studies indicate that approximately 98% mortality of North American *Fraxinus* (ash) trees will be killed in stands where emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB) becomes established. . However, EAB host preference, or the ability of trees to resist colonization, varies among North American ash species. To date, the survival of *F. quadrangulata* (blue ash) in forest settings has not been investigated although it was recognized early on as a "less preferred" North American host species. In a 2004-05 study in southeast Michigan, Anulewicz et al. (2006) showed larval density and woodpecker activity was higher on white ash (*F. americana*) than blue ash in two woodlots where both species co-occurred.

We revisited these same woodlots in 2009 and 2010 to assess survival of *F. americana* and *F. quadrangulata*. In a second project, we evaluated feeding preference and survival of adult EAB and larval density of wild EAB on trees in a common garden at the Michigan State University's Tree Research Center.

Woodlot study: Twelve plots (50 X 50 m each) were established in a woodlot in Plymouth and a second woodlot in Superior Township. We thoroughly surveyed all living and blue ash trees (DBH > 2.5 cm) in spring 2010. Species, canopy class (dominant, intermediate and suppressed), DBH, canopy dieback and transparency, and GPS locations were recorded for all living trees. When trees were dead (standing or fallen), we recorded species, DBH, and GPS location. Variable radius plots were also used to determine the relative importance of *Fraxinus* trees within each woodlot.

Results showed *Fraxinus* spp. was 8% of the total overstory basal area in both woodlots. White ash trees were more vulnerable to EAB than blue ash trees in both sites. Blue ash was represented by trees in all canopy classes at both locations, but no dominant white ash trees survived in either site. A majority (82% at Superior Township, 60% at Plymouth) of blue ash basal area remains alive. In contrast, living white ash was completely absent from the Superior Township site and only 1% of white ash basal area remained alive at the Plymouth site (intermediate and suppressed trees only). These results indicate that dominant blue ash trees will likely persist as an overstory species. Whether remaining intermediate *F. americana* trees at Plymouth will reach the overstory remains unknown.

Common Garden Study: Feeding and mortality bioassays with adult EAB were conducted in summer 2010. Leaves collected from blue and white ash trees were transported in a cooler to the laboratory where a section of leaflets from each shoot was removed, weighed, scanned and placed in a petri dish. Newly emerged beetles (two males + two females) were caged with each leaf and allowed to feed for four days. Mortality was recorded on a daily basis. After four days, foliage was removed, weighed and re-scanned to determine area and weight of tissue consumed. Surviving beetles were allowed to feed for an additional 10 days. Beetles were provided with fresh foliage and mortality was recorded every other day. In October 2010, the blue and white ash trees that had been exposed to wild EAB populations for one year were harvested. Each tree was carefully debarked to expose EAB galleries in the phloem. Larval instar, adult exits, woodpecks, and parasitoid attacks were recorded for each tree.

Preliminary results indicate that adult EAB consumed a similar amount of blue and white ash leaf tissue during 4 day feeding preference bioassays. However, more adult EAB died when fed blue ash (58%) than white ash (33%) over 14 days. Larval density on debarked trees was higher on white ash (121.6 ± 3.06 galleries/m^2) than blue ash (10.8 ± 1.57 galleries/m^2). White ash trees were fully colonized after a single year of exposure. In contrast, even though blue ash trees were colonized, larvae density was relatively low.

Our results indicate that blue ash may persist in areas where EAB has become established and that it is less vulnerable to EAB infestation than other North American species tested to date. Our study is the first to document that a North American *Fraxinus* species is able to survive and persist in areas that have experienced the high density EAB populations that characterize the peak of the invasion wave.

LINGERING ASH POPULATION DYNAMICS IN MICHIGAN AND OHIO

Kathleen S. Knight,[1] Daniel A. Herms,[2] Reid Plumb,[3] Eileen Sawyer[3] Daniel Spalink,[3] Karen Menard,[3] Bernadette Wiggin[3] Rachel Kappler,[3] and Elizabeth Pisarczyk[3]

[1]USDA Forest Service Northern Research Station
ksknight@fs.fed.us

[2]Ohio State University, Ohio Agricultural and Research Development Center

[3]Metroparks of the Toledo Area

ABSTRACT

In some areas where EAB has caused almost complete mortality of mature ash trees, a small number of healthy ash trees intermingled with the dead ash trees have been discovered, sparking interest in these "lingering" ash trees. We conducted surveys in 2010 and 2011 of two populations of surviving ash trees in southeast Michigan and northwest Ohio, where the vast majority of ash trees had died by 2008. A 2010 survey of ash trees ≥10 cm d.b.h. (diameter at breast height, 1.4 m) along 10 km of floodplain forest in northwest Ohio found 2.6 percent of the ash trees were alive and 1 percent of the ash trees were healthy in an area where most of the ash trees had died by 2008. The canopy condition and EAB symptoms of these surviving trees were recorded. A repeated survey in 2011 found that most of the surviving trees that had healthy canopies in 2010 remained healthy in 2011, while trees with unhealthy canopies declined or died by 2011. In southeast Michigan, a population of living ash trees was discovered in 2010, at which time 39 trees were tagged and their canopy condition and EAB symptoms were recorded. When the trees were re-surveyed in 2011, the same pattern observed in northwest Ohio was evident: most of the trees that had healthy canopies in 2010 remained healthy in 2011, while trees with unhealthy canopies declined or had died by 2011. At both sites, some of the trees with healthy canopies show evidence of past EAB infestation, while others had no symptoms. Research is ongoing to determine whether these "lingering" ash trees express resistance or tolerance to EAB, or are simply the last to die.

REFERENCES

Knight, K.S.; Herms, D.A.; Plumb, R.; Sawyer, E.; Spalink, D.; Menard, K.; Wiggin, B.; Kappler, R.; Pisarczyk, E. 201_. Dynamics of surviving ash (Fraxinus spp.) populations in areas long infested by emerald ash borer (Agrilus planipennis). In: Proceedings of the disease and insect resistance in forest trees conference; 2011 August 1-5; Eugene, OR. *In press*

UPDATE ON BREEDING FOR THE DEVELOPMENT OF EAB-RESISTANT NORTH AMERICAN ASH

Jennifer L. Koch,[1] David W. Carey,[1] Kathleen S. Knight,[1] Therese Poland[2] Daniel A. Herms,[3] and Mary E. Mason[3]

[1]USDA Forest Service, Northern Research Station, Delaware, OH 43015
jkoch@fs.fed.us

[2]USDA Forest Service, Northern Research Station, East Lansing, MI 48823

[3]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691

ABSTRACT

We have initiated a breeding program that employs two strategies to incorporate EAB resistance into North American ash species: hybrid and traditional breeding. In the hybrid breeding approach, we are looking for EAB resistance in Asian species of ash from the EAB's region of origin (sympatric resistance). EAB-resistant Asian ashes will be crossed with native North American species to create hybrids. The hybrids will be subjected to subsequent rounds of testing, selection, and backcrossing with native species until only the resistance genes from the Asian species are carried into the native population while traits from the native species are retained, similar to the breeding program of the American Chestnut Foundation. For a more traditional breeding approach, we are searching for rare native individuals with resistance or tolerance to EAB (allopatric resistance).

To identify additional EAB-resistant species that may be used as parents in a hybrid-breeding program, we have been accessioning Asian ash species across a wide geographical and ecological range. Our current exotic ash collection includes 85 independent accessions, including 11 Asian species and 3 European species. To determine what Asian species may successfully produce F1 hybrids with North American species, we performed controlled cross-pollinations using as many combinations as possible (dependent on the availability of viable male and female flowers). In 2010, 42 different species combinations were crossed and 15 successfully produced seed. Five of the species combinations produced seedlings, with family sizes ranging from 11 to 104. Backcrosses were also performed between the commercially available F1 hybrid Northern Gem and both *F. mandshurica* and *F. nigra*, the parent species of the hybrid. These crosses have produced seed, and the seed is beginning to germinate. These new germinants represent the most advanced *Fraxinus* interspecies pedigree and demonstrate that F1 hybrids are reproductively competent, providing evidence that the hybrid/backcross breeding approach has potential to be successful in ash. All resulting F1 and backcross seedlings will be screened for EAB resistance.

We are also identifying surviving North American ash in natural stands where over 99 % of ash trees have been killed by EAB. These "lingering ash" that have a healthy crown, are in the dominant or co-dominant crown class (or open grown), and large enough to have been exposed to EAB during the main infestation are selected for propagation and further study. Currently we have identi-

fied and grafted 33 lingering ash trees (both green and white ash). We are testing the trees to confirm their EAB phenotype by conducting bioassays and establishing plantings of grafted ramets. For the bioassay testing, eggs were affixed to the bark of some of the lingering ash selections, and preliminary results from this experiment indicate that there are larvae being killed by host responses. Two of the grafted lingering ash genotypes flowered in 2010 and controlled cross-pollinations were performed, resulting in two small full-sibling families that can be analyzed for segregation of EAB tolerance/resistance phenotypes and will give an indication of the potential for enhancing native resistance through breeding.

CHANGES IN ASH FOLIAR CHEMISTRY IN RESPONSE TO EAB ADULT AND LARVAL FEEDING

Yigen Chen,[1,2] Therese Poland[3]
Justin Whitehill[4,5], and Pierluigi Bonello[4]

[1]Department of Entomology, Michigan State University, East Lansing, MI 48824
ygchen@msu.edu

[2]Current address: Department of Entomology, University of California, Davis, CA 95616

[3]USDA Forest Service, Northern Research Station, East Lansing, MI 48823

[4]Department of Plant Pathology, Ohio State University, Columbus, OH 43210

[5]Current address: Michael Smith Laboratories, University of British Columbia
Vancouver, BC Canada V6T 1Z4

ABSTRACT

The emerald ash borer (EAB; *Agrilus planipennis* Fairmaire; Coleoptera: Buprestidae), has killed tens of millions of ash trees (*Fraxinus* spp.) since its discovery in Michigan and Ontario in 2002 and it threatens the ash resource throughout North America. In an effort to understand ash responses that may influence susceptibility or resistance, we investigated the phytochemical responses of the three most common North American ash species (black, *F. nigra;* green, *F. pennsylvanica;* and white ash, *F. americana*) in northeastern USA to EAB adult foliar feeding. Two-year old ash seedlings (30-50 cm tall) of each species were grown in a greenhouse. Ten EAB adults were caged on a branch of 8 trees per species and allowed to feed for 48h on mature foliage (>4 weeks after flushing). Eight control trees of each species were similarly caged with no beetles. Branches were aerated and foliage was sampled for chemical analyses. Black ash was the least responsive to EAB adult feeding in terms of induction of volatile compounds and emission of only two (indole and benzyl cyanide) of the 11 compounds studied. In green ash, two [(*E*)-β-ocimene and indole] of the 11 volatile compounds studied were elevated while two green leaf volatiles [hexanal and (*E*)-2-hexenal] decreased. White ash showed the greatest response with an increase in 7 [(*Z,E*)-α-farnesene, α-humulene, β-caryophyllene, cubenene, (*E*)-β-ocimene, indole, benzyl cyanide] of the 11 compounds studied. Ligustroside was the only phenolic compound detected in all three ash species. Oleuropein aglycone and 2 unidentified compounds were found only in black ash, coumaroylquinic acid and feruloylquinic acid were detected only in green ash, and verbascoside hexoside was detected only in white ash. EAB adult feeding did not elicit or decrease concentrations of any selected individual phenolic compounds and levels of total phenolics were not affected in green or black ash. However, levels of total phenolics decreased significantly in white ash. EAB adult feeding elevated chymotrypsin inhibitors in black ash.

We also investigated foliar phytochemical responses of black ash to EAB larval feeding in the main stem. Two-year old black ash seedlings (50-70 cm tall) were grown in a greenhouse. Six EAB eggs with a small piece of bark excised from ash sticks where females had oviposited were pinned to the trunk of 36 trees. Small pieces of bark with no eggs were pinned to 36 control trees. Six trees per

treatment were aerated and leaves sampled every 10 days after egg hatch. (*E*)-β-Ocimene was the only volatile compound out of 11 consistently detected in foliage that was elevated by EAB larval feeding in the trunk. Emission of the green leaf volatile, (*Z*)-3-hexenyl acetate, decreased. Levels of carbohydrates and the defensive compounds, polyphenols, increased, while levels of proteins and most amino acids decreased. The decrease of proteins and amino acids was probably due to a combination of decreased synthesis and accelerated export of these compounds from foliage to a new nutrient sink below the EAB larval feeding site. An increase in carbohydrates and a decrease of proteins resulted in reduced foliar nutrient balance. The nutritional quality of foliage in trees fed upon by EAB larvae further deteriorated as noted by an elevation of polyphenols. Twenty three amino acids were consistently detected in the foliage of black ash. The three most abundant amino acids were aspartic acid, glutamic acid, glutamine, and the four least abundant were α-aminobutyric acid, β-aminoisobutyric acid, methionine, and sarcosine. Most (16) foliar free amino acids decreased in response to EAB larval feeding. Changes in ash foliar volatiles, nutritional compounds, and polyphenols in response to EAB adult and larval feeding may be associated with host tree defense responses.

Chemical and Microbial Control

EVALUATION OF NEONICOTINOID CLASS INSECTICIDES FOR CONTROL OF THE EMERALD ASH BORER

F. Miller

Department of Entomology, The Morton Arboretum, 4100 Lincoln Ave., Lisle, IL 60532
fmiller@jjc.edu

ABSTRACT

The emerald ash borer (*Agrilus planipennis*) continues to spread throughout the Midwestern and eastern United States and potentially threatens all North American ash (*Fraxinus* spp.) species. To date, chemical insecticides have been the primary pest management tactic for saving and preserving ash trees however, this tactic is not economically or environmentally sustainable over the long term. Other management tactics, such as biological control and host plant resistance have potential, but will take some time before they are adopted.

Beginning in 2007, a series of field studies were initiated to evaluate neonicotinoid class insecticides for use in protecting small (DBH<15 inches) and large (>15 inches) diameter class ash trees growing in typical urban forest settings where EAB had been recently detected.

The objectives of these studies were:

- To evaluate the efficacy of neonicotinoid class insecticides against the emerald ash borer at various dosage rates, application timing, and delivery methods for both small and large diameter class ash trees
- To compare the relative affordability, cost effectiveness, and utility of neonicotinoid class insecticides for control of the emerald ash borer

Results presented here will be for the 2007-2011 field seasons. Formulations evaluated consisted of various formulations and rates of neonicotinoid class insecticides applied via soil injection, micro-infusion and basal soil drenching. Visual dieback ratings and tree mortality are used to measure treatment efficacy. All study sites are located in the greater Chicago metropolitan area utilizing green ash (*F. pennslyvanica*) parkway trees.

Treatments of Imidacloprid + Clothianidin SC basal soil drench (BSD) at the 1X rate with two spring applications; Imidacloprid + Clothianidin SC BSD at the 2X rate with one spring application; Xytect 75WSP BSD at the 1.5X and 2X rates with one spring application each; Xytect 75 WSP 1X rate + Soil Penetrant BSD with one spring application; Merit 2F BSD at one spring application; and Merit 2F BSD with a spring and fall application; and Aracinate 2% infusible root flare injection (RFI) with one spring application) are all showing to be effective against moderate to heavy (<30% canopy dieback) EAB pressure.

Xytect 75WSP BSD at the 1X rate with one spring application); Xytect 5% Infusible RFI with one spring application; Lepitect 97.4% Systemic BSD with three monthly applications; Acelepryn 18.4% BSD with one spring application; and Imidacloprid + Clothianidin G at two spring applications are failing to provide effective control (31%-40% dieback) under moderate to heavy EAB pressure.

There appears to be a dose response effect for Xytect 75WSP with ash trees treated at the higher rates having less dieback (mean=6%) compared to lower rates and the untreated controls (mean=18%). Bayer Advanced products are also indicating a dose response based on the number of applications and application timing. Time will tell whether these products continue to provide protection of ash trees growing in areas with heavy EAB beetle pressure.

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

Shawn Bernick[1], Dr. Fredric Miller[2], Dr. Bal Rao[3], Grant Jones[4] and Karl Persons[5]

[1] Rainbow Treecare Scientific Advancements, Minnetonka, MN 55343
sbernick@treecarescience.com

[2] Morton Arboretum, 4100 Illinois Route 53 Lisle, IL 60532

[3] Davey Institute, Kent, OH, 1500 N. Mantua St. Kent, OH 44240
[4] Davey Institute, 396 Fenton Lane Suite 608 West Chicago, IL 60185

[5] Village of Hazel Crest, 3000 W. 170th Place Hazelcrest, IL 60429

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 safe for humans and the environment. Systemic neonictinoid insecticides are commonly applied by landscapers and tree care professionals to manage numerous insect pests on woody ornamentals. Insecticides containing the neonictinoid active ingredients imidacloprid and diontefuran are labeled for use on EAB and can be applied as either a 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.

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. 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 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 neonictinoid insecticides

- Determine the efficacy of fall vs. spring soil injection treatments
- Evaluate the performance of annual soil injection neonictinoid treatments with a TREE-age treatment.
- Evaluate the performance of soil injection treatments of imidacloprid when applied at the new maximum labeled dosage rate.

Table 1. Preventative EAB treatments applied to green ash trees in Hazel Crest, IL

Treatment	Dosage Rate	Applied Annually Since
Spring Xytect 75 WSP (a.i. imidacloprid)	1.42 g/in DBH	2008
Spring Transtect 70 WSP (a.i. dinotefuran)	3.40 g/in DBH	2008
Spring Xytect + Transtect	1.42 g + 3.4 g/in DBH	2008
Spring Xytect 2x	2.84 g/in DBH	2009
Fall Xytect	1.42 g/in DBH	2008
Fall Transtect	3.40 g/in DBH	2008
Fall Xytect + Transtect	1.42 g + 3.4 g/in DBH	2008
Fall Xytect 2x	2.84 g/in DBH	2009
TREE-äge (a.i. emamectin benzoate)	0.648 g/in DBH	2008*
Untreated Control		

* TREE-äge was applied in 2008 and will be reapplied in 2012

Treatments were applied to green ash (*Fraxinus pennsylvanica*) at two sites Hazel Crest, IL (Table 1). The Fountainbleau and Village Drive sites are approximately 1 mile apart. Mean canopy loss was used as an indicator of EAB pressure and canopy loss was significantly greater at the Fountainbleau site than the Village Drive site. Therefore treatment comparisons were made by site and not size class as originally intended. Over time we expect EAB pressure to increase at both sites, making treatment comparisons between size classes possible.

Canopy loss has increased each year at the Fountainbleau site. Treatments differed significantly in 2011 (Table 2). The Spring Xytect 2x treatment resulted in significantly less canopy loss than the untreated control. Other treatments were not significantly different from each other. Mean canopy loss was less in the spring treatments compared to the fall treatments; however, this was not always significant. Canopy loss at the Village Drive site continues to increase each year; however, at present there are no significant differences between treatments.

It is too early to make conclusions about treatment efficacy. The project will continue at least through 2013 and longer if necessary. We expect differences among treatments will become more apparent as EAB pressure increases.

Table 2. 2011 mean canopy loss of green ash (*Fraxinus pennsylvanica*) trees growing at the Fountainbleau site in Hazel Crest, IL

Treatment	Mean Canopy Loss %
Untreated Control	63.8 a
Fall Xytect + Transtect	50.7 ab
Fall Xytect	45.0 ab
Fall Transtect	41.0 ab
Spring Xytect	38.2 b
Spring Xytect + Transtect	38.7 bc
Spring Transtect	27.1 bc
Spring Xytect 2x	14.0 c

Columns with a different letter were significantly different at $\alpha = 0.05$, LSD

MULTIYEAR EVALUATION OF EFFICACY OF SOIL AND TRUNK APPLIED SYSTEMIC INSECTICIDES FOR ASH CANOPY CONSERVATION

Daniel A. Herms

Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691
herms.2@osu.edu

ABSTRACT

In 2006, studies were initiated 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 applied annually in spring or fall, and (2) to determine the number of years of control provided by different rates of a single emamectin benzoate (Tree-äge) trunk-injection.

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

The following imidacloprid soil drench treatments were applied from 2006-2011 to a green ash (*Fraxinus pennsylvanica*) street tree planting ranging in size from 11-21 in DBH (mean = 15 in):

(1) untreated control
(2) Merit 2F, 1.42 g ai / inch DBH applied in May
(3) Merit 2F, 1.42 g ai / inch DBH, applied in October
(4) Xytect WSP, 1.42 g ai / inch DBH, applied October
(5) Xytect WSP, 2.84 g ai / inch DBH, applied in May
(6) Xytect WSP, 2.84 g ai / inch DBH, applied in October

No trees showed visible symptoms of canopy decline in 2006 when treatments began. Untreated trees began to decline in 2008 and by 2010, all were dead. Trees treated in spring or fall with the high rate of Xytect, or in spring with Merit, maintained healthy canopies through 2011 with percent canopy thinning less than 20% in all cases. Trees treated in the fall with the low rate (1.42 g ai/ inch dbh) exhibited significantly greater canopy decline (averaging 40-60%).

These results suggest that when applied annual applications of imidacloprid soil drenches can effectively protect trees in the 14-20 inch DBH size class even under intense pest pressure, and at lower rates that spring applications are more effective than fall applications.

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. Pest pressure was intense at the study site and canopy decline of untreated trees averaged 53% and 96% in 2008 and 2009 respectively, and all untreated trees had died by 2010. All rates provided excellent control through two years (less than 10% canopy decline in 2008). However, in the third year, canopy decline increased significantly in all treatments, indicating that control was beginning to relax. In 2009, canopy decline averaged 25%, 55%, 60%, and 13% in the 0.1, 0.2, 0.4, and 0.8 g ai/in DBH treatments, respectfully. These results indicate that trees in the 20-25 inch DBH size class can be protected effectively for two years by a single application of emamectin benzoate. However, under intense pest pressure, treatment efficacy begins to decline in the third year.

SUCCESSFUL CONTROL OF AN EMERALD ASH BORER INFESTATION IN WEST VIRGINIA

Phillip A. Lewis[1] and Richard M. Turcotte[2]

[1]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542
Phillip.A.Lewis@aphis.usda.gov

[2]USDA Forest Service, Forest Health Protection, Morgantown, WV 26505

ABSTRACT

Project Background. Emerald ash borer (EAB) 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. An integrated 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) at varying dosages depending on tree diameter.

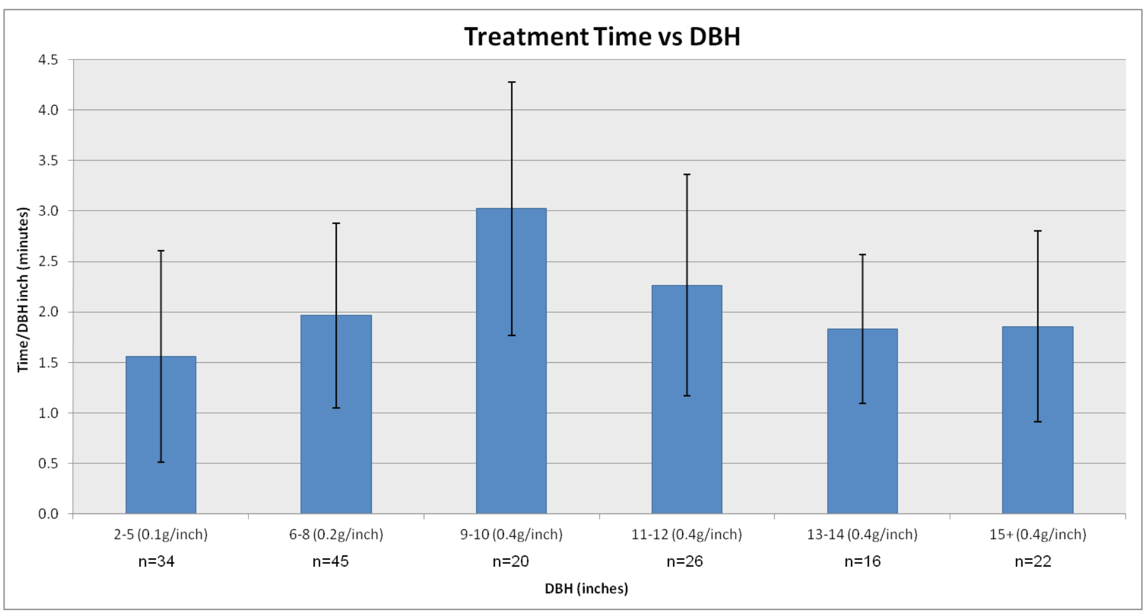


Figure 1. Average time to treat 163 ash trees, grouped by DBH class and treatment rate. The 0.1 g rate equals 2.5 mL of undiluted product.

Baseline monitoring. Foliar samples from a 31 of the treated trees (~20%) were collected and analyzed for pesticide residue from 2008 to 2010. The ELISA analysis being employed to determine emamectin content in leaf tissue was confirmed as accurate when compared with an 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 amount of residue found in the foliage ranged between 5 and 8 ppm. Figure 2 shows that average residue in 2009 was 4-5% of the previous year, dropping further to between 2 and 3% of the 2008 values by 2010.

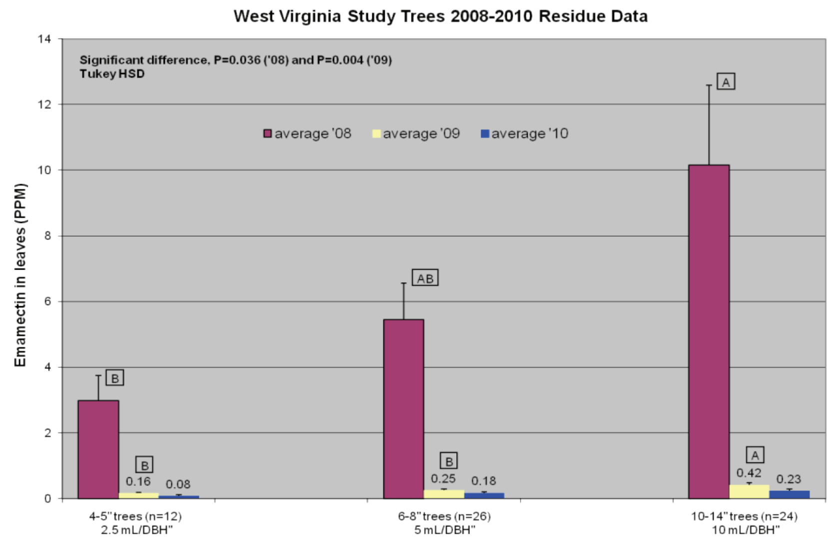


Figure 2. Pesticide residue data from foliage of treated trees.

Sample trees were also assessed for D-shaped exit holes, woodpecker feeding and general tree health by rating the tree canopy crown. This was conducted twice during each summer over the last four growing seasons. 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. The trends continued through the current year assessment, with no woodpecker attacks and no visible exit holes for 2011 (Figure 3).

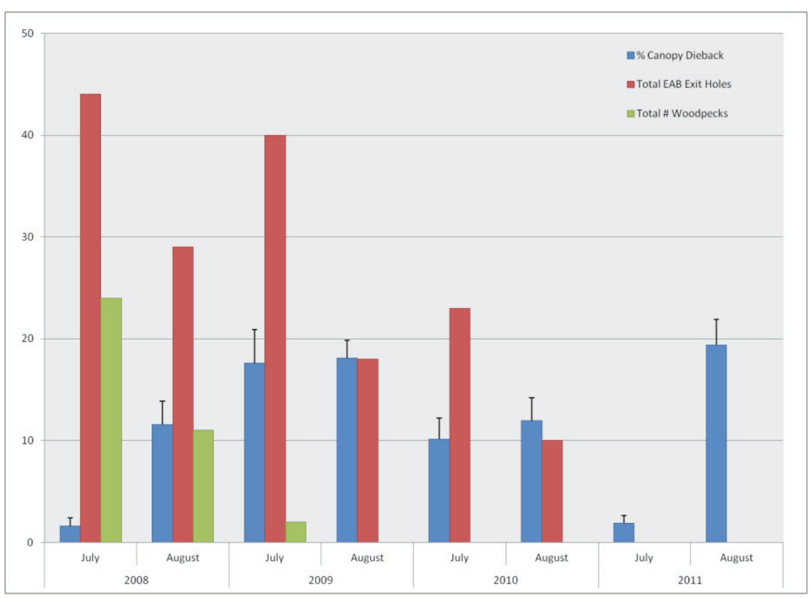


Figure 3. Tree health and infestation level ratings post application.

Trapping. A 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 first identified in the early spring of 2009 (red dots North of Ace, Fig. 4). All traps were EAB program panel traps green in color, initially baited only with Phoebe oil, but from 2009 onward the traps were also baited with Manuka oil. Ninety-four trapping sites were established in 2008 with an additional 111 sites in 2009. In 2010 a total of 274 traps were placed for an increase of 65 traps from the previous year. In 2011 only the Ace management area was trapped and 40 traps were set out to monitor EAB adults.

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. 4). In 2009 a total of 95 insects were captured on 27 traps, half of which captured only one insect each. Five of the traps had between 13-18 beetles (red triangles, Fig. 4). For 2010, there were 36 positive traps, many again had only single or small numbers of EAB, but 7 traps ranged between 12-42 beetles. For the limited trapping that was done this year within Ace, 12 of 40 traps caught 27 total beetles.

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 (inner red circle, Fig. 4). In 2010 there was one trap within Ace that caught 29 EAB adults, and this trap was located within the management area on the eastern edge. The ash tree the trap was placed in had not been identified prior to the 2008 treatments, but subsequently was used as a trap tree. It is interesting to note that while the trap tree was noted to be dead in 2010, within a few hundred meters of this tree several of the treated trees remain healthy and have good canopy cover. This information along with the 27 beetles caught on traps within the Ace management area demonstrates that strong insect pressure is present on the ash trees within this area.

Assessments. Twelve of the study trees were felled and examined in the fall of 2011 for a more complete assessment of treatment impact and EAB infestation levels. Four trees from each of the three application rates were selected, felled and debarked to characterize the EAB population within the treated trees. Four control trees from North of Ace that had obvious signs of EAB infestation were used as positive controls by which to compare.

A visual check for exit holes was made of entire trunk, which was then debarked up to 10 m, with alternate meter sections debarked beyond that. Visual checks were made on all branches > 2" in diameter for bark cracking and exit holes. For the control trees the infestation was so heavy that only a visual check of the trunk was performed for EAB exits and woodpecker attacks, along with a visual check on branches >2" in diameter. For the treated tress, 129 m^2 of log area were processed. No exit holes were found and only 7 woodpecks ($<0.1/m^2$). From all of the bark scraping, only 2 galleries (one a year old) were noted on one of the trees and no live EAB larvae were found. In contrast, control trees had 36 m^2 of log area processed and 392 EAB exit holes were identified ($11/m^2$) and there were 2,232 woodpecks ($62/m^2$).

The results of this study demonstrate that there are situations where EAB populations can be managed. An integrated approach of phloem reduction (removal of small diameter trees and felling and surface treatments of infested trees) and insecticide treatment can provide multi-year protection of the ash resource from EAB attack. Although the ultimate outcome of EAB on ash may not change, integrated approaches using currently available tools can provide land managers with options to remove and retain trees as desired. Thinning, timber stand improvement and crop tree techniques used in concert with an integrated management approach can be used to control the mortality trajectory of EAB, manage canopy disturbance and treefall.

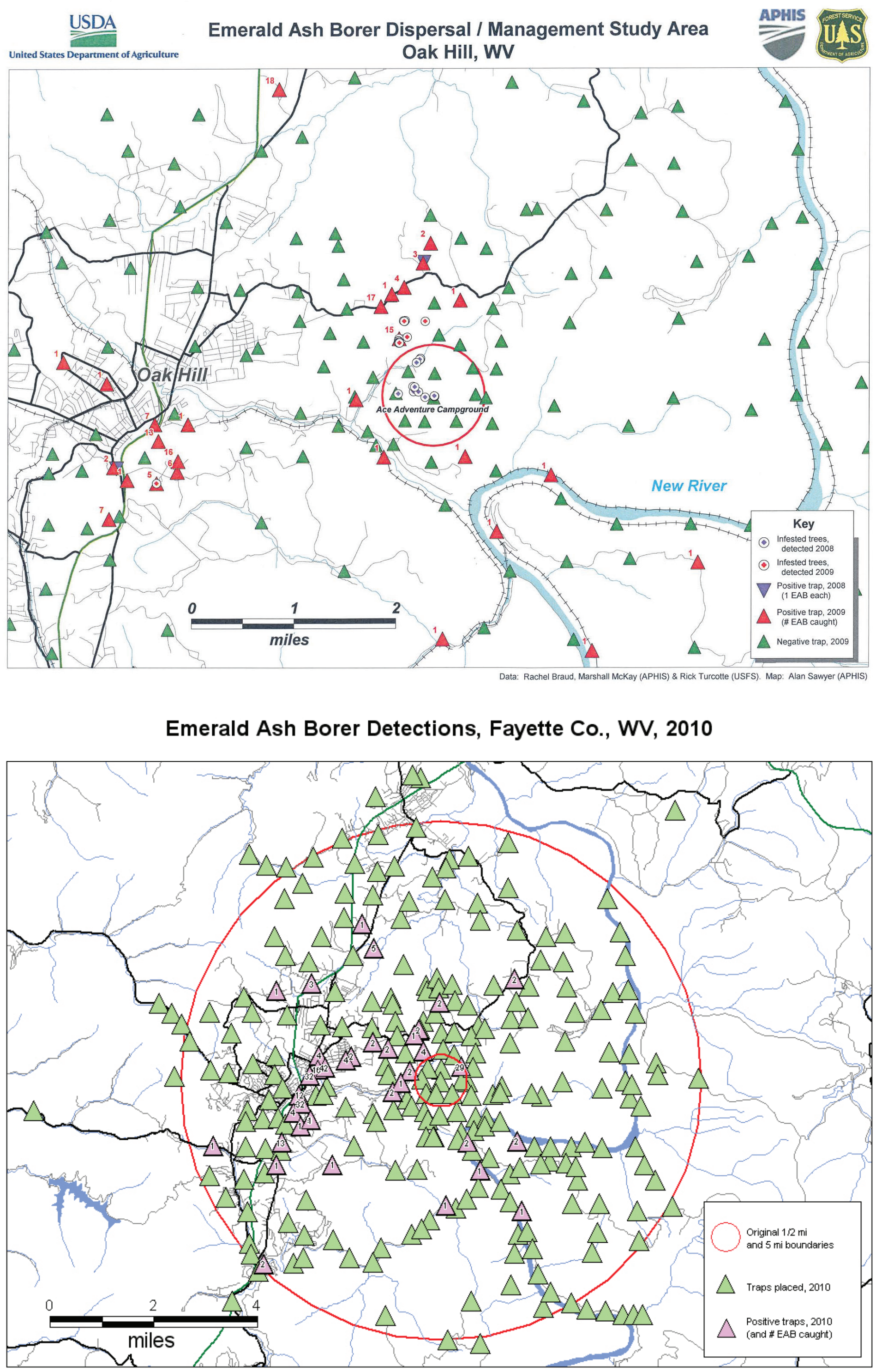


Figure 4. Trapping results and infested trees found near Ace Adventure, 2009-2010.

SLAM IN AN URBAN FOREST: CAN WE OPTIMIZE STRATEGIES FOR PROTECTION LANDSCAPE ASH TREES FROM EMERALD ASH BORER?

Deborah G. McCullough[1] and Rodrigo J. Mercader[2]

[1]Department of Entomology and Department of Forestry
Michigan State University, East Lansing, MI 48824
mccullo6@msu.edu

[2]Department of Biology, Washburn University, Topeka, KA 66621

ABSTRACT

Emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB) has already killed tens of millions of North American ash (*Fraxinus* spp.) trees growing in forests, riparian areas and urban areas. Loss of ecosystem services and the economic costs associated with widespread ash mortality in forested setting will likely be profound but are difficult to quantify. Economic costs resulting from mortality of landscape ash trees is a serious problem for many municipalities. Unlike lost ecosystem services, costs incurred for removal or insecticide treatment of landscape ash can be quantified. Recent projections of EAB distribution between 2009 and 2019 indicated that if approximately 45% of landscape trees were removed and replaced or treated at 2-year intervals with insecticide, discounted economic costs were likely to exceed $10.5 billion USD.

Management options are now available to reduce EAB population growth, which will slow the onset and progression of ash mortality in a given area. A pilot project was initiated in 2008 in northern Michigan to develop, implement and evaluate an integrated approach to EAB management. This project, called SLAM (SL.ow A.sh M.ortality) is underway in an area that is dominated by forestland, with some rural and urban areas. Scientists from MI State University, MI Tech University, the MI Dept. of Agriculture, the MI Dept. of Natural Resources, the USDA Forest Service and USDA APHIS are participating in SLAM. In the SLAM pilot project, systematic grids of girdled ash trees are used to intensively evaluate EAB distribution, density and development rate. Purple prism traps are used when ash trees are not available. Clusters of 2-4 girdled trees are also used as EAB "population sinks." These trees attract ovipositing females but are destroyed before larvae can develop. Selected ash trees are treated with emamectin benzoate, a systemic insecticide that provided nearly 100% EAB control for two years in major field studies. Timber sales have been used to reduce the overall availability of ash in the project area.

As part of the evaluation of the SLAM pilot project, a simulation model has been developed to compare the EAB distribution and ash condition with and without management. Major components of this model include functions for adult EAB emergence, adult dispersal, population growth and phloem consumption by larvae. The model was parameterized with empirical data from more than 25 field sites and spatially explicit information about EAB presence and ash abundance can be incorporated into the model.

Under the SLAM approach, the management tactics employed to reduce EAB population growth should be site specific. Tactics for suburban and residential areas are likely to be different than options available for use in forests. In urban areas, for example, girdling ash trees will rarely be acceptable. On the other hand, urban trees are highly accessible for systemic insecticide injections. Our simulation model provides a framework that allows the potential effectiveness of management options to be evaluated for different types of sites.

Using a modified version of the simulation model developed for the SLAM pilot project, we evaluated the effectiveness of using emamectin benzoate (sold as TREE-äge®), a systemic insecticide, to SL.ow A.sh M.ortality (SLAM) in a hypothetical residential area. We simulated local EAB spread and damage in neighborhoods consisting of 3200 lots, each of which could have a tree in the front and a tree in the back. Ash distribution was determined using Bernouli trials where the probability of an ash tree occupying each potential spot was 0.3. Tree diameter ranged from 6 to 30 inches. We assumed 400 adult EAB were introduced into the neighborhood. Each female could visit up to 10 trees and produce up to 20 offspring. If >60% of the phloem was consumed by EAB larvae, we assumed the tree would be severely declining and require removal. Trees that were treated with the emamectin benzoate insecticide remained toxic to EAB for 2 years. Average costs of removing and replacing the trees or treating trees with emamectin benzoate were acquired from municipal foresters in areas where EAB populations are present. We projected EAB distribution, spread, ash condition and costs over ten years.

In our simulations, if no trees were treated with insecticide, ash removals began in Year 3, accelerated in years 5-7 and by Year 10, all trees were gone. This pattern is very consistent with real-life scenarios many communities have experienced during peak years of the EAB invasion. If 20% of trees were treated annually with the insecticide beginning one year after introduction, 99% of the trees remained in Year 10. When 20% of trees were treated annually, targeting trees near the introduction was highly effective but when treatment began four years post- introduction, randomly selecting trees for treatment was most effective. When no insecticide was used, cumulative costs of removing and replacing trees over the 10-year time frame were $1.9 million USD. Costs of optimal scenarios were approximately $265,000 (if treatment began in Year 1) or $365,000 (if treatment began in Year 4).

Along with the economic benefits, treating 20% of trees annually protected nearly all ash in our neighborhood, retaining the values associated with large, mature landscape trees. Treatment costs are predictable and can be included in budgets and workplans, allowing municipalities to avoid the catastrophic tree mortality associated with peak EAB populations. Ash trees can be replaced gradually over time, while maintaining the overall health of the urban forest. There are also reasons to expect that using a systemic insecticide to slow EAB population growth will be compatible and may even enhance success of native and introduced biological control agents.

THE DEVELOPMENT OF AN AUTOCONTAMINATION/ AUTODISSEMINATION OF TRAP SYSTEM TO MANAGE POPULATIONS OF EMERALD ASH BORER WITH THE NATIVE ENTOMOPATHOGENIC FUNGUS, *BEAUVERIA BASSIANA*

D. Barry Lyons,[1,2] Robert Lavallée,[3] George Kyei-Poku[1]
Kees van Frankenhuyzen,[1] Johny Shajahan,[1] Joseph Francese[4]
Gene Jones,[1] Martine Blais,[3] and Claude Guertin[5]

[1]Natural Resources Canada, Canadian Forest Service, 1219 Queen Street East
Sault Ste. Marie, ON, Canada P6A 2E5
Barry.Lyons@NRCan-RNCan.gc.ca

[3]Natural Resources Canada, Canadian Forest Service, Sainte-Foy, QC, Canada

[1]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay
MA 02542, United States

[5]INRS-Institut Armand-Frappier, Laval, QC, Canada

ABSTRACT

Herein, we describe studies to develop an autocontamination / autodissemination trapping system to disseminate the fungal entomopathogen, *Beauveria bassiana* to control emerald ash borer (EAB) populations. In such a system, beetles contaminate themselves when passing through a non-lethal trap and subsequently spread the fungus to other beetles in the population. Laboratory contaminated beetles usually succumbed to the pathogen within 5-8 days after exposure. Two fungal isolates (INRS-CFL and L491AA), that were tested, were collected from the same ecozones as our field tests. The standard trap for detecting and delimiting EAB populations in Canada is a light-green prism trap covered in insect adhesive (lethal) and baited with (Z)-3-hexenol. In 2010, we demonstrated that EAB adults could be attracted to and captured in a green panel trap (non-lethal) baited with (Z)-3-hexenol and the adults could acquire a lethal dose of the fungus by passing through the trap. In 2011, we compared green Lindgren traps with the standard green prism trap as well as the green panel traps tested in 2010 and found the former to be significantly more efficient at collecting EAB adults especially when coated with the slippery substance, fluon. Field tests were conducted which involved luring the beetles to a baited green Lindgren funnel trap, to which a prototype autocontamination chamber was attached. The beetles acquired fungal conidia from a fungal culture in the chamber, exited the trap and were recaptured on pestick-coated traps. Low recaptures (~7%) of fungal contaminated beetles suggested that the selected fungal isolate used in the field (L491AA) was not growing well on the medium in the autocontamination chamber or had experienced detrimental storage conditions. Concurrent tests using the second fungal isolate (INRS-CFL) in field-exposed autocontamination traps and laboratory bioassays indicated that the latter

isolate was more virulent under these conditions. We also evaluated the longevity of the fungal cultures in traps under field conditions using culturing (conidial germination) and beetle bioassay (fungal virulence) techniques and determined that the fungal colonies survived at least two months throughout the flight period of the beetle. Using PCR techniques, DNA markers have been developed to differentiate our released fungal isolate from other natural *B. bassiana* isolates in the field.

Survey and Trapping

EMERALD ASH BORER ATTRACTION TO ARTIFICIAL TRAPS: INFLUENCE OF TRAP DESIGN AND COLOR AT DIFFERENT POPULATION DENSITIES

Therese M. Poland[1,2] and Deborah G. McCullough[2,3]

[1]USDA Forest Service, Northern Research Station, East Lansing, MI 48823
tpoland@fs.fed.us

[2]Department of Entomology, Michigan State University, East Lansing, MI 48824

[3]Department of Forestry, Michigan State University, East Lansing, MI 48824

ABSTRACT

Effective methods to detect, delimit and monitor low density populations of emerald ash borer (EAB, *Agrilus planipennis* Fairmaire) remain vitally important for operational programs. Various trap designs and lure formulations for EAB have been developed and evaluated in field trials; however, most field studies have been conducted in sites with moderate to high EAB densities. In such sites many ash trees exhibit canopy decline and dieback and emit stress-related volatiles, all of which may affect either the visual response of beetles to traps or the olfactory response of beetles to the lures under evaluation. We hypothesized that attractiveness of different trap types and colors may vary depending on EAB density and conditions within a site. Relative attractiveness of different trap designs in sites where EAB populations are low and ash trees are generally healthy is most relevant for operational programs.

To test our hypothesis, we evaluated two trap designs (canopy traps and double-decker traps) and two trap colors (purple and dark green) in 2011 at five sites selected to represent a range of EAB population levels. Canopy traps were the standard 3-sided prism traps, coated with clear Pestick® and suspended in the canopy of ash trees used by the USDA Animal and Plant Health Inspection Service (APHIS) EAB National Survey Program. Double-decker traps consisted of 2 prisms mounted on a 2.4 m tall, 10 cm diameter PVC pipe slid over a T-post placed in full sun, approximately 10-15 m away from ash trees with canopy traps. Both trap designs were tested with prisms made of the standard shade of purple used in the USDA APHIS EAB National Survey Program and a new shade of dark green (Francese et al. 2010). All traps were baited with two *cis*-3-hexenol bubble caps (7.4 mg/day, Contech Enterprises, Inc., Delta, B.C.) attached near the top and one Manuka oil pouch (50 mg/day, Synergy Semiochemical, Burnaby, B.C.) attached near the bottom. Twenty replicates of each trap type and color were installed, with 3 to 6 replicates per site.

Two of the field sites were classified as low EAB density with very little canopy dieback and few EAB symptoms, one site had a moderate EAB infestation level, and two sites had high populations as evidenced by ash dieback, EAB exit holes, woodpecker attacks and epicormic shoots. Traps were installed in mid May and beetles collected biweekly through August. Lures on all traps were replaced in mid July. Species identification was verified in the laboratory where beetles were counted and

sexed. A 3-way ANOVA was used to evaluate effects of site, trap color (purple versus green) and trap type (canopy versus double-decker) and significance of interactions on the number of EAB captured (log-transformed) using the GLM procedure in SAS. When ANOVA results were significant ($P < 0.05$), least square means multiple comparison tests were employed.

There was a significant interaction between site and trap type, indicating the relative effectiveness of trap types and colors varied depending on the EAB infestation level. Differences among traps were pronounced in sites with low to moderate EAB populations where the number of EAB captured per trap differed among trap types and between trap colors. Purple double-decker traps captured more beetles than green canopy traps. The number of EAB captured on green double-decker and purple canopy traps were intermediate. Trap design was not important in the two sites with high EAB densities, where all traps captured many EAB beetles. Traps for EAB detection would be unnecessary in these sites where symptoms of EAB were visibly evident. These results show the need to evaluate traps or other EAB detection tools in sites where EAB populations are at low densities.

REFERENCES

Francese, J. A., D. J. Crook, I. Fraser, D. R. Lance, A. J. Sawyer, and V. C. Mastro. 2010. Optimization of trap color for emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 103: 1235-1241.

EFFICACY OF MULTI-FUNNEL TRAPS FOR CAPTURING EMERALD ASH BORER: EFFECT OF COLOR, SIZE AND TRAP COATING

Joseph A. Francese[1], Michael L. Rietz[2], David R. Lance[1] Ivich Fraser[2], and Victor C. Mastro[1]

[1]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542
joe.francese@aphis.usda.gov

[2]USDA APHIS PPQ CPHST, Brighton Laboratory, Brighton, MI 48116

ABSTRACT

Tens of thousands of adhesive-coated purple prism traps are deployed annually in the United States to survey for the invasive emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). A re-usable, more user-friendly trap is desired by program managers, surveyors and researchers. Field assays were conducted in southeastern Michigan in 2009 and 2010 to ascertain the feasibility of using non-sticky traps as survey and detection tools for emerald ash borer. In subsequent color comparison assays, green and purple painted multi-funnel (Lindgren) traps (and later, plastic versions of these colors) performed as well or better than the prism traps. Multi-funnel traps coated with spray-on adhesive caught more beetles than untreated traps. The increased catch, however, occurred in the traps' collection cups, and not on the trap surface. In a separate assay, there was no significant difference detected between glue coated traps and Rain-X (normally a glass treatment) coated traps, but both caught significantly more *A. planipennis* adults than untreated traps (Francese et al. 2011).

The standard size multi-funnel trap we have used since 2009 is a 12-unit model. Miller and Crowe (2009) found that funnel trap length (number of funnels) played a role in capturing some species of bark- and woodboring beetles. We tested different sizes of traps (number of funnels) to multi-funnel trap size plays a role in capture of EAB. We tested green and purple versions of 4-unit, 8-unit, 12-unit and 16-unit traps. All traps were coated with Rain-X. There was no significant interaction between color and number of funnels per trap, so green and purple traps were combined for statistical analysis. Twelve-unit multi-funnel traps were more effective than other traps as they caught more beetles than the smaller traps, and were not as unwieldy as the 16-unit traps. Sex ratio was not affected by number of funnels per trap.

Flu-on has been shown to increase captures of cerambycids in baited intercept panel traps over Rain-X treated and control traps (Graham et al. 2010). While Flu-on is clear when applied to a surface, it dries white. Concerned that this could change the attractiveness of the trap to EAB, we added pigments to a Flu-on base to change the color of the liquid and the dried residue. The pigments had previously been used to create the green and purple funnel trap plastic color matches. The addition of the green pigment to Flu-on was unnecessary as non-tinted (white) flu-on applied to green plastic funnel traps produced the highest catch of all trap treatments. There was no significant dif-

ference among Rain-X and Flu-on treated purple multi-funnel traps and green-tinted Flu-on treated traps. Treating the traps was necessary as uncoated traps were out-caught by Flu-on and Rain-X coated traps, regardless of color.

REFERENCES

Francese, J.A., I. Fraser, D.R. Lance and V.C. Mastro. 2011. Efficacy of multifunnel traps for capturing emerald ash borer (Coleoptera: Buprestidae): effect of color, glue, and other trap coatings J. Econ. Entomol. 104(3): 1235-1241.

Graham, E. E., R. F. Mitchell, P. F. Reagel, J. D. Barbour, J. G. Millar and L. M. Hanks. 2010. Treating panel traps with a flouropolymer enhances their efficiency in capturing cerambycid beetles. J. Econ. Entomol. 103 (3): 641-647.

Miller, D.R. and C.M. Crowe. 2009. Length of multiple-funnel traps affects catches of some bark and wood boring beetles in a slash pine stand in northern Florida. Florida Entomol. 92 (3): 506-507.

ATTRACTION OF *AGRIULS PLANIPENNIS* FAIRMAIRE (COLEOPTER: BUPRESTIDAE) TO A VOLATILE PHEROMONE: EFFECTS OF RELEASE RATE, HOST VOLATILE AND TRAP PLACEMENT

Krista L. Ryall,[1] Peter, J. Silk,[2] Peter Mayo,[2] Damon Crook[3]
Allard A. Cossé,[4] Jon Sweeney,[2] and Taylor Scarr[5]

[1]Natural Resources Canada, Great Lakes Forestry Centre, 1219 Queen Street East
Sault Ste Marie, ON P6A 2G3 Canada. 506

[2]Natural Resources Canada, Atlantic Forestry Centre, 1350 Regent St
Fredericton, NB E3B 5P7 Canada
Peter.Silk@NRCan-RNCan.gc.ca

[3]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542

[4]United States Department of Agriculture, Agricultural Research Service, NCAUR
Peoria, IL 61604

[5]Ontario Ministry of Natural Resources, 70 Foster Drive, Suite 400
Sault Ste Marie, ON P6A 6V5 Canada

ABSTRACT

Attraction of emerald ash borer, *Agrilus planipennis* Fairmaire, to a volatile pheromone was explored through a number of field experiments using baited green sticky traps. A dose-response curve was generated for male *A. planipennis* to increasing release rates of (3*Z*)-lactone in combination with the green leaf volatile, (3*Z*)-hexenol. Only the lowest release rate (<150 µg/day) of (3*Z*)-lactone (Bartelt et al. 2007; Silk et al. 2011) significantly increased captures of male *A. planipennis*. Effect of trap height on the attraction of the (3*Z*)-lactone + (3*Z*)-hexenol combination was explored by placing traps within the ash canopy (where most mating is thought to occur) vs. well below the canopy. As predicted, the (3*Z*)-lactone + (3*Z*)-hexenol combination was only attractive when traps were hung high in the canopy. These data increase our understanding of the pheromone ecology of the invasive emerald ash borer, provide further confirmation of the behavioural activity of the female-produced lactone pheromone, and provide valuable information towards the development of a baited trap for early detection of *A. planipennis* infestations.

REFERENCES

Bartelt, R., A. A. Cossé, B.W. Zilkowski, and I. Fraser. 2007. Antennally active macrolide from the emerald ash borer Agrilus planipennis emitted predominantly by females. J. Chem. Ecol. 33:1299-1302.

Silk, PJ, Ryall K, Mayo P, Lemay M, Grant G, Crook D, Cossé A, Fraser I, Sweeney JD, Lyons DB, Pitt D, Scarr T, and Magee D (2011) Evidence for a volatile pheromone in Agrilus planipennis Fairmaire (Coleoptera: Buprestidae) that increases attraction to a host foliar volatile. Environ. Entomol, 40(4): 904-916.

TRAPPING OF EUROPEAN BUPRESTID SPECIES IN HUNGARIAN OAK FORESTS

Michael J. Domingue,[1] Gyuri Csoka,[2] Miklos Toth,[3] Zoltan Imrei,[3] Jonathan P. Lelito,[4] Victor Mastro,[5] and Thomas C. Baker[1]

[1]Chemical Ecology Laboratory, Pennsylvania State University
University Park, PA 16802
mjd29@psu.edu

[2]Hungarian Forest Research Institute
Matrafured Hungary

[3]Plant Protection Institute, Hungarian Acadamy of Sciences, Budapest, Hungary

[4]USDA APHIS, USDA-APHIS, EAB Rearing Facility, Brighton MI

[5]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542

ABSTRACT

We applied a variety of techniques developed for trapping *Agrilus planipennis* in an effort to capture other *Agrilus* species found in European Oak forests. These species included *Agrilus sulcicollis,* a recently detected exotic pest in North America (Haack et al., 2009), as well as *Agrilus biguttatus*, a more aggressive pest that is known to kill trees within its native range (Moraal and Hilszczanski, 2000). The behaviors that we attempted to evoke included the visual mating approaches first observed for *A. planipennis* (Lelito et al., 2007), but also observed for several *Agrilus s*pecies, including the oak feeding species of interest (Domingue et al., 2011). We baited traps with chemical formulations of tree-produced volatiles such as manuka oil and Z3-hexen-1-ol, which have been shown to be attractive to *A.planipennis* (Crook et al, 2008, de Groot et al., 2008). We also used fly lures consisting of Z9-tricosene, which is similar to *A.planipennis* cuticular hydrocarbons such as Z9-tricosane, which has been shown to be a contact pheromone.

Novel sticky traps were devised that rested over the leaves in the canopy. These traps provided parallel surfaces that slope slightly downward away from each other (Fig. 1). In one experiment exploring the optimum visual characteristics of such traps, they were deployed covered by one of the following materials 1) green plastic 2) purple plasitic 3) cut leaves 4) no covering (white cardboard surface). In each case the traps were either provided with *A.planipennis* visual decoy baits to evoke male visual mating approaches, or left blank. The traps were then covered with Tanglefoot. These traps were compared to simple preparations of Tanglefoot directly applied to leaves *in situ*, with or without beetle decoys. Another experiment was performed with odor dispensers including either 1) manuka oil, 2) Z-3-hexen-1-ol, 3) Z9-tricosene, or 4) no odor. Green or purple plastic-covered traps, with and without decoy beetles, were employed within 0.5m of each lure. Sticky leaves with and without visual decoys were also prepared within 0.5 m of each lure. For 17 days traps were checked at one to two day intervals, depending on the weather conditions. The different color and odor treatments were rotated within the design every three or four days.

In the first experiment examining the effects of the visual qualities of the trap surfaces, a total of 483 *Agrilus* beetles were caught in 12 traps. Three of these beetles (0.6%) were *A. biguttatus*, while 16 % were *A. sulcicollis*, and the remaining 83% have yet to be identified to species level. The green plastic-covered traps performed most efficiently with a mean (± S.E.) of 2.7 ± 0.7 total *Agrilus* captured per day without a visual decoy, and 4.2 ± 0.7 *Agrilus* per day with a visual decoy. All other trap designs were much less effective, with white or leaf covered traps capturing only 0.5 to 0.6 *Agrilus* per day regardless of the use of a visual decoy. Purple plastic-covered traps and sticky leaves with or without decoys caught only 0.1 to 0.2 beetles per day. The results were proportionately similar if only the *A. sulcicollis* captures were considered.

The second experiment, which involved odor lures, led to the capture of 1462 *Agrilus* beetles in 128 traps. Similarly only ten (0.7%) were *A. biguttatus*, while 13 % were *A. sulcicollis*, and the remaining 86% have yet to be identified to species level. Again the green plastic-covered traps were much more effective than the purple plastic-covered traps or the sticky leaf traps. Odorless green traps captured 1.9 ± 0.3 beetles per day without decoys or 2.5 ± 0.3 beetles per day with decoys. Greater captures in the green traps were achieved with any of the odor treatments, with more beetles per day caught when decoys were employed. (Z3-hexen-1-ol: 2.4 ± 0.3 without decoy, 2.9 ± 0.3 with decoy; Manuka oil: 3.0 ± 0.6 without decoy, 3.9 ± 0.8 with decoy; Z9-tricosene: 3.8 ± 0.7 without decoy, 4.1 ± 0.6 with decoy). Trap captures were always less than 0.5 beetles per day when purple plastic traps or sticky leaves were employed. The results were again proportionately similar if only the *A. sulcicollis* captures were considered.

Of the 13 *A. biguttatus* captures observed in both experiments, 12 were on green plastic traps (9), or sticky leaves (3), of which 11 had *A. planipennis* visual decoys. The other trap catching this species was a white trap with a visual decoy. Because equal numbers of decoy baited versus unbaited traps were deployed, the effect of the decoy on *A. biguttatus* capture is highly significant ($C^2 = 9.3$, d.f. = 1, $p = 0.002$).

This experiment has determined that our green plastic traps are an effective and inexpensive tool for detecting multiple *Agrilus* species in a forest setting. Both odor lures and visual decoys increase captures, and further research is needed to determine which blends of odors might provide optimal capture rates. The visual decoys were especially important for *A. biguttatus*, which was only detected once on a trap without a decoy. Further research envisions refinement of the physical characteristics of the traps, as well as calibration of the efficiency of these small economical traps with established detection tools such as prism traps.

REFERENCES

Crook D.J., Khrimian A., Fraser I., Francese J.A., Poland T.M. and Mastro V.C. 2008. Electrophysiological and behavioral responses of *Agrilus planipennis* (Coleoptera: Buprestidae) to host bark volatiles. *Environmental Entomolology* 37: 356–365.

de Groot P., Grant G.G., Poland T.M., Scharbach R., Buchan L., Nott R.W., Macdonald L. and Pitt D. 2008. Electrophysiological response and attraction of Emerald Ash Borer to green leafvolatiles (GLVs) emitted by host foliage. *Journal of Chemical Ecology* 34: 1170–1179.

Domingue, M.J., Csóka Gy., Tóth, M., Vétek, G., Pénzes, B., Mastro, V. and Baker, T.C. (2011). Field observations of visual attraction of three European oak buprestid beetles toward conspecific and heterospecific models. *Entomologia Experimentalis et Applicata* 140(2): 112–121.

Haack, R.A., Petrice, T.R. and Zablotny, J.E. 2009. First report of the European Oak Borer, *Agrilus sulcicollis* (Coleoptera: Buprestidae), in the United States. *Great Lakes Entomologist* 42: 1-7.

Lelito J.P., Fraser I., Mastro V.C., Tumlinson J.H., Boroczky K. and Baker T.C. 2007. Visually mediated "paratrooper copulations" in the mating behavior of *Agrilus planipennis* (Coleoptera: Buprestidae), a highly destructive invasive pest of North American ash trees. *Journal of Insect Behavior* 20: 537–552.

Moraal L.G. and Hilszczanski J. 2000. The oak buprestid beetle, *Agrilus biguttatus* (F.) (Coleoptera: Buprestidae), a recent factor in oak decline in Europe. *Journal of Pest Science* 73:134-138.

DEVELOPMENT OF A SAMPLING PLAN FOR DETECTION AND DELIMITATION OF *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA: BUPRESTIDAE) INFESTATIONS IN URBAN AREAS

Krista L. Ryall, Jeff Fidgen, Ron Fournier, and Jean Turgeon

Natural Resources Canada, Great Lakes Forestry Centre, 1219 Queen Street East
Sault Ste Marie, ON P6A 2E5 Canada
Krista.Ryall@NRCan-RNCan.gc.ca

ABSTRACT

Delimitation of infestations of *A. planipennis* is important for effective and timely management of this invasive pest. It was shown recently that sampling two 50 cm branches from the mid crown of asymptomatic ash trees was a highly reliable method to detect *A. planipennis*-infested trees (Ryall et al. 2011). We used this method to delimit the geographic extent of the infestation in Sault Ste Marie, Ontario, Canada. We tested the usefulness of using a 1 km triangular grid-based survey and varying intensity of sampling per survey cell (numbers of tree sampled). As predicted, the number of trees sampled in each hexagonal cell of the grid affected the outcome of this survey: only 8 cells were classified as positive for *A. planipennis* when 5 trees were sampled, compared to 20 cells when 15 trees were sampled. Preliminary results suggest that in the epicentre of the infestation, a minimum of 30-40% of the trees were infested with an average gallery density of 16 galleries/m^2. Prior to data collection for this research, however, a number of heavily-infested trees had been removed; thus, values in the epicentre underestimate actual percentage of trees infested and gallery density. In the surrounding peripheral cells, all trees were asymptomatic, and only 10% of trees were infested with an average density of less than 2 galleries/m^2. Given the low percentage of trees infested and low gallery density, it was typically necessary to sample 10-15 trees in these peripheral plots in order to classify them as infested. Preliminary results from kernel density estimation mapping suggest plots could have been spaced 2-km apart, and would have detected roughly the same area of infestation, but with considerably less sampling effort. This information is essential to establish optimal grid spacing and tree-sample size requirements for given levels of precision in detectability. These requirements and precision levels will prove useful in the development of detection and delimitation survey programmes for urban landscapes. The accuracy and usefulness of this approach for sampling *A. planipennis* will be validated with similar data sets from other urban centres.

CERCERIS IDENTIFICATION AND AWARENESS (C.I.A. FOR EAB)

F. Miller and D. Krafka

Department of Entomology, The Morton Arboretum, 4100 Lincoln Ave., Lisle, IL
fmiller@jjc.edu

ABSTRACT

Since its accidental introduction into the United States in 2002, the emerald ash borer(EAB), *Agrilus planipennis* has established itself as a destructive pest to shade trees and forest species of North American ash (*Fraxinus spp.*). Presently in the Eastern United States there is only one native hunting wasp, *Cerceris fumipennis*. *Cerceris* is a solitary ground nesting wasp that uses buprestids to feed their young. Although solitary, they build their nests in close proximity to others forming neighborhoods. Their nest entrances can easily be identified as glorified ant holes with small circular mounds of earth over the pencil width in size holes. They can be found in compact sandy soil such as baseball diamonds, volleyball courts, gravel parking lots and campground fire pits. *Cerceris* is easily recognized for its wings and body. It is large in size comparable to common yellow jacket wasps. It has dark, smoky, blue/black wings. Their bodies are mainly black with one creamy yellow band on its abdomen. To find and monitor *Cerceris*' activity throughout Illinois and its relation to EAB, a program was created called *Cerceris* Identification and Awareness for the Emerald Ash Borer (C.I.A. for EAB). C.I.A. for EAB is a two part biosurveillance project, in which we monitor the intake of buprestids. The first part of the project includes confirming *Cerceris* nest sites and the second is a citizen science portion. C.I.A. is an important monitoring tool because *Cerceris* can detect EAB much faster than the human eye aiding the early detection efforts and it is a great way to observe and learn behavioral patterns of EAB.

Beginning in 2010, a series of field surveys were launched to seek and confirm *Cerceris* nest sites throughout Illinois.

The objectives of these surveys were to:

- locate, identify and confirm *Cerceris* nest sites.
- monitor the intake and variety of buprestids.
- study the spread of EAB through Illinois.
- launch the citizen science project.

Baseball fields were selected from the following criteria: within/outside of the EAB quarantine zone, near a river, accessibility and use. In the summers of 2010 and 2011, each field was visited and evaluated for the presence of *Cerceris*. If confirmed a weekly sampling was conducted by placing clear plastic cups over entrance holes and netting encumber females. Samplings lasted 30 minutes and each beetle was placed in a clear plastic vial with method of collection, date and location labels. Beetles will be identified at a later date.

To date, 19 sites, spanning 7 counties, have been identified in Illinois and the beetles are currently being identified.

In the future, there will be a continuing effort to identify *Cerceris* colonies and large launch of the citizen science portion, where volunteers will monitor and collect beetles. This project is scheduled to begin the summer of 2012.

Biology, Behavior and Ecology

IDENTIFICATION OF ODOR-PROCESSING GENES IN EMERALD ASH BORER

Praveen Mamidala,[1] Asela Wijeratne,[2] Saranga Wijeratne,[2] Therese Poland [3], and Omprakash Mittapalli[1]

[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691
mamidala.2@osu.edu

[2]Molecular and Cellular Imaging Center, The Ohio State University, Ohio Agricultural and Research Development Center, 1680 Madison Ave., Wooster, OH 44691

[3]USDA Forest Service, Northern Research Station, Michigan State University
East Lansing, MI 48823

ABSTRACT

Olfaction is the primary sensory perception in insects which guides them to locate food (host cues), conspecifics (mating), suitable oviposition sites (access to nutritious and digestible food for off-springs), predators and toxic compounds. *Agrilus planipennis* Fairmaire (Emerald Ash Borer, EAB) a serious invasive insect pest has killed billions of North American ash (*Fraxinus* sps) trees and threatens its very existence. To date, little to no molecular knowledge exists on *A. planipennis* olfaction process, which is crucial for its survival and adaptation. Hence, we undertook antennal-specific transcriptomic study to identify repertoire of odor processing genes involved in *A. planipennis* olfaction.The 454 pyrosequencing of *A. planipennis* antenna resulted in significant number of transcripts (139,085) of reads which were assembled into 30,615 expressed sequence tags (ESTs). A suite of odor processing genes (odor binding proteins, odorant receptors, sensory neuron membrane proteins and odorant degrading enzymes) were identified. The quantitative real-time PCR analysis of candidate genes revealed development- and sex-specific expression patterns. The developed antennal ESTs are rich molecular sources for decoding olfaction process in *A. planipennis* involved in detecting host- and mate-cues. This study could potentially form the basis for developing pest management practices (chemical lure traps), for efficient control of *A. planipennis*.

CHARACTERIZING ADULT EMERGENCE PATTERNS OF EMERALD ASH BORER ACROSS A LATITUDINAL GRADIENT

**Samuel A. Discua,[1] Robin A.J. Taylor,[1] William Ripley[2]
Kathleen S. Knight,[3] Amy K. Stone,[4] Elly Maxwell,[5] and Daniel A. Herms[1]**

[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691
discua.1@osu.edu

[2]Architectural Landscape Design, Cincinnati, OH 45244

[3]USDA Forest Service Northern Research Station, Delaware, OH 43015

[4]The Ohio State University Extension, Education, Outreach, and Communications Toledo, OH 43615

[5]The Dow Gardens, Midland, MI 48640

ABSTRACT

Timely forecasting the start and end of emerald ash borer (EAB) adult emergence is required to inform regulatory decisions and control practices. Brown-Rytlewsky (2004) determined the cumulative number of growing degree-days (GDD) required for first EAB emergence in southeastern Michigan. However, full emergence phenology period, and especially the end of emergence, has not been adequately characterized, nor has EAB phenology been characterized in other regions.

Phenological models are an important tool for predicting pest activity and timing pesticide applications. Akers and Nielsen (1984) developed degree-day models to predict adult emergence of the native bupestrid bronze birch borer at three locations in Ohio, concluding that a different model was most accurate at each location. The objectives of this study were (1) to characterize adult emergence patterns of EAB across latitudinal gradient; and (2) to use adult emergence and temperature data to generate degree-day models for predicting key phenological events, including first, 10%, 50%, 75%, and 99% adult emergence.

In 2011, we monitored EAB emergence at five locations across a north to south latitudinal gradient extending from Midland, MI at the north extreme progressing south to Toledo, Wooster, Delaware, and Cincinnati, OH. Infested ash trees felled at each location were cut into bolts ca 2m in length. Bolts were held in shade under ambient conditions, and EAB adult emergence was monitored by marking all preexisting exit holes, and then counting new emergence holes weekly from first adult emergence until three consecutive weeks of no new emergence.

As expected, emergence commenced earliest in Cincinnati, OH and latest in Midland, MI. Adult emergence period was larger in Cincinnati, OH (12 weeks) than Midland, MI (7 weeks). These results provide evidence of the effect of latitude and different climatic conditions on EAB adult phenology. Start of emergence in Delaware, Wooster, and Toledo, OH, was only 2 days apart, but ended a week later in Toledo, OH. We will repeat this experiment in 2012 adding Lexington, KY as a study site to further extend the gradient to the south. Adult emergence and average daily temperature data will be used to generate a GDD models for predicting EAB adult emergence across much of its current north-south distribution.

REFERENCES

Akers R.C., and D.G. Nielsen. 1984. Predicting *Agrilus anxius* Gory (Coleoptera: Buprestidae) adult emergence by heat unit accumulation. *Journal of Economic Entomology.* 77: 1459-63.

Brown-Rytlewski, D.E., and M.A. Wilson. 2005. Tracking the emergence of emerald ash borer adults. In V. Mastro and R. Reardon, compilers: *Proceedings of 2004 Emerald Ash Borer Research and Technology Development Meeting,* p. 13-14. USDA Forest Service Forest Health Technology Enterprise Team FHTET-2004-15, 83 pp.

MOVEMENT AND IMPACT OF EAB IN MARYLAND

Holly Martinson,[1] Chris Sargent,[1] Dick Bean[2]
Alan Sawyer,[3] and Michael Raupp[1]

[1]Department of Entomology, University of Maryland, College Park, MD 20742
hmartins@umd.edu

[2]Maryland Department of Agriculture, 50 Harry S. Truman Parkway, Annapolis, MD 21401

[3]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542

ABSTRACT

The emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) was introduced to Maryland in 2003 when infested nursery stock was received by a nursery in southern Prince Georges County. The Maryland Department of Agriculture has collected data since that time on positive detections of EAB from naturally-occurring and landscape ash trees. From 2003 to 2008, EAB spread approximately 1 km per year (Sargent et al. 2010).

The objectives of the current study were to 1) test whether the linear rate of spread was a good predictor of EAB occurrence in subsequent years (2009 – 2011), 2) identify the potential economic impact of EAB to select municipalities in the region, and 3) highlight management options for municipal forest managers.

The linear rate of spread model consistently under-predicted the leading edge dispersal of EAB from 2009-2011. Through the use of polynomial regression, we found evidence for an increasing rate of spread starting in 2010. Preliminary analysis of 2011 trap data suggests an even faster rate of spread than was detectable based on the 2010 data. Random street tree surveys using iTree Streets (http://www.itreetools.org/streets/index.php) were conducted to determine ash tree inventories in several municipalities surrounding the original quarantine zone. Using the polynomial rate of spread model, the year of arrival of EAB can be estimated and municipal arborists can then use tools such as iTree Streets and the EAB Cost Calculator (Sadof 2009; http://extension.entm.purdue.edu/treecomputer/) to develop timely and effective management programs to lessen both the economic and environmental impact of EAB infestation.

REFERENCES

Sadof, C.S. 2009. The emerald ash borer cost calculator. <http://extension.entm.purdue.edu/treecomputer/index.php> (Accessed 12/21/2010).

Sargent, C.; Raupp, M.; Bean, D.; Sawyer, A.J. 2010. Dispersal of emerald ash borer within an intensively managed quarantine zone. Arboriculture and Urban Forestry. 36(4): 160-163.

THE RISE AND FALL OF EMERALD ASH BORER POPULATIONS AND THE ASH OVERSTORY AT THREE STAGES OF THE INVASION WAVE

Stephen J. Burr[1] and Deborah G. McCullough[1, 2]

[1]Department of Entomology, Michigan State University, East Lansing, MI 48824
burrstep@msu.edu

[2]Department of Forestry, Michigan State University, East Lansing, MI 48824

ABSTRACT

Emerald ash borer (EAB), *(Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) is a phloem-feeding beetle native to Asia. First discovered in Detroit, Michigan and Windsor, Ontario in 2002, dendrochronological studies indicated EAB first became established in North America at least six to ten years earlier in the metro Detroit area. Since its arrival, EAB has attacked native ash (*Fraxinus spp.)* trees in forest, rural and urban settings. As ash resources are depleted, beetle populations disperse and colonize new areas. The resulting infestation wave appears to expand in all directions.

Questions consistently arise as to the status of EAB populations and the persistence of ash in forested settings, particularly in the original core of the EAB infestation in southeast Michigan. We sampled EAB populations and canopy ash in 24 green ash (*F. pennsylvanica*) sites, each 1 ha in size in 2010 and 2011. Eight sites were located in each of three areas of southern Michigan representing (1) the original EAB Core in the southeast; (2) the Crest where EAB populations are currently peaking and (3) the Cusp of the invasion in southwest Michigan where EAB has recently become established.

Adult beetles were captured using a variety of trapping techniques. In each field site, we placed two purple double-decker panel traps coated in Pestick and baited with cis-3-hexenol and an 80:20 mix of manuka and phoebe oil in 2010 and manuka oil only in 2011. Three uninfested, green ash nursery trees, (2-3 cm diam), were planted in each site. A sticky band consisting of 30 cm wide band of plastic wrap coated in Tanglefoot was wrapped around each planted tree. Two naturally occurring ash trees in each site were girdled (1 m high; 15 cm wide). Girdling elicits a chemical stress response that attracts EAB. Girdled trees had a sticky band placed immediately above girdles. Two untreated control trees growing on site were also chosen and a sticky band was applied using the same methods. Beetle captures were standardized per m^2 of trap area. Larval density was determined by cutting down trap trees, removing sections of bark and estimating larva per m^2 of exposed phloem.

Canopy ash trees (DBH $\geq$ 10 cm) were counted along two belt-transects run diagonally across sites in an X-formation. Sites were also divided into 4 quadrats. Overstory ash (live and dead) were counted and DBH measured in one macro-plot per quadrat. Dead trees were assumed to be killed by EAB if external symptoms such as woodpecks, exit holes, epicormic shoots and stump sprouts were present. Canopy dieback (EAB-related) of ash was assessed in the belt transects and macro-plots.

We found EAB populations continue to persist in Core sites but densities were low in both 2010 and 2011. Captures of adult EAB and larval density were highest in the Crest sites in central

Michigan. Populations of EAB are building in Cusp sites, where adult captures nearly doubled from 2010 to 2011. As expected, overstory ash mortality is highest in the Core, lowest in the Cusp and increasing in Crest sites where EAB is at peak densities. Live ash basal area is highest in Cusp sites and lowest in the Core. Dead ash basal area is highest in the Core but increased 7-fold in Cusp sites from 2010 to 2011. While overstory ash persists in some of the Core sites, canopy condition of most ash was generally poor. In the Crest, ash continues to decline and dieback increased from 2010 to 2011. In Cusp sites, trees showed little to no signs of infestation in 2010 but dieback is increasing as the EAB populations build.

ARTHROPOD ASSOCIATES OF THE EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) IN NORTHERN ILLINOIS

E. Mitchener[1] and F. Miller[2]

[1]Illinois Department of Agriculture, Springfield, IL

[2]Research Associate-Entomology, The Morton Arboretum, 4100 Lincoln Ave., Lisle, IL

ABSTRACT

The emerald ash borer (EAB) is a phloem feeding wood boring insect that kills its host by destroying the vascular system of the tree. As with any opportunistic organism, competition for limited resources is a major factor population dynamics. One area of EAB biology that needs further investigation is to identify and quantify the impact of other Arthropod species associated with EAB tree colonization and what impact, if any, these species have on the phloem resource within a given ash tree.

Beginning in the winter of 2007, a study was initiated to address the above questions with the following objectives to:

- identify what arthropods are commonly found in healthy ash trees.
- identify the arthropod complex ("EAB associates") associated with EAB infested trees.
- determine what impact, if any, competition from EAB associates might have on the available phloem resource and development of EAB life stages.

Bolts from healthy and EAB infested trees were collected from 18 central and northern Illinois counties during 2007-2011. The bolts were placed in rearing containers and held under ambient conditions. All Arthropods were allowed to emerge and then were placed in glass vials with 70% alcohol for future identification. At the end of the rearing season, the bolts were peeled and the relative percentage of EAB and non-EAB galleries were visually estimated (nearest 5%) for each bolt. Available phloem surface area was estimated by measuring the diameter of each end of the bolt and the length of the bolt.

In 2008, the eastern ash borer (EABB) consumed just over half (53%%) of available phloem in non-EAB infested ash trees. A reversal occurred for 2009 and 2010. Ash trees suspected and/or known to be infested with EAB experienced little if any colonization by EABB with 43% of available phloem consumed by EAB larvae. These preliminary results suggest that trees not infested with EAB are primarily colonized by the eastern ash bark beetle (EABB) (*Hylesinus varius*) along with a few ash clearwing borers (*Podosesia sygringae*) and members of the Buprestidae and Cerambycidae beetle families. In contrast, EAB infested trees have minimal gallery formation by EABB. Based on the results presented here, it appears that there is very little direct competition between EAB and EABB for the available phloem resource.

The red-headed ash borer was present in all sampling years, but not in large numbers and is not considered a major phloem competitor. It is found associated with dead wood (firewood) and does not typically infest living phloem tissue.

The only significant predator collected was a very small number (<1% of total insects collected) of clerid beetles (Cleridae). This family of beetles includes common predators of bark beetles.

Hymenopteran belonging to the Eurytomidae, Ichneumonidae, and Pteromalidae made of 28%, 29%, and 31% of all insects reared from ash tree logs, respectively. The remaining 12% of insects collected included members of the Braconidae, Eupelmidae, and Torymidae Hymenopteran families. All of the aforementioned Hymenopteran families are known to contain parasitoids of wood-boring insects. Positive identification is in progress to determine if any or all of these insects might be potential EAB biological control agents.

DIRECT AND INDIRECT ECOLOGICAL IMPACTS OF EMERALD ASH BORER AND ASH MORTALITY

Daniel A. Herms,[1] John Cardina,[2] Kathleen S. Knight[3]
Kamal J.K. Gandhi, [1,4] Wendy Klooster,[2] Kevin Rice,[1] Catherine P. Herms[2]
Diane Hartzler,[1] Annemarie Smith,[1,5] and Deborah G. McCullough[6]

[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691
herms.2@osu.edu

[2]Department of Horticulture and Crop Science, The Ohio State University
Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691

[3]USDA Forest Service, Northern Research Station, Delaware, OH 43015

[4]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602

[5]Ohio Department of Natural Resources, Division of Forestry
2045 Morse Road, Building H-1, Columbus, OH 43229

[6]Departments of Entomology and Forestry, Michigan State University, East Lansing, MI 48824

ABSTRACT

We have monitored patterns and subsequent effects of ash (*Fraxinus* spp.) mortality due to emerald ash borer (EAB, *Agrilus planipennis*) in 38 forest stands in the Upper Huron River watershed of southeastern Michigan since 2004. Black (*F. nigra*), green (*F. pennsylvanica*), and white (*F. americana*) ash were the most common ash species in hydric, mesic, and xeric stands, respectively. As of 2010, ash mortality of trees with trunk diameters greater than 2.5 cm at breast height was 99.7% across all plots.

Ash were the most common woody species in the seedling and sapling layers of these stands, 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 saplings are killed as they become large enough to be colonized? We measured densities of four demographic classes of ash: newly germinated seedlings (cotyledons present), established seedlings (at least one-year-old but less than 30 cm tall), saplings (30 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 suggest that the ash seed bank in these stands was rapidly depleted as over story trees died. Small numbers of seeds were found in 2005 and 2006; however, no ash seeds were found in 2007 or 2008. 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 2008 and 2009, respectively. No new ash seedlings were observed in 2010 or 2011 inside or outside the monitoring plots.

Established seedlings and saplings are now the only demographic classes of ash living in these stands. However, purple panel traps indicate that EAB adults continue to persist at low levels, 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 be locally extirpated as this orphaned cohort of juvenile ash is gradually depleted via EAB mortality. Alternatively, a dynamic equilibrium may establish in which some trees reach reproductive maturity before succumbing to EAB, especially if native and introduced biological control agents prove capable of regulating EAB at very low densities.

Nearly simultaneous mortality of ash has resulted in wide spread formation of canopy gaps and a dramatic increase in accumulation of coarse woody debris on the forest floor. Studies are finding that these ecological impacts of the emerald ash borer invasion are having pervasive direct and indirect effects on successional trajectories, establishment and spread of invasive plants, soil invertebrate communities, and phytophagous arthropods.

POTENTIAL REPLACEMENTS FOR NORTHWOODS BLACK ASH IN A CHANGING CLIMATE: THE CONFLUENCE OF TWO CHALLENGES

Louis Iverson,[1] Anantha Prasad,[1] Kathleen S. Knight[1]
Daniel A. Herms,[2] Stephen Matthews,[1,3] Matthew Peters[1]
Annemarie Smith,[4] and Robert Long[5]

[1]Northern Research Station, USDA Forest Service, Delaware, Ohio 43015
liverson@fs.fed.us

[2]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison, Ave., Wooster, OH 44691

[3]School of Natural Resources, The Ohio State University, Columbus, OH 43210

[4]Division of Forestry, 2045 Morse Road, Building H-1, Columbus, OH 43229-6693

[5]Northern Research Station, USDA Forest Service, Delaware, Ohio 43015

ABSTRACT

Introduction By most indications, the fate of all North American native and non-urban ash (*Fraxinus* spp.), in the wake of emerald ash borer (EAB), is bleak. This study is concerned with creating a better understanding of the potential replacements of black ash (*Fraxinus niger*) in native stands in the Northwoods (northern Minnesota, Wisconsin, and Michigan) as the ash dies out.

Research Approach We evaluated the current distribution and abundance of black ash and its co-occurring species in the eastern United States, according to US Forest Service Forest Inventory Data. With this information, along with 38 environmental variables, we create suitable habitat models for the species using the RandomForest statistical modeling tools (see http://www.nrs.fs.fed.us/atlas). We then model the suitable habitat for each species under various scenarios of climate change for ~2040, 2070, and 2100. The model outputs are intended to give some indication of potential changes in species composition under climate change.

Coupled with this effort is an analysis of Forest Inventory and Analysis (FIA) plots for Minnesota, and a field assessment of current co-occurring species within black ash ecosystems in Ohio and Michigan. With FIA, a total of 9427 FIA plots were assessed, with 23% of them recording black ash. A total of 182 plots in Ohio and 93 in Michigan were evaluated prior to, or in some cases, during, EAB invasion for seedlings, saplings, and overstory trees to assess potential species mixes in the next forest.

Results The climate change suitable habitat models, even when excluding the direct impacts of EAB, provide evidence that black ash (*F. nigra*) would lose much of its suitable habitat over the eastern US by 2100. In this study, we focus on species which presently or potentially in the future may co-occur with black ash, and how their habitats may change by the end of this century. The primary

species presently co-occurring with black ash in Minnesota included quaking aspen (*Populus tremuloides*), balsam fir (*Abies balsamea*), paper birch (*Betula papyrifera*), and balsam poplar (*Populus balsamifera*). Major co-occurring tree species in the Ohio-Michigan, black ash-dominated plots included primarily elms (*Ulmus* spp.) and maples (*Acer rubrum, A. saccharinum, A. saccharum*) in Ohio, and maples, oaks (*Quercus* spp.), and basswood (*Tilia* sp.) in Michigan. These latter species may be potential replacements for black ash under a changed climate situation.

Discussion The short-term disturbance of EAB infestation will drastically overwhelm longer term impacts of climate change. By coupling plot-level (FIA) information with the overall trends in suitable habitat for co-occurring species, we provide new analyses on the possible future composition of these ecosystems. For example, current co-occurring species with black ash in Minnesota are the likely replacements initially, but the future northward expansion of the potential range of some non-ash tree species currently from Ohio or lower Michigan may allow them to move into areas where they currently do not co-occur with ash. Or, should managers and researchers decide to do so, this information provides a basis for potential species approprate for experiments in assisted migration. Very subtle variations in topography, soils and genetics can greatly influence which species may be appropriate, so further studies and transplanting experiments are recommended.

NATIVE AND INVASIVE PLANT GROWTH RELATED TO LIGHT LEVELS IN EAB-IMPACTED FORESTS

Wendy S. Klooster,[1] Catherine P. Herms[1]
Daniel A. Herms,[2] and John Cardina[1]

[1]Department of Horticulture and Crop Science, The Ohio State University, Ohio Agricultural and Research and Development Center, 1680 Madison, Ave., Wooster, OH 44691
klooster.2@osu.edu

[2]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison, Ave., Wooster, OH 44691

ABSTRACT

Invasive plants can disrupt the natural succession of forest understories by inhibiting native plant growth and regeneration. Disturbed areas, such as canopy gaps, are highly susceptible to plant invasions. Emerald ash borer (EAB; *Agrilus planipennis*) has killed thousands of ash (*Fraxinus* spp) trees throughout the eastern United States, and has the potential to spread throughout North America. A total of 129 18-m radius circular plots were established in 7 State or Metroparks in southeast Michigan to study the ecological impacts of EAB. In this region, ash tree mortality exceeds 95%. Plots were classified according to soil moisture condition (hydroclass) on a scale of 1 (xeric) to 5 (hydric). Plots also span a gradient of density of ash trees and time since EAB infestation.

Our objective was to monitor the growth of invasive and native plants in the understory of EAB-impacted forests. We focused on 10 woody invasive species that had been previously identified in the area: *Rosa multiflora*, *Lonicera* spp, *Berberis thunbergii*, *Elaeagnus umbellata*, *Celastrus orbiculatus*, *Rhamnus cathartica*, *Frangula alnus*, *Ligustrum vulgare* and *Euonymus alatus*. Native plants were limited to species that were growing in the vicinity of the invasive plants, with *Fraxinus* spp., *Lindera benzoin*, and *Carpinus caroliniana* being the most common.

Beginning in 2008, we established over 500 native and invasive plant pairs in 53 plots. Typically, we selected one plot per transect, but in some transects we used more than one plot and some transects did not contain any invasive plants. Within each plot we performed a visual survey to locate up to 5 individual woody invasive plants per species present. We measured the length × width × height of each plant and labeled it with a metal tag to ensure that the same plant was measured each consecutive year. Within 1 m of the invasive plant we located, measured, and labeled a native plant. Native plants were selected to be roughly the same size and of the same growth habit (tree, shrub or vine) as the invasive plant whenever possible. Hemispherical photographs were taken above each pair and analyzed using WinSCANOPY software to determine the gap size, and indirectly the light availability, associated with the plants. Individual pixels were labeled as either "sky" or "canopy"; canopy gap fraction was calculated as a ratio of sky pixels to total pixels.

We hypothesized that plant growth would be related to canopy gap fraction, and growth of invasive species would be greater than growth of native species.

In a comparison of relative growth rates among the paired native and invasive plants we found no consistent trend of invasive plants outgrowing native plants. Furthermore, no trends were evident when analyzed by hydroclass. For future analyses, we will focus on the situations where the invasive plant outgrew the paired native plant since that is the situation which poses problems for land managers and threatens natural ecosystems.

To determine if the invasive species were taking advantage of the light resource more efficiently than native species we looked at growth rates in response to canopy gap fraction. Canopy gap fractions above native and invasive pairs ranged from 1.3 to 21.5. We found no relationship between plant growth and canopy gap fraction when species and plots were analyzed together. When we compared growth to canopy gap fraction separately by hydroclass we still found no clear trend. Future analyses will involve comparing growth and gap fraction separately for each growth habit and by species.

CASCADING ECOLOGICAL IMPACTS OF EMERALD ASH BORER: TRITHROPHIC INTERACTIONS BETWEEN PRICKLY ASH, GIANT SWALLOWTAIL BUTTERFLY LARVAE AND LARVAL PREDATORS

Kevin B. Rice,[1] John Cardina,[2] and Daniel A. Herms[1]

[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison, Ave., Wooster, OH 44691
rice.467@osu.edu

[2]Department of Horticulture and Crop Science, The Ohio State University
Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691

ABSTRACT

Extensive ash tree mortality caused by emerald ash borer (EAB, *Agrilus planipennis*) has generated widespread canopy gaps, resulting in increased light penetration to the understory. Foliage of the native shrub prickly ash (*Zanthoxylum americanum*) contains furanocoumarins, which are secondary metabolites that deter most herbivores, especially as they become more toxic when photoactivated by UV light. Furthermore, furanocoumarin biosynthesis is energy intensive, and their concentration increases when photosynthesis is enhanced by increased light availability. Female plants typically invest more resources in defense, while males allocate more towards growth. Therefore, male and female prickly ash located in canopy gaps may differ in their furanocoumarin concentrations, growth rates, and reproductive effort.

Giant swallowtail butterfly larvae (*Papilio cresphontes*) are specialist herbivores on prickly ash capable of detoxifying furanocoumarins. Energetic costs of furanocoumarin detoxification can slow larval development, and thus increase exposure to natural enemies (slow growth high mortality hypothesis). Therefore, *P. cresphontes* larvae feeding on prickly ash in canopy gaps may experience decreased growth and increased predation pressure. In a series of field and lab experiments, we examined the effects of EAB-induced canopy gaps on resource allocation of prickly ash, and growth and survival of *P. cresphontes*.

Prickly ash located in canopy gaps had lower specific leaf area (a trait often negatively correlated with secondary metabolite concentrations), increased growth, and increased thorn densities compared to shaded plants. Male prickly ash grew faster than females, but females produced more flowers and fruits. We hypothesize that the slower growth of females results from higher allocation to reproduction and defense.

In laboratory bioassays, *P. cresphontes* larvae fed foliage from plants in gaps had lower growth rates than larvae feeding on shaded plants. There was no difference in survival of larvae placed on plants in gaps compared to understory plants, with mortality over 48 hours close to 70% in both habitats. We conclude that larval survival may be lower in gaps because decreased growth rates may increase the amount of time larvae are exposed to natural enemies.

IMPROVING THE ARTIFICIAL DIET THAT DOES NOT CONTAIN HOST MATERIAL AND METHODS DEVELOPED FOR REARING THE EMERALD ASH BORER

Melody A. Keena,[1] Pierluigi (Enrico) Bonello,[2] and Hannah Nadel[3]

[1]USDA Forest Service, Northern Research Station, 51 Mill Pond Rd., Hamden, CT 06514-1703
mkeena@fs.fed.us

[2] Department of Plant Pathology, Ohio State University, 201 Kottman Hall,
2021 Coffey Road Columbus, OH 43214

[3]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542

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. Rearing larvae for research and parasitoid production currently relies heavily on host material. Methods exist to rear all stages of this beetle, but the only artificial diet in use today to rear larvae contains ash phloem, which is time consuming to obtain and process. The goal of this research is to develop an artificial diet that does not contain ash phloem.

We evaluated EAB larval survival and development in several artificial diets without host material. Each series of new diets was evaluated against the best diet we had at the time. Fifty to 100 larvae (equal numbers from a minimum of 10 females) were placed on each diet, either as eggs (within a few days of hatch) on a small piece of paper or as newly hatched larvae. Single (3 mm) or double (6 mm) thick diet pieces were placed in tight-fitting Petri dishes (50 x 9 mm), leaving a small area without diet ("cut-out" on one side). We oriented the dishes in the rearing boxes either horizontally with the larvae/eggs inserted in a slit in the middle of the top of the diet, or vertically with the single larva/egg inserted next to the dish bottom (which is now a side) through the cut-out space which was oriented at the top. The larvae were then kept at 25 °C, 65% relative humidity (RH), and 16 h:8 h (light:dark) cycle.

We analyzed and compared the nutritional composition of an earlier formulation of the EAB-artificial diet with phloem samples from three North American ash species [*F. americana* L. (white ash), *F. pennsylvanica* Marsh (green ash), and *F. nigra* Marsh (black ash)] and an EAB-resistant species from northeastern Asia [*F. mandshurica* Rupr. (Manchurian ash)]. Analysis of moisture content revealed that the EAB artificial diet was higher in percent moisture than that of the ash phloem samples. The artificial diet was ~7-9 times higher in total protein than the ash species, whereas sugars and starch contents were similar. Of the mineral nutrients, the artificial diet had 10 times more phosphorous than did ash species; the diet had the lowest calcium level; and some ash species had higher potassium levels than did the diet. The diet had iron levels that were similar to those of the more tolerant ash species. These findings were used as clues to changes that might be necessary in the diet.

The thirty-fourth host-free artificial diet is the best formulation evaluated to date, with about 80% of the larvae developing to the pre-pupal stage in about 10 weeks (survival to adult data not yet available on this diet). This diet seems to have the correct nutritional and moisture contents to allow larvae to burrow in, survive, and complete their development. Several modifications were needed to reach this level of survival and development. First, the antimicrobial components in the diet were reduced to the lowest, functional level as EAB larvae seem to be sensitive to either the antimicrobials or to the lower pH environment that these ingredients created (and at which they are most effective). Second, the sources of protein in the diet were modified. Wheat germ in the diet promoted fungal and bacterial growth and provided extra iron that the larvae do not need. Replacing wheat germ with soy flour maintained the right protein level, reduced the iron, and reduced the microbial growth problem. Another source of protein in the diet was brewer's yeast; replacing it with potato starch reduced the protein content and improved larval development. The third source of protein in the diet, casein, was maintained because the other changes in protein were sufficient and the casein also supplied calcium, which is otherwise low in the diet. Third, the moisture content of the diet was critical, and dropping the percentage from 60% (content of freshly made diet) to 50% kept first-instar larvae from drowning, thus improving survival rates. We are completing a moisture-content diet trial designed to optimize moisture content of the EAB diet. Fourth, the EAB larvae naturally tunnel downward; when the diet dishes were oriented horizontally, larvae placed on the surface had only a short distance to travel before hitting the plastic at the bottom (where several got stuck). Orienting the dishes vertically and setting eggs or larvae on top of the cut-out allowed the larvae sufficient space to tunnel downward and reach a size that could turn around without getting stuck. Larvae then tunneled either along the plastic-diet interface on the side (originally the bottom) or inside the diet. Finally, EAB larvae seem to have some problems establishing new tunnels when extracted from the diet and therefore should not be disturbed until they have reached the desired size. We now provide the larvae with either a layer of diet that fills the dish (double thickness) or a single sheet (3 mm thick) to which we can add a second sheet after 5 weeks so that the larvae can complete their development with no diet change and only minor disturbance when we check for prepupae.

Future research will include improving the pupal holding methods, assessing the parasitoid acceptance of larvae reared on artificial diet without host material, evaluating methods to rear more than one larva per container, determining the phenology of EAB using the artificial diet, and testing allelochemicals of ash origin in other studies aiming to understand mechanisms of ash resistance to EAB.

Biological Control

PHEROMONES AND ATTRACTANTS FOR THE DETECTION OF EXOTIC AND NATIVE PARASITOIDS OF THE EMERALD ASH BORER

Allard Cossé,[1] Bruce Zilkowski,[1] Richard Petroski,[1*] Jonathan Lelito[2] Juli Gould,[3] and Miriam Cooperband[3]

[1]USDA ARS, Peoria, IL
allard.cosse@ars.usda.gov

[2]APHIS PPQ, Brighton, MI

[3]USDA-APHIS-PPQ CPHST Otis Laboratory
1398 W. Truck Rd., Buzzards Bay, MA 02542
*retired

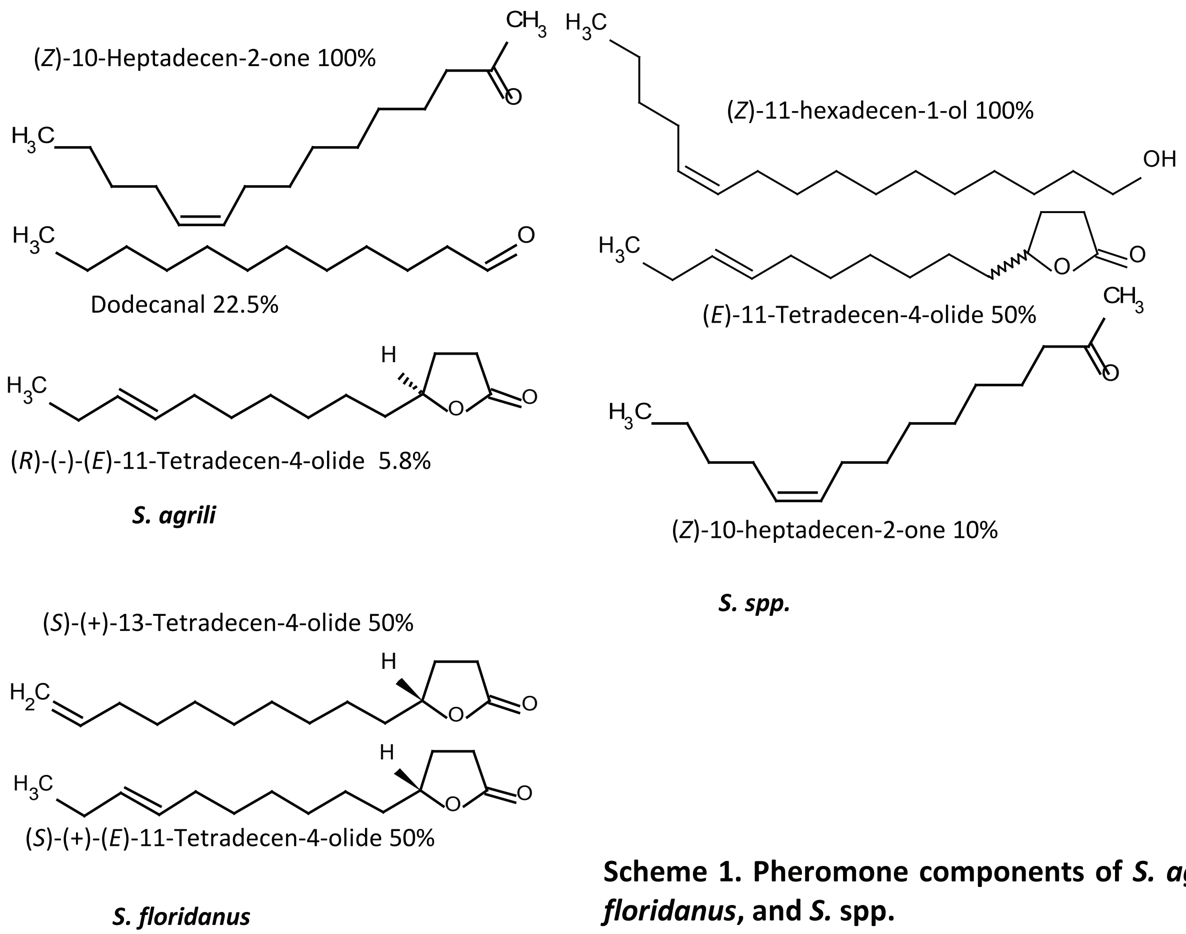
ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* (Buprestidae) is one of the most destructive invasive insect species to impact U.S. forests. Native to Asia, it was discovered in the U.S. in 2002. Since then EAB has been found in 15 states and killed tens of millions of ash trees (*Fraxinus* spp.), destroying 99.9% of ash trees in an area. At this stage of the EAB infestation biological control with natural enemies is the only sustainable method for managing EAB at the landscape level in forests, woodlots, and riparian zones. Three species of parasitic Hymenoptera originally reared from EAB in China have been released in the USA as possible biological EAB control agents, *Spathius agrili* Yang (Braconidae), *Tetrastichus planipennisi* Yang (Eulophidae), and *Oobius agrilus* Zhang & Huang (Encyrtidae (Bauer et al., 2006; Yang et al., 2006; Zhang, et al., 2005). Recently, Cappaert and McCullough (2009) reported significant parasitism of EAB by *Atanycolus cappaerti* Marsh and Strazanac (Hymenoptera: Braconidae), a newly described, indigenous North American parasitoid. The parasitism rates at two sites near Fenton, Michigan ranged from 9-71%, suggesting *A. cappaerti* may also be an effective EAB biocontrol agent. While harvesting EAB larvae for the mass rearing of exotic EAB parasitoids, two more indigenous EAB parasitoids where discovered, *S. floridanus* and a yet unidentified *Spathius* species (Jonathan Lelito, personal communications). Fuester et al. (2009) postulated that with more than 160 species of *Agrilus* in the North American fauna (Arnett 1985), the likelihood of one or more indigenous natural enemies switching over to EAB might be substantial. The authors also reported several newly discovered indigenous EAB parasitoids. This host switching by indigenous parasitoids may become an important part of the EAB biocontrol effort.

Identification of long-range pheromones and/or kairomones for EAB parasitoids could be used in monitoring systems to evaluate the establishment and spread of newly released populations of EAB biocontrol agents. Current practices require a laborious process of felling EAB infested ash trees in the vicinity of parasitoid release sites and the removal of EAB larvae, in the hope of detecting the presence of the biological control agents.

Only limited methods are available to determine indigenous parasitoid densities and dispersal. Semiochemical attractants would be extremely useful for monitoring indigenous parasitoids populations at sites of EAB infestations, allowing for an assessment whether indigenous parasitoids could make an impact with the EAB biocontrol program. In addition, indigenous parasitoids with relatively high rates of EAB parasitism may be mass reared for release and semiochemical attractants could be useful, monitoring the parasitoid populations at release sites.

Recently, we have identified the male-emitted aggregation pheromones of the exotic EAB parasitoid *S. agrili* (Cossé et al., 2009, 2011) and the male-emitted aggregation pheromones of two native *Spathius* species; *S. floridanus* and a unidentified *S.* spp. Male *S. agrili* emit a blend of 7 compounds three of which are pheromone compounds (see scheme 1) as determined by wind tunnel behavioral assays.



Scheme 1. Pheromone components of *S. agrili*, *S. floridanus*, and *S.* spp.

S. floridanus males emit a two component pheromone blend and males of the unidentified *Spathius* species emit a three component pheromone blend. Both males and female wasps responded to natural and synthetic pheromone blends in wind tunnel and field cage studies. All three species share a lactone, (*E*)-11-tetradecen-4-olide, as a component in their pheromone blend, but the chirality (by chiral GC) of the *S. agrili* and *S. floridanus* lactone are different. The chirality of the unidentified *S.* spp. has still to be determined. We developed syntheses for racemic and chiral (*E*)-11-tetradecen-4-olide as well as racemic and enantiomeric forms of a second lactone, 13-tetradecen-4-olide, which is the second component in the *S. floridanus* pheromone blend. Blends containing racemic materials performed similarly to enantiomeric pure blends in attracting all three parasitoid species using flight tunnel experiments and field trapping in large screen cages. Substituting the (*S*)-(+)-*E*-11-tetradecen-4-olide of *S. floridanus* by the (*R*)-(-)-*E*-11-tetradecen-4-olide of *S. agrili* in the *S. floridanus* blend resulted in a complete shutdown of the *S. floridanus* behavioral response, suggesting that *S. floridanus* will not be attracted to the *S. agrili* pheromone.

REFERENCES

Arnett, R. H. 1985. American insects: A handbook of the insects of America north of Mexico. Van Nostrand Reinhold Company, New York.

Bauer, L.S., Liu, H.P., Gao, R.T., and Zhao, T.H.. 2006. Egg and larval parasitoids of emerald ash borer from China: potential for biocontrol in North America. Pp. 48-49, *In* Proceedings of the 2005 Emerald Ash Borer Research and Technology Development Meeting. FHTET-2005-16.

Cappaert, D.; McCullough, D. G. Occurrence and seasonal abundance of *Atanycolus cappaerti* (Hymenoptera: Braconidae) a native parasitoid of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae).Great Lakes Entomologist 2009 Vol. 42 No. 1/2 pp. 16-29.

Cossé, A.A., Lelito, J., and Gould, J. 2009. Semiochemicals for *Spathius agrili*: a preliminary look. Emerald Ash Borer Research and Technology Development Meeting, 20-21 October, 2009, Pittsburgh, PA.

Cossé, A. A., Petroski, R., Zilkowski, B., Lelito, J., and Gould, J. 2011. Pheromones of *Spathius agrili* and *S. floridanus*: Exotic and native parasitoids of the invasive Emerald Ash Borer. 27th Meeting of the International Society of Chemical Ecology, July 24-28, 2011, Burnaby, Canada.

Fuester, R. W., Duan, J. J., Wildonger, J., and Taylor, P. B. 2009. Biological Control of Emerald Ash Borers: The Role of Indigenous North American Parasitoids. Emerald Ash Borer Research and Technology Development Meeting, 20-21 October, 2009, Pittsburgh, PA.

Yang, Z-Q., J.S. Strazanac, Y-X. Yao, and X-Y. Wang. 2006. A new species of emerald ash borer parasitoid from China belonging to the genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae) parasitizing emerald ash borer from China. *Proc. Entomol. Soc. Wash.* 108:550-558.

Zhang, Y.Z., D.W. Huang, T.H. Zhao, H.P. Liu, and L.S. Bauer. 2005. Two new egg parasitoids (Hymenoptera: Encyrtidae) of economic importance from China. *Phytoparasitica* 33:253-260.

HOST-HABITAT LOCATION USING SEMIOCHEMICALS BY *PHASGONOPHORA SULCATA* WESTWOOD (HYM: CHALCIDIDAE), A NATIVE PARASITOID OF *AGRILIS PLANIPENNIS* FAIRMAIRE (COL: BUPRESTIDAE)

Roscoe, Lucas E.,[1] D. Barry Lyons,[2] and Sandy M. Smith[1]

[1]Faculty of Forestry, University of Toronto, Toronto, Ontario, Canada
l.roscoe@utoronto.ca

[2] Natural Resources Canada, Canadian Forest Service – Great Lakes Forestry Center
Sault Ste. Marie, Ontario, Canada

ABSTRACT

The Emerald Ash Borer (EAB) *Agrilus planipennis* is an alien invasive wood-boring pest of native and imported ash (*Fraxinus* spp.) in North America. It was first found in Detroit, MI and soon after in Windsor, Ont. in 2002. EAB is believed to have arrived approximately 10 years earlier via wood packing materials from Asia (Poland and McCullough 2006). Since 2002, EAB has killed millions of ash trees in 13 states and 2 provinces. The damaging stage of EAB is the larvae which enter through the bark of affected trees and create serpentine galleries in the cambium. Continued infestation in affected trees disrupts the movement of nutrients within the tree causing canopy dieback and eventual tree death. Due to the hidden nature of the damaging stage, and the inability of infested trees to show signs of attack until one or more years after infestation, detection of EAB can be difficult (Poland and McCullough 2006). The difficulties in EAB detection as well as the wide ash host range and ability to aggressively attack healthy trees makes the continued spread into and destruction of ash reserves throughout North America a distinct possibility.

In Canada, management of EAB consists of short- and long-term plans. Short-term plans involve chemical injections into infested trees, while long-term management will involve biological control by either fungal pathogens or by wasp parasitoids. While classical biological control using parasitoids from China has been studied in the United States, researchers in Canada have been studying the potential for augmentative biological control using North American parasitoids. One species, *Phasgonophora sulcata* Westwood, has been reared from EAB in high numbers, and could thus be a potential biological control candidate. This wasp has been recorded as a solitary endoparasitoid of North American *Agrilus* spp., but has recently been seen to incorporate EAB into host range.

In studying the biology of this parasitoid in relation to EAB, we determined the role of two ash-produced volatiles and one EAB-produced volatile in host orientation. Parasitoids will use olfactory cues to locate hosts or host-plants where hosts may be present (Vinson 1985). As these volatiles can attract parasitoids, it is possible that they can be used in applied roles that benefit augmentation programs. These can include baits for parasitoid survey traps, and as attractants for aggregating parasitoids to host-rich areas that are devoid of effective parasitoid populations. To determine which volatiles elicited a positive behavioral response from parasitoids, we used a choice-test that was carried out

within a Y-tube olfactometer. We observed that female 1-3 day-old virgin *P. sulcata* adults chose a host-plant produced volatile, Z-3 hexenol, and 3-Z lactone, an EAB-produced volatile, significantly more than a volatile control. Our results indicate that Z-3 hexenol and 3-Z lactone elicit positive behavioral responses in female 1-3 day-old virgin parasitoids. Therefore, it would be recommended that either of these compounds, or a combination of the two, be used in baited survey traps for *P. sulcata* adults or as part of a *P. sulcata*-specific aggregation compound. Our results also indicate a surprising example of a native parasitoid detecting and orienting to volatile sources produced directly by a non-native pest. This is an important result as this parasitoid-host association has only recently been developed.

REFERENCES

Poland, T.M., and McCullough, D.G. 2006. Emerald ash borer: Invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry. 104: 118-124.

Vinson, S.B. 1985. The behaviour of parasitoids. In: Comprehensive Insect Physiology, Biochemistry and Physiology Eds.: G.A. Kerkut, and L.I. Gilbert. Pegamon Press, New York. pp. 417-469

METHODS FOR STUDYING EMERALD ASH BORER PARASITOIDS IN THE FIELD

Leah Bauer,[1,2] Jian Duan,[3] Juli Gould,[4] Kristopher Abell[2,5] Deborah Miller,[1] Jason Hansen,[2] and Roy Van Driesche[5]

[1]USDA Forest Service (FS), Northern Research Station, E. Lansing, MI 48823
lbauer@fs.fed.us

[2]Department of Entomology, Michigan State University, E. Lansing, MI 48824

[3]USDA ARS, Beneficial Insects Introduction Research Unit, Newark DE 45433

[4]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542

[5]Department of Plant, Soil and Insect Science, University of Massachusetts, Amherst, MA 01003

ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), is an invasive phloem-feeding beetle from Asia that attacks ash (*Fraxinus* spp.) trees. EAB was determined to be the cause of extensive ash tree mortality throughout southeast Michigan and nearby Ontario in 2002. For several years, regulatory agencies attempted eradication of EAB, but these efforts were unsuccessful and were later abandoned in favor of management.

Classical biological control is considered the only sustainable method for long-term management of EAB in forested ecosystems. In areas of China where EAB is native, three hymenopteran parasitoid species were discovered parasitizing EAB eggs [*Oobius agrili* (Encyrtidae)] or larvae [*Tetrastichus planipennisi* (Eulophidae) and *Spathius agrili* (Braconidae)]. After completing studies on EAB population dynamics in China, parasitoid biology and host ranges, and preparation of an Environmental Assessment, USDA APHIS issued permits for releases of these parasitoid species in Michigan in 2007. Parasitoid releases were expanded to include study sites in Ohio and Indiana in 2008, and Illinois and Maryland in 2009. In 2010, an APHIS EAB-parasitoid rearing facility in Brighton, Michigan became operational, and parasitoid releases expanded to most of the EAB-infested states in 2011.

Until recently, determination of parasitoid overwintering or establishment in the field involved destructive sampling of EAB-infested ash trees. Using this method, one or more parasitoid species have been confirmed as established in Michigan, Ohio, Maryland, Indiana, and Illinois. Because ash trees are increasingly scarce at our field sites, we developed alternate methods for detecting these introduced parasitoids.

Methods for recovery of the egg parasitoid *O. agrili*: The usual methods to detect overwintering or establishment of *O. agrili* at field sites are to 1) search bark for EAB eggs and rear out egg parasitoids in the laboratory (see symptoms of egg parasitism below) or 2) to fell EAB-infested ash trees at or near the original release epicenter in late winter or early spring and hold logs or bark for rearing. Logs or bark samples are then placed in dark cardboard-rearing tubes at room temperature

for 6 to 8 weeks and emerging insects are collected every few days from a clear plastic emergence cup attached to the end of each tube and held for identification. Logs and bark samples (stored in paper bags) can be refrigerated (4°C) for up to three months. Although *O. agrili* successfully emerge from ash log and bark samples, this method requires a large amount of laboratory space and does not provide data on parasitoid prevalence as the number of EAB eggs in the sample is unknown.

An alternate method for recovering *O. agrili* in the field includes hanging small ash logs with EAB eggs (egg-sentinel logs) on ash trees. Egg-sentinel logs (ESLs) were made in the laboratory by exposing small ash logs (~5-cm diameter × 25-cm long) to gravid EAB females and fresh ash foliage in 3.8-L ventilated plastic jars until ≥50 eggs were laid on each log (2 to 3 days). Before exposure, the logs' ends were dipped in paraffin and each log was wrapped with a spiral of curling ribbon to stimulate EAB to lay eggs beneath the ribbon. After the eggs were counted and marked, the ribbon was placed back over the eggs, and ESLs were hung on ash trees in the field for one to two weeks. To determine percent egg parasitism, ESLs were returned to the laboratory and the eggs on the log observed under a dissecting microscope for signs and symptoms of egg parasitism. These signs include 1) emergence of *O. agrili* from EAB eggs when held in a vial or Petri dish; 2) circular emergence-holes on exposed surfaces of eggs from which adult wasps emerged earlier; 3) dark coloration of EAB eggs; or 4) presence of *O. agrili* life stages inside eggs when dissected. The prevalence of egg parasitism in collections of naturally occurring eggs tends to be underestimated because EAB-egg shells remain on ash bark for several years. On the other hand, natural egg parasitism rates on ESLs may be overestimated if the density of the eggs offered on the log is too high. Thus, deployment of more ESLs with fewer eggs/ESL on more trees may better estimate the prevalence of *O. agrili* in the field.

ESLs are useful for detecting changes in *O. agrili* parasitism over time and space. For example, at one Michigan biological control release plot where *O. agrili* adults had previously been released over a three-year period (2007-2009), we deployed ESLs from on each of five or six ash trees in the last three years. We found 3.9% egg parasitism on ESLs deployed in 2009, 6.1% on ESLs deployed in 2010, and 20.4% in 2011. Moreover, in 2011 we made the first detection of *O. agrili* parasitism in a control plot (0.2% egg parasitism) using ESLs placed ~800 m away from the point of initial release. In 2011, we also used ESLs to assess the phenology of *O. agrili* at this release plot over the course of the full season (see Abell et al., in these proceedings).

Methods for recovery of the larval parasitoids *T. planipennisi and S. agrili*: The usual method to recover EAB larval parasitoids in the field is destructive sampling of infested ash trees. The sampled trees are debarked in the field or laboratory. Immature EAB and associated parasitoids are extracted and reared in the laboratory to obtain adults for identification or dissected to obtain endoparasitoids. Another option is to return ash logs to the laboratory and hold them in cardboard-rearing tubes at room temperature (~25°C) and wait for parasitoids to emerge. With the latter method, however, the parasitoid host species is unknown.

Detection of larval parasitoids in the field may also be achieved by hanging small ash logs containing EAB larvae (larval-sentinel logs) on ash trees. Larval-sentinel logs (LSLs) were made by cutting small ash logs (~5 cm dia × 18 cm long), inserting five 3rd- or 4th-instar EAB larvae in chambers cut under bark flaps, sealing the ends of the logs with Parafilm, and hanging them on ash trees for one week. The LSLs were returned to the laboratory, the parasitoids removed and reared to the adult stage for identification. In 2010 and 2011, we used LSLs to detect parasitism by *T. planipennisi* at three sites where this species had been released over a two year period (2008-2009). In 2010, EAB-larval parasitism rates in the LSLs by *T. planipennisi* were 7%, 45%, and 23% at the three sites. At these same sites in 2011, parasitism rates by *T. planipennisi* in sentinel logs were 3%, 22%, and 32%, respectively. Interestingly, at the two sites where *T. planipennisi* parasitism of EAB larvae declined in 2011, parasitism by native parasitoids in the genus *Atanycolus*, exceeded 50%. Although *S. agrili* was also released at these sites, it was not detected using the LSLs.

We also tested the use of yellow pan traps (YPTs), which are known to be attractive to certain Hymenoptera, including some parasitoid species. YPTs were made from yellow plastic disposable bowls (~12 cm dia) containing soapy water or non-toxic antifreeze. These bowls were mounted horizontally on ash trees using shelf brackets. After three to five days, the contents of these traps were collected, returned to the laboratory, stored in ethanol, and EAB parasitoids removed under a dissecting microscope. Of the introduced EAB biocontrol agents, *T. planipennisi* was more commonly captured by this trap than *S. agrili*; however, no *O. agrili* were recovered using this method. YPTs are a simple method to detect establishment of larval parasitoids at release sites. To optimize parasitoid catch, however, we recommend YPTs be placed 1) at sites no sooner than two years after the last parasitoid release; 2) near the original point of release; and 3) during late summer or early fall when EAB parasitoid population densities are high.

Summary. At our EAB biological study sites in central Lower Michigan, most of the larger ash trees died off within one or two years of initiation of our studies. Survival and continued growth younger ash trees in the stands provide us with host material to destructively sample. The numbers of such trees in and around our field sites, however, are limited due to limited recruitment of ash seedling into the stands. Loss of ash trees at our study sites will adversely affect parasitoid populations and the results of our research. We have determined that larval- and egg-sentinel logs and yellow pan traps are useful for determining the presence or absence of EAB parasitoids in the field.

UPDATE ON RECOVERY AND ESTABLISHMENT OF PARASITOIDS OF THE EMERALD ASH BORER

Juli Gould,[1] Leah Bauer,[2] and Jian Duan[3]

[1]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542
juli.r.gould@aphis.usda.gov

[2]USDA Forest Service (FS), Northern Research Station, E. Lansing, MI 48823

[3]USDA ARS, Beneficial Insects Introduction Research Unit, Newark DE 45433

ABSTRACT

Biological control of the emerald ash borer (EAB) started in Michigan in 2007 with the release of three EAB parasitoid species native to China: an egg parasitoid (*Oobius agrili*) and two larval parasitoids (*Spathius agrili* and *Tetrastichus planipennisi*). However, relatively few parasitoids were released the first year because the permits were granted late in the field season and our laboratory colonies were small. Limited numbers were also released in 2008 at field sites in three states, however, the numbers of parasitoids and sites have increased steadily since 2009 when the EAB Biocontrol Rearing Facility became operational and rearing methods improved. As of 2011, over 375,000 parasitoids have been released at sites in 12 states. More *T. planipennisi* and *S. agrili* have been released to date because both species are gregarious and can be reared in larger numbers compared to *O. agrili*, which is a solitary parasitoid.

This report discusses two important milestones following parasitoid release: overwintering and establishment. If insects are found to overwinter, it documents that they can reproduce at the field site and survive the conditions of at least one winter. Establishment, recovery one or more years following the final release, documents that overwintering parasitoids emerge in synchrony with the host and can continue to persist at the site. Three methods were used to sample field sites for the presence of the introduced EAB parasitoids: 1) felling trees and debarking trees, although destructive and labor intensive, has been quite effective. Larval parasitoids are collected from the EAB galleries, and the bark is put in emergence canisters to recover *O. agrili* from EAB eggs. Sentinel logs, either artificially or naturally infested with EAB larvae or eggs, can be hung at field sites and also successfully recovered the three parasitoid species. Yellow pan traps (YPTs) are less destructive than tree felling, they are relatively easy to deploy, and one can spread them throughout an area, increasing the chances of detecting parasitoids, which tend to have a patchy distribution in the field. Unfortunately, *O. agrili* was not attracted to YPTs. Parasitoids were found to overwinter and establish using all three methods, although no one method worked consistently better than another (Bauer et al. in press).

In 2011, YPT kits were distributed to cooperators in four states because in earlier studies we confirmed these traps captured the adult EAB-larval parasitoids, and they are inexpensive to make and easy to deploy (Bauer et al. in press). Fifteen traps were set up at eighteen parasitoid-release sites in a grid around the release epicenter. The traps were sampled once in August and once in September. Many other adult parasitic hymenopteran species were captured in these traps, with 22 individuals belonging to the genus *Spathius*. Only two of the *Spathius* were identified as *S. agrili*, but both recoveries provided evidence of establishment (in MI and IL). The traps also captured 37 *Atanycholus* sp., but no *T. planipennisi* were recovered.

It generally takes several years after parasitoids are released to recover them in the field. Earlier this year, we reported on parasitoid recoveries from three states (MI, OH, and MD) (Gould et al. in press). Now, with increasing sampling efforts and more methods available, we recovered parasitoids from two additional states (IL and IN). These are, naturally, the five states where releases occurred earliest (2007, 2008, 2009) and, in some cases. for two years consecutively. *Spathius agrili* has overwintered at nine sites and is considered established (recovered at least one year after the last release) at six of these sites (Figure 1). *Tetrastichus planipennisi* has established at six of the ten sites where it has overwintered (Figure 2). Options for sampling *Oobius agrili* are more limited, thus fewer recovery samples have been taken, yet we have determined establishment at seven sites and overwintering at two more sites (Figure 3).

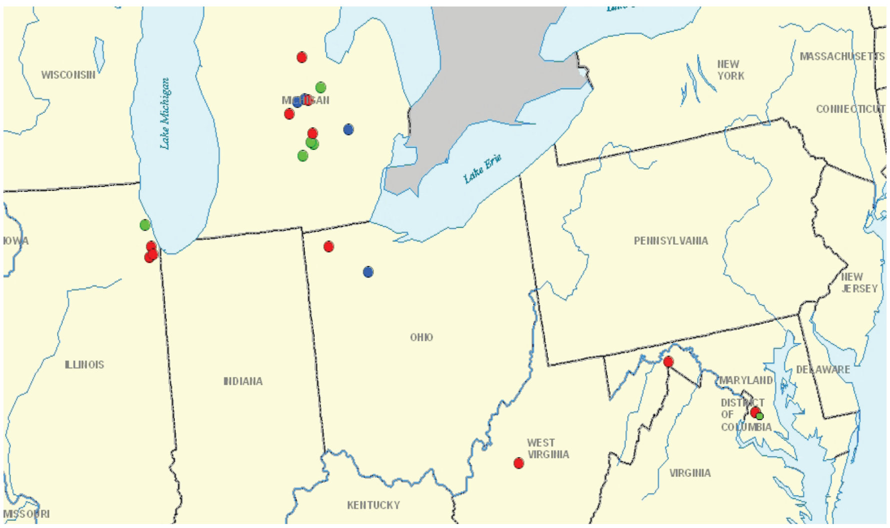


Figure 1. *Spathius agrili* overwintered at sites represented by blue dots and established (was found one or more years after the last release) at sites with green dots. No parasitoids were recovered at sites marked with red dots.

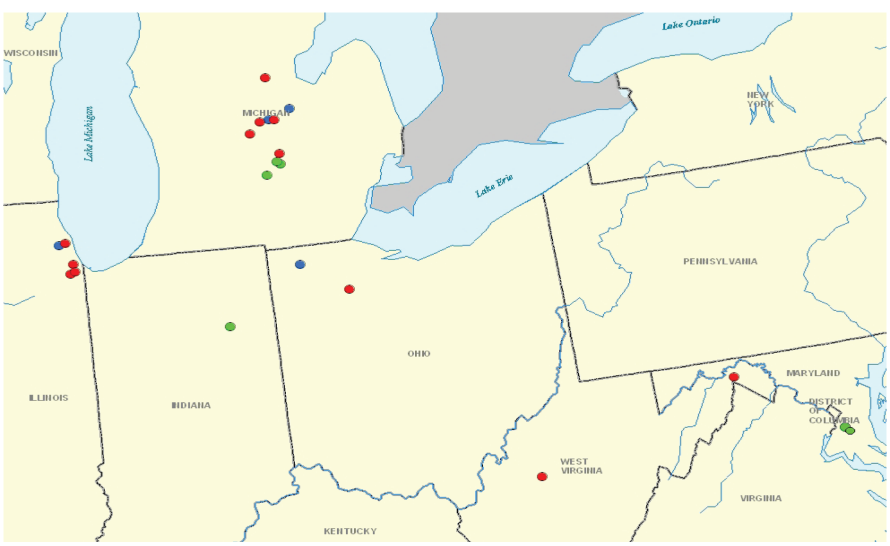


Figure 2 *Tetrastichus planipennisi* overwintered at sites represented by blue dots and established (was found one or more years after the last release) at sites with green dots. No parasitoids were recovered at sites marked with red dots.

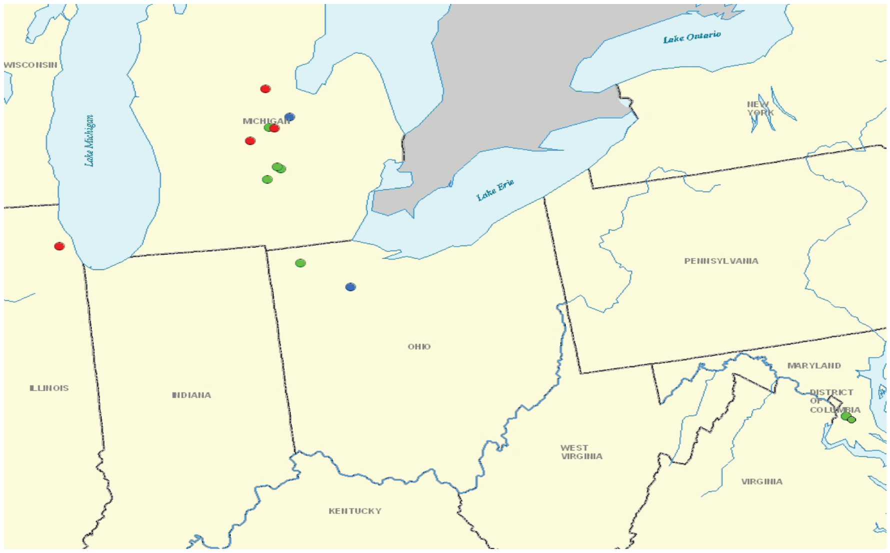


Figure 3. *Oobius agrili* overwintered at sites represented by blue dots and established (was found one or more years after the last release) at sites with green dots. No parasitoids were recovered at sites marked with red dots.

REFERENCES

Bauer, L., J. Gould, J. Duan, J. Hansen, A. Cossé, D. Miller, K. Abell, K. J., R. Van Driesche, J. Lelito, T. Poland. Sampling methods for recovery of exotic emerald ash borer parasitoids after environmental release. *In*: McManus, K., Gottschalk, K. eds. Proceedings, U.S. Department of Agriculture Interagency Research Forum on Invasive Species, 2011 January, Annapolis, MD, USDA Forest Service, Gen. Tech. Rep. (*in press*).

Gould, J., L. Bauer, J. Duan, I. Fraser, J. Hansen, M.Ulyshen, J. Lelito. Release and recovery of parasitoids of the emerald ash borer in Michigan, Ohio, and Maryland. . *In*: McManus, K., Gottschalk, K. eds. Proceedings, U.S. Department of Agriculture Interagency Research Forum on Invasive Species, 2011 January, Annapolis, MD, USDA Forest Service, Gen. Tech. Rep. (*in press*).

POPULATION RESPONSES OF NATURAL ENEMIES TO THE EMERALD ASH BORER (COLEOPTER: BUPRESTIDAE) IN RECENTLY INVADED AREAS IN MICHIGAN

Jian J. Duan,[1] Kristopher J. Abell,[2] Leah S. Bauer,[3] and Roy van Driesche[2]

[1]USDA ARS, Beneficial Insects Introduction Research Unit, Newark, DE 19713
jian.duan@ars.usda.gov

[2]Department of Plant, Insect and Soil Sciences, University of Massachusetts, Amherst, MA

[3]USDA Forest Service, Northern Research Station
East Lansing, MI 48823

ABSTRACT

Populations of natural enemies attacking larval stages of the invasive emerald ash borer (EAB) *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) were surveyed in 2009 and 2010 in the recently invaded areas in Michigan (USA), where two introduced EAB larval parasitoids, *Tetrastichus planipennisi* Yang and *Spathius agrili* Yang were released for classical biological control. During our two-year study following the introduction of two EAB larval parasitoids in forested areas of central Michigan, we found that EAB populations were heavily attacked by woodpeckers, undetermined biotic factors (such as pathogens and/or host plant resistance) and a diverse group of hymenopteran parasitoids, including the introduced biocontrol agent *T. planipennisi*. Population densities of EAB appeared to have been reduced by those mortality agents across different study sites from 87.1 - 126.5 in 2009 to 63.9 - 90.2 (larvae /m^2 phloem) in 2010.

Woodpeckers consumed 32 – 42% of the immature EAB stages present at our study sites, while undetermined biotic factors (such as microbial disease and host tree resistance) caused 10 – 22% mortality of the observed EAB larvae. Among the Hymenoptera parasitoids, the gregarious species *T. planipennisi* was the most abundant, accounting for 93% of all parasitoid individuals collected in 2009 (immediately after field release) and 58% in 2010 (a year later after field releases); low levels (1 – 5%) of parasitism of EAB larvae by *T. planipennisi* were consistently detected at survey sites in both years. Separately, the abundance of the native parasitoid, *Atanycolus* spp., increased sharply, resulting in an average parasitism rate of EAB larvae from <0.5% in 2009 to 19% in 2010. Other parasitoids such as *Phasgonophora sulcata* Westwood, *Spathius* spp., *Balcha indica* Mani & Kaul, *Eupelmus* sp., and *Eurytomus* sp. were much less abundant than *T. planipennisi* and *Atanycolus* spp., and each caused <1% parasitism. Relevance of these findings to the potential for biological control of EAB in the invaded areas of North America is discussed.

THE THIRD INSTAR OF EAB BIOLOGICAL CONTROL: 2011 RELEASES AND PROGRESS ON MASS-REARING TECHNIQUES

Jonathan P. Lelito

[1]EAB Biological Control Facility, USDA APHIS PPQ
5936 Ford Court, Suite 200, Brighton, MI 48116
jonathan.lelito@aphis.usda.gov

ABSTRACT

The Brighton, MI EAB Biological Control Facility is now in its third season of operation and releases of the three exotic parasitoids of EAB have been conducted in 12 of the EAB-infested states. During the past two seasons, the facility has increased production of all three species of biological control agents dramatically from the last report in this publication. Production has allowed releases to occur at more than seventy-five sites in total, including sites in each of the following states: IL, IN, KY, MD, MI, MN, NY, OH, PA, VA, WI, WV. Four of these states are new states for 2011: NY, with 8 sites and a total of 4800 females each of *Spathius* and *Tetrastichus* wasps released; PA, with 3 sites and a total of 4000 *Spathius*, 6000 *Tetrastichus*, and 2500 *Oobius* females released; WI, where 1800 *Spathius*, 2700 *Tetrastichus*, and 1400 *Oobius* females were released at one site, and VA, where 200 *Spathius*, 600 *Tetrastichus*, and 400 *Oobius* females were released at one site. Work ongoing at the facility with native species of parasitoids found to be attacking EAB has also revealed at least one previously undescribed native species in the genus *Spathius*, which attacks EAB at sites across lower MI and is very similar to the more commonly encountered member of the genus, *Spathius floridanus*. This new species is morphologically similar to *S. floridanus*, but differs in several aspects, including the pheromone blend produced by the males. Work with ARS and FS cooperators to identify gene sequences unique to this new species, as well as continuing work with the pheromone to identify more sites at which this wasp occurs, is ongoing.

Significant parasitoid production increases have been realized following several investigations during the 2010 production season and the winter of 2010-2011. These improvements have so far focused primarily on the larval parasitoids, because to that point no viable long-term storage method had yet been devised. The facility has developed methods to store quantities of both *Spathius* and *Tetrastichus* during the winter season, with low mortality even after six months of cold storage. By exposing adults of both species to differing photoperiod and temperature regimes during their own larval development as well as during their oviposition period, and subsequent development of the progeny, the variables most critical to inducing diapause (or a cold-tolerant state of torpor in the case of *Tetrastichus*) and then ensuring the ability to endure prolonged cold temperatures were identified. In both cases, decreased temperature during development appears to be the critical factor for the induction of diapause, but a photoperiod duration decrease during development appears to aid survival independent of temperature. We also revealed a difference in behavior at cooler temperatures between the species: while *Spathius* females decrease brood size but continue to oviposit regularly, *Tetrastichus* fe-

males drastically reduce the rate of oviposition. In the case of *Tetrastichus*, the reduction in propensity to oviposit can be significantly offset if the adult females used are reared in a short-photoperiod environment before being exposed to lowered temperatures. In both species, cool temperatures combined with short photoperiod during development combine to produce juvenile stages capable of enduring six months or more at refrigeration temperatures (4°C). While *Spathius* larvae will remain at the prepupal phase if returned to warm, long-day conditions, *Tetrastichus* larvae will continue to develop and emerge as viable adults. Therefore, we conclude that *Spathius* have a true diapause, and in fact must be cold-stored at 4°C for at least three months to break this condition, while *Tetrastichus* do not appear to enter diapause but rather enter torpor and initiate development immediately upon return to favorable conditions. Even in torpor, *Tetrastichus* larvae can endure at least ten months at 4 ° C. Work with both species is ongoing to determine how these traits may influence the timing of releases in the field.

In total, the facility released over 105,000 female *Tetrastichus* in 2010, and that number has increased to over 147,000 in 2011. Similarly, *Spathius* production rose from just over 55,000 females in 2010 to over 92,000 females in 2011. *Spathius* production has hit the maximum given current resources; but, given the improvements in storage technology, *Tetrastichus* production may be able to be increased further even with current resources. Interestingly, in recent months, the rearing facility has recovered small numbers of *Tetrastichus* wasps from sites in SE MI where bolt material for parasitoid rearing exposures is harvested – this supports the growing body of evidence that *Tetrastichus* is establishing in the field. Therefore, the facility will continue to work toward improving *Tetrastichus* production significantly.

In 2011, improvements were made by facility staff to the methods used to rear adult emerald ash borers, and this allowed an increase in our production of *Oobius* wasps. This also facilitated an investigation of the *Oobius* wasps themselves that led to modified protocols for rearing this wasp as well, and future production is expected to be significantly higher than in previous years. In 2009, the facility maintained a colony of *Oobius* but did not release any to cooperating agencies, whereas in 2010 a total of 5452 females were provided to ARS, FS, and CPHST collaborators, primarily for research releases. In 2011, 26,276 female *Oobius* were released, fully supporting several large-scale research projects and providing numerous state cooperators with wasps for program releases as well.

In collaboration with ARS researchers, the facility has developed a protocol for mass-rearing of *Spathius* and *Tetrastichus* wasps using green ash bolts harvested from natural habitats and artificially infested with EAB ova in the laboratory. Precise timing of the development of the larvae within these bolts allows the presentation of the proper host EAB stage to each species of larval parasitoid, including the native parasitoids. We can also manipulate the development of the EAB within these bolts to produce larvae over a desired period of time. Perhaps even more important to the long-term sustainability of parasitoid mass-rearing, the facility has developed a protocol for using these bolts to produce adult EAB in large numbers, opening the possibility of self-sustaining, lab-reared colony of EAB for the first time. The limited production of EAB ova, even with production improvements made over the past year, is the primary factor preventing this system from surpassing the current system of production in terms of raw production power, and this can be offset to a degree by increased greenhouse space and foliage production in the future should the need arise.

A WEB-ENABLED GEOSPATIAL FRAMEWORK FOR THE MONITORING, MANAGEMENT, AND EVALUATION OF EMERALD ASH BORER BIOLOGICAL CONTROL AGENTS

Amos Ziegler and Gabriel Carballo

Applied Spatial Ecology and Technical Services Laboratory, Department of Entomology
Michigan State University, East Lansing, MI 48824
ziegler2@msu.edu

ABSTRACT

The quantification of any applied environmental science practice such as the biological control of arthropods is essential to its evaluation of success, obstacles and risks (Warner 2009). We find that the tasks associated with biological control lend themselves to systematic collection. It is this systematic collection that ultimately supports the necessary quantification which is so important to the documentation and analysis of program successes. This is in turn only possible when a complete record is maintained regarding the release of control agents.

With the initiation of the 2010 mapBioControl Project (http://www.mapbiocontrol.org), which supports the tracking of EAB parasitoid release, recovery and evaluation researchers at the Applied Spatial Ecology and Technical Service (ASETS) laboratory at Michigan State University (MSU) began to define a geospatial data management framework that would allow for the evaluation of success with regard to the biological control of EAB (Figure 1).

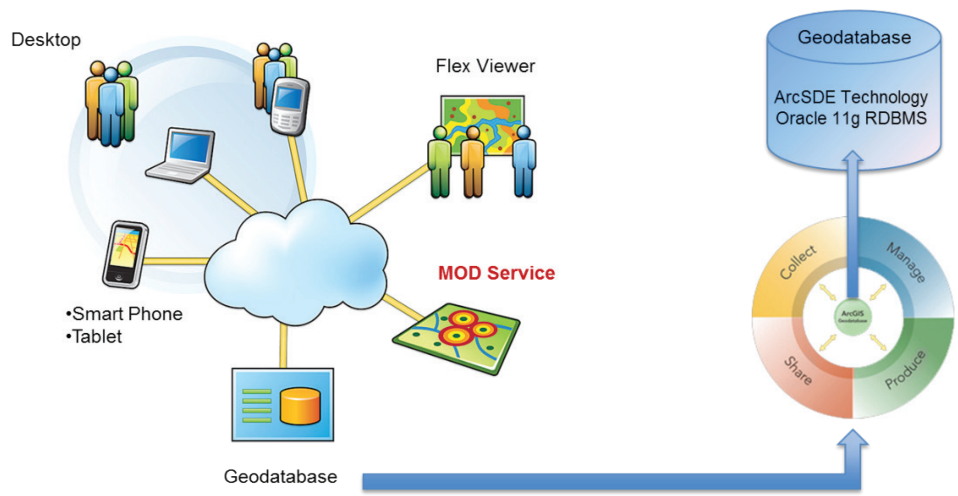


Figure 1. The MapBioControl.org geospatial infrastructure.

The tracking of the release and recovery of biological control agents is essential to the evaluation of their success as a management strategy. The initial version of the mapBioControl geospatial data management framework provides an initial platform for the management of data that will allow for the future modeling of site characteristics which contribute to the greatest success of released control agents. With a more thorough understanding of optimal site characteristics the mapBioControl framework can be enhanced to aid in site selection for the release of future control agents.

REFERENCES

USDA–APHIS/ARS/FS. 2010. Emerald Ash Borer, Agrilus planipennis (Fairmaire), Biological Control Release Guidelines. USDA–APHIS–ARS-FS, Riverdale, Maryland.

Warner K. D., C Getz, S Maurano and K. Powers. 2009. An analysis of historical trends in classical biological control of arthropods suggests need for a new centralized database in the USA. Biological Control Science and Technology. 19(7): 675-688.

Regulatory, Management and Outreach

GIRDLING AND PEELING TO KNOW: DELIMITATION AND MANAGEMENT OF EMERALD ASH BORER IN AN OUTLIER INFESTATION IN SOUTHWESTERN NEW YORK STATE

Melissa K. Fierke,[1] John Vandenberg[2]
Mark Whitmore,[3] and Jerry Carlson[4]

[1] College of Environmental Science and Forestry, State University of New York
1 Forestry Drive, Syracuse, NY 13210
mkfierke@esf.edu

[2] USDA ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853

[3] Natural Resources Department, Cornell University, Ithaca, NY 14853

[4] New York Department of Environmental Conservation, Albany, NY 12201

ABSTRACT

Objectives of this research were to develop an effective delimitation technique and implement and evaluate management in a newly discovered outlying EAB infestation in New York State. The infestation was delimited using 118 girdled trap trees, or "sentinels". Deployment and weekly monitoring of purple prism traps (PPTs) on sentinel trees facilitated early detection and a quick management response. Thirteen PPTs were positive for EAB based on weekly checks through the summer. One hundred three sentinel trees were cut and debarked in late winter to further inform the delimitation (15 trees were beyond several negative sentinels and so were not peeled). PPTs deployed on sentinel trees were effective for monitoring as five trees positive for EAB on PPTs did not harbor larvae. Five trees that were negative for EAB on PPTs did harbor larvae. Management included deployment of clusters of girdled trap trees, or population "sinks", to attract ovipositing EAB and concentrate larvae for targeted removal. Five clusters were initially deployed adjacent to the original infestation area. Thirteen more were deployed throughout the summer as infested trees were identified and removed or EAB adults were found on PPTs in sentinel trees. Three trees were cut and completely debarked from each cluster (n = 54). Seven sinks on the outer edges of the infested area were negative for larvae in trees and so deployment of these were not effective or an efficient use of manpower and time. Efficacy of initial sinks and those closer to the initial infested area were evaluated by comparing trap trees from nine of the sinks to 26 control trees taken within 20-50 m of the sinks. Data analysis indicated there were significantly more larvae in girdled sink trees (mean 42.4 ± 11.6 (SE)) than in ungirdled control trees (10.0 ± 6.03). This research provides an enhanced delimitation protocol using girdled "sentinel" trees and confirms that clusters of girdled trap trees, "sinks", are an effective management tool for reducing numbers of EAB.

IMPACT OF SANITATION ON PUTATIVE NUMBERS OF EMERALD ASH BORER IN AN URBAN AREA

Mark Abrahamson,[1] Robert Venette,[2] and Brian Aukema[3]

[1]Minnesota Department of Agriculture, St. Paul, MN
mark.abrahamson@state.mn.us

[2]USDA Forest Service, St. Paul, MN

[3]University of Minnesota, St. Paul, MN

ABSTRACT

Emerald ash borer (EAB) was discovered in St Paul, Minnesota in May 2009 and subsequently in adjacent areas of the cities of Minneapolis and Falcon Heights. Since the infestation was discovered, the primary control tactic taken by the affected cities has been sanitation, i.e., removal of EAB-infested trees. Trees have been identified for sanitation through visual inspection during the winter and early spring for signs of woodpecker activity and / or cracking bark with EAB galleries beneath. Trees displaying these symptoms have been considered "EAB suspect" and are removed and destroyed prior to adult emergence. Both public and privately owned trees have been handled in this manner, although a higher burden of proof has been required for privately owned trees – i.e., EAB galleries must be observed for a tree to be condemned.

As trees were removed, sections of trees were sampled to estimate the density of EAB larvae. In addition to trees that had been woodpecked (EAB suspect), some trees in the same areas that were not woodpecked were also removed and sampled. A complete census was made of standing ash trees in the infested area each winter. Each tree was evaluated for woodpecking and EAB galleries, DBH was measured and tree location recored or verified. The recorded DBH was then used to estimate the amount of surface area in each standing tree (McCullough and Siegert, 2007). This figure was combined with the estimated EAB densities for trees with woodpecking or without woodpecking depending on how the tree had been rated (woodpecked or not woodpecked). These data were used to estimate how many EAB were present in the infested area each year after sanitation had been implemented and how many EAB would have been present if sanitation had not been implemented.

Sanitation activities removed more than 200 trees during the winter periods of 2009 and 2010, resulting in a dataset where we could analyze more than 1,600 of 2,300 trees left standing in the core infestation area. Using 1,000 Monte Carlo simulations to consuct confidence intervals about the estimated larval densities in the trees with and without woodpecking activity, we estimated that removal of 28% of the trees in the core area (639/2307) had reduced numbers of EAB between 32 and 41% from September 2009 to May 2011.

(Note that we simply estimate total insect numbers, and this preliminary work does not control for overwintering mortality or semivoltine development. Current work is focusing on improved detection using branch sampling, integrating other control efforts such as girdling trap trees, and biological control.)

OBTAINING COMMUNITY SUPPORT FOR EAB RESPONSE PLANS THROUGH TARGETED OUTREACH

Clifford Sadof, Jodie Ellis, and Melissa Shepson

Department of Entomology, Purdue University, Smith Hall, 901 West State Street
West Lafayette, IN 47907-2087
csadof@purdue.edu

ABSTRACT

The development of chemical controls for EAB has made it possible for communities to protect many of the ash trees that are at risk of being killed by this pest. Unfortunately, many urban ash trees are unlikely to receive these treatments because communities fail to adopt and implement a management plan before EAB has damaged trees to the point where treatment is no longer effective. Delays by communities are caused by two factors. First, treatment is can be expensive. In these days of dwindling municipal budgets, urban forestry programs simply do not have the funds to treat treas. Second, the appearance of EAB symptoms is often too subtle to catch the attention of the public until a substantial portion of the ash forest has declined beyond the point of where treatment can be successful. For this reason we have focused our outreach to garner local support from communities by helping them visualize the resources so they can implement a plan to treat ash trees while they can still be saved. This effort, called Neighbors Against Bad Bugs creates a partnership between local communities, Master Gardeners and local arborists that is dedicated to

NABB is a program that works in communities to place informative green tags on ash trees in rights of way to help neighborhoods visualize the value that healthy ash trees bring to their neighborhoods. By working with the media and leaving tags on the trees communities are encouraged to get estimates from arborists to develop a management plan for conserving treatable ash and replacing those not worth treating. By working as a group, arborists can pass on savings associated with high volume work to these communities. Generating a competitive bidding process is crucial to helping communities stretch their management dollars. We have found costs to be quite variable for EAB treatment. For example costs for emmamectin benzoate injections ranged from \$5-\$20/inch DBH.

To help arborists provide more realistic estimates of treatment costs and resulting forest size during the initial infestation wave for a particular community we have revised our web-based EAB Cost Calculator. Arborists can stage the existing EAB infestation in their community or use a guess as to when EAB is likely to start damaging trees in this area. Through this visualization process arborists can demonstrate to communities how advanced planning can help preserve the forest and lower annual out of pocket costs.

EMERALD ASH BORER OUTREACH IN NEW YORK STATE: LAYERS OF GOVERNMENT AND NO TIME TO LOSE; THE USE OF LOCAL EAB TASK FORCES TO FOSTER INTER-AGENCY AND INTER-MUNICIPAL COOPERATION AND MOBILIZE VOLUNTEERS

Mark C. Whitmore[1] and J. Rebecca Hargrave[2]

[1]Department of Natural Resources, Cornell University, Ithaca, NY 14853
mcw42@cornell.edu

[2]Cornell Cooperative Extension of Chenango County
99 North Broad St., Norwich, NY 13815.

ABSTRACT

Emerald Ash Borer (EAB) was first detected in New York just over two years ago. Since that time EAB outreach has been an important focus of the New York Department of Environmental Conservation and Cornell Cooperative Extension (CCE). As more infestations have been found demand for outreach assistance has increased and resources have been stretched thin. One of the projects initiated by CCE has been the training of volunteers, especially Master Gardeners and Master Forest Owners, to conduct outreach in their own communities. This program has been well received but participation has been inconsistent across the state. As a result different groups have become engaged in EAB issues in different localities, not just CCE. Examples include Planning Departments, City Arborists, County Environmental Management Councils, and some regional Partnerships for Regional Invasive Species Management (PRISM's). This year the CCE EAB outreach program decided to focus on the formation of Local EAB Task Forces in order to coordinate the activities of this diverse constituency, identify local resources, and mobilize volunteers. The hardest part of Task Force establishment has been the identification of a core group to create organizational capacity. Once established, one of the most important tasks is to educate volunteers so they can assume some of the outreach responsibilities and help communities initiate planning. We have been and continue to develop training materials for this purpose and are working with the Northeast Region of the National Plant Diagnostic Network to develop a curriculum for EAB First Detectors similar to the program recently developed in Minnesota.

Posters*

Biological Control

*Posters appear in alphabetical order according to title.

ARE IMPACTS OF NATIVE ENEMIES ON EMERALD ASH BORER LARVAE INCREASING?

Andrew R. Tluczek[1] and Deborah G. McCullough[1,2]

[1]Department of Entomology, Michigan State University, East Lansing, MI 48824
tluczek@msu.edu

[2]Department of Forestry, Michigan State University, East Lansing, MI 48824

ABSTRACT

Since emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB)*,* was discovered in southeastern MI and Essex County, Ontario in June 2002, scientists have worked to identify native natural enemies that might mitigate the impacts of EAB. In the fall of 2007, we observed *Atanycolus* sp. parasitism of EAB larvae in two sites near Fenton, MI at rates as high as 70% in a single tree. The wasps were initially identified as *Atanycolus hicoriae*. Recent morphological and molecular evidence, however have established that it is a new species, which was only recently described as *Atanycolus cappaerti* nr. sp. *hicoriae* Marsh (Marsh et. al. 2010, Great Lakes Entomologist). We are continuing to monitor the range, hosts and factors influencing the rate of parasitism of EAB by *A. cappaerti*.

Adult wasps identified as *A. cappaerti* have been reared from logs or from cocoons collected from debarked ash logs in at least 13 Michigan counties (Barry, Clinton, Genesee, Gratiot, Ingham, Isabella, Kalamazoo, Mackinac, Midland, Oakland, Shiawassee, Washtenaw, and Wayne Counties). Adult *A. cappaerti* wasps or cocoons have also been collected from native phloem or wood-borer species including *Agrilus lirugus* (poplar borer)*, Agrilus bilineatus* (twolined chestnut borer)*,* and *Neoclytus sp.* (redbanded ash borer). *A. cappaerti* parasitizes the larvae when they are feeding in the phloem.

Our observations suggested *A. cappaerti* is most often found in or near areas of high density EAB populations. Populations of this wasp may be changing behavior to search ash trees for potential hosts. To evaluate effects of EAB density and tree vigor on parasitism rates, *A. cappaerti* and EAB larval density were sampled and standardized by area (m^2) at sites with varying EAB population levels. Sites at Maple River Game Area (MR) and Lincoln Brick Park (LB) had moderate but building EAB populations. At both sites, 12 ash trees were girdled in May 2010 and 12 ash trees were left un-girdled. In 2010, there was a high density population of EAB at Sleepy Hollow State Park (SH). At Seven Lakes State Park (SL), EAB populations peaked at least two years ago and in 2010, were relatively low. At the SH and SL sites, 15 ash trees were marked in spring for sampling. In fall 2010, trees from all sites were felled and alternate 1 m long sections were debarked. Phloem consumption, EAB larval density and parasitism rates were recorded.

Overall, *A. cappaerti* parasitized 8-25% of EAB larvae in all four sites, regardless of EAB density. In the MR and LB sites, the highest rates of parasitism were on trees with high densities of EAB larvae where little phloem remained intact. Girdling trees had no significant effect on parasitism rates. In the high density SH site and the post-outbreak SL site, parasitism rates were not sig-

nificantly related to EAB larval density but were related to phloem consumption. Parasitism rates were highest in trees where little phloem remained. Native hosts of *A. cappaerti* are usually associated with severely declining or dying trees. We hypothesize that these wasps may be attracted to volatiles associated with ash trees in similarly poor condition.

ASSESSMENT OF *OOBIUS AGRILI* PHENOLOGY USING EGG SENTINEL LOGS

Kristopher J. Abell[1,3], Leah S. Bauer[2,3], Deborah L. Miller[2]
Jian Duan[4], and Roy Van Driesche[1]

[1]University of Massachusetts, Amherst, MA

[2]USDA FS NRS, East Lansing, MI

[3]Michigan State University, East Lansing, MI

[4]USDA ARS BIIRU, Newark, DE

ABSTRACT

Biological control is being used in the United States for long-term management of the emerald ash borer (EAB) (*Agrilus planipennis*), an invasive buprestid introduced from China to North America via international trade. EAB was first discovered in 2002 in southeast Michigan and nearby Ontario causing widespread mortality of ash trees (*Fraxinus* spp.). EAB biological control started in 2007, with introductions of three species of parasitic Hymenoptera that attack EAB in northeast China: an egg parasitoid *Oobius agrili* (Encyrtidae) and two larval parasitoids *Tetrastichus planipennisi* (Eulophidae) and *Spathius agrili* (Braconidae) (Federal Register 2007). The first parasitoid releases were done in central Lower Michigan in 2007, and establishment of these species has been confirmed using several labor intensive methods (Bauer et al. 2011). The objective of this study was to determine the phenology of *O. agrili* in the field to develop a better detection method for *O. agrili*.

Various methods have been used to recover *O. agrili* in the field, including: 1) looking for evidence of *O. agrili* parasitism in EAB eggs sampled from the bark of EAB-infested ash trees, 2) placing field-collected ash logs or bark samples in cardboard-rearing tubes and holding for emergence of *O. agrili* adults, and 3) placing egg sentinel logs (ESLs) (ash logs artificially infested with EAB eggs) on EAB-infested ash trees and assessing eggs for *O. agrili* parasitism (Duan et al. 2010, 2012).

In this study, we used the ESL method at a biocontrol study site in Okemos, MI where *O. agrili* is now known to be established. An ESL is made by spiraling ribbon around a small green ash (*Fraxinus pennsylvanica*) log (18-cm long × 5- to 7-cm diameter) and exposing it to gravid EAB females in a laboratory container. After 2-3 days, each ESL had an average of 115 eggs. From May through September 2011, one ESL was placed on each of six living ash trees for a two-week exposure period and then replaced with a new ESL. After the exposure period, the eggs on each ESL were assessed for parasitism in the laboratory using a dissecting microscope.

Oobius agrili-parasitized EAB eggs were first detected in late June and early July 2011, when parasitism averaged ~10%. Egg parasitism increased through the summer and peaked in early August at ~25% and declined thru September. The distribution of *O. agrili* was patchy between trees with egg parasitism ranging from 0% to as high as 60% on different ESLs on a given date at this site. These results

suggest that ESLs can be an effective monitoring tool for *O. agrili*, and that *O. agrili* is capable of achieving parasitism rates in areas in Michigan comparable to those in its native China (Liu et al. 2007). Our results also suggest that *O. agrili* remains highly localized or prefers a narrow set of host finding conditions.

REFERENCES

Bauer, L. S., J. Gould, J. Duan, J. Hansen, A. Cossé, D. Miller, K. Abell, R. Van Driesche, J. Lelito, and T. Poland. 2011. Sampling methods for recovery of exotic emerald ash borer parasitoids after environmental release. pp. 2-5. *In* McManus, K., K. Gottschalk (eds), Proceedings of the 22nd USDA Interagency Research Forum on Invasive Species 2011. Annapolis, MD. USDA Forest Service Gen. Tech. Rep. NRS-P-92. http://nrs.fs.fed.us/pubs/39810

Duan, J. J., L. S. Bauer, L.S., M. D. Ulyshen, J. Gould, J., and R. Van Driesche. 2010. Development of methods for the field evaluation of *Oobius agrili* (Hymenoptera: Encyrtidae) in North America, a newly introduced egg parasitoid of the emerald ash borer (Coleoptera: Buprestidae). Biol. Control 39: 170-174.

Duan, J. J., L. S. Bauer, J. A. Hansen, K. J. Abell, R. Van Driesche. 2012. An improved method for monitoring parasitism and establishment of *Oobius agrili* (Hymenoptera: Encyrtidae), an egg parasitoid introduced for biological control of the emerald ash borer (Coleoptera: Buprestidae) in North America. Biol. Control 60: 255-261.

Federal Register. 2007. Availability of an environmental assessment for the proposed release of three parasitoids for the biological control of the emerald ash borer (*Agrilus planipennis*) in the continental United States:. Federal Register 72: 28947 – 28948 [Docket No. APHIS-2007-006]. http://www.regulations.gov/#!docketDetail;D=APHIS-2007-0060

Liu, H-P., L.S. Bauer, D. L. Miller, T-H. Zhao, R-T. Gao, L. Song, Q. Luan, R. Jin, and C. Gao. 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biol. Control 42: 61-71.

LONG-TERM COLD-STORAGE OF *TETRASTICHUS PLANIPENNISI*: PROGRESS IN MASS-REARING

Bethany G. Coggins and Jonathan P. Lelito

EAB Biological Control Facility, USDA APHIS PPQ
5936 Ford Court Suite 200, Brighton MI 48116
jonathan.lelito@aphis.usda.gov

ABSTRACT

The PPQ EAB Biological Control Facility in Brighton, MI, along with our FS and ARS cooperators, has been recovering *Tetrastichus* wasps from local APHIS PPQ research plots where joint releases of EAB biological control have been performed. In order to successfully establish *Tetrastichus* on a national scale, production of the wasps must be increased despite rearing limitations such as larval host availability and the lack of a long-term storage protocol for the parasitoids. Through a series of experiments, we have developed a technique to improve parasitism rates in pre-pupal EAB hosts and extend the duration of cold storage that is possible with this species of parasitoid wasp.

To rear *Tetrastichu*s using EAB "J-larvae" (the pre-pupal phase of the host), the EAB hosts are placed into smaller-diameter (1-2.5 cm) ash bolts and positioned in "V" shaped grooves made with a Dremel tool fitted with a routing bit. J-larvae are chosen on specific criteria, and along with groove depth, length, and width, it is the most critical aspect when rearing *Tetrastichus* using this method. The goal is to get the host larva into a groove narrow enough so it has to chew in order to create a pupal chamber no matter which way it is able to move, while the groove remains deep enough as to not damage it when closing the bark flap to secure the host EAB larva. The more the hosts have to chew to position themselves properly for pupation, the more the *Tetrastichus* are alerted to the presence of the host. This increases the likelihood of parasitism as compared to using the same host larva in a standard rearing bolt that is much larger in diameter and/or constructed with differently configured grooves in the wood.

Increased parasitism using J-stage EAB has allowed us to maintain a larger and more diverse colony of *Tetrastichus* throughout the winter months, and enabled us to test and implement a reliable cold storage procedure. Additionally, cold storing individual *Tetrastichus* at the pre-pupal stage yields the highest return of successful emergence once warmed. The duration of the pupal stage of *Tetrastichus* grown at our standard rearing conditions of 26.5°C for 16 hours of daylight and 22.5°C for 8 hours of darkness is approximately 14 days. At that time, we move the entire exposure into 10°C for 48 hours as a step to the eventual 4°C long-term storage temperature. We have successfully stored *Tetrastichus* wasps in this manner for up to 40 weeks at 4°C. Even at this duration of cold-storage, mortality remains fairly low (<20%). Mortality at the more typical storage duration of 3 to 6 months remains insignificant (<10%) to mass-production as well.

With this newly developed technique and the associated larger production of *Tetrastichus* wasps, we have endeavored to understand the effect of photoperiod duration and temperature changes on *Tetrastichus* wasps during their development and oviposition. In order to determine if there was a cue that triggered diapause, we exposed pre-treated wasps to combinations of shorter photoperiod and temperature reduction. With an emergence of over 90% occurring in every treatment once warmed, we've concluded *Tetrastichus* wasps do not enter diapause, but simply remain in suspended development until the appropriate conditions (warmer temperatures) return. These experiments are currently in progress at the Brighton facility and we anticipate future improvements to the method to increase production potential in the coming release seasons. Implications of this phenology for the window of release in the field, as well as timing of releases to match suitable host stages, will be tested this winter at Brighton.

Utilizing EAB-J larvae has now become a standard practice for our rearing facility and produces reliable levels of parasitism (50% or greater) and sufficient brood sizes (mean = 42.5 female wasps per parasitized host). This season, over 3,000 female wasps that were stored for 3-6 months at 4°C were emerged and integrated into our production stream. Our facility will continue to improve rearing methods for *Tetrastichus* wasps using EAB J-larvae in order to increase parasitism rates and maintain a healthy, diverse colony over the winter months when optimal stages of host larvae remain unavailable in suitable quantities. This permits support of research and methods development projects both at Brighton and at cooperating laboratories.

UNDERSTANDING DIAPAUSE IN *SPATHIUS AGRILI*: THE ROLES OF PHOTOPERIOD AND TEMPERATURE

Vincent M. Belill and Jonathan P. Lelito

EAB Biological Control Facility, USDA APHIS PPQ
5936 Ford Court Suite 200, Brighton MI 48116
jonathan.lelito@aphis.usda.gov

ABSTRACT

One of the major challenges to the mass-rearing of *Spathius agrili* is the current lack of reliable and standardized methods for diapause induction and recovery. Without diapause or other cold storage procedures, the window for rearing *Spathius* nearly mirrors the window for program release. This makes stockpiling difficult and vastly decreases the overall production potential of a rearing operation. This research attempted to evaluate several components of the diapause procedure in hopes of improving current diapause protocols. Two experimental designs were constructed towards this aim. The first experiment was a highly randomized procedure whereby *Spathius*-parasitized EAB larvae within ash sticks were placed into cold storage and allowed to store at random intervals before being warmed. This was done to provide an insight into the ideal length of time that *Spathius* should be stored to maximize survival and emergence. The second experiment attempted to document generational environmental effects that could inhibit or promote successful diapause. This study used a factorial design to assess what effect, if any, these effects have on rates of parasitism and diapause, as well as brood size, mortality, and survivorship. Treatments included Warm/Short Day (WS), Warm/Long Day (WS), Cool/Short Day (CS), and Cool/Long Day (CL) conditions, which were constructed in paired groups (i.e., W/S to W/L). The first set of conditions refers to the environment into which parental wasps were born. The second set of conditions represents the environment of oviposition and larval development. The larvae resulting from this oviposition were stored for three months, six months, or warmed immediately.

The overall mean emergence for all bolts stored during the first experiment was 17.41 ± 1.84 adult wasps (95% CI). The highest emergence recorded from any single bolt was 72 adults, having been stored for 99 days. A raw data model predicted ideal emergence after approximately 134 days of cold storage. When grouped into month-long cohorts, average emergence appeared to peak between 90 and 120 days of cold storage. Average emergence decreases thereafter. In the second experimental protocol, the Warm/Short to Cold/Short regime caused 92.54 ± 2.08 % (95% CI) of *Spathius* larvae to enter diapause. This was the highest percentage of diapause induction witnessed among treatments. The least successful treatment for inducing diapause was Warm/Long to Warm/Long, which resulted in only 9.28 ± 4.15 % (95% CI) of larvae to diapause. Treatments that began with Warm/Short conditions entered diapause at a greater proportion than treatments beginning with Warm/Long conditions ($p=0.0026$). A significant difference in brood size, however, could not be found based on preliminary conditions ($p=0.27$).

Treatments in which oviposition occurred at cool temperatures produced a higher percentage of diapausing *Spathius* than warm treatments. These cool treatments, however, also produced smaller brood sizes when compared to warm treatments. Both of these relationships held true among all

statistical comparisons between warm and cool treatments ($p<0.0001$). Varying light exposure had no significant effect on percent diapause ($p=0.025, 0.05$) or brood size ($p=0.085, 0.25$). Pre-diapause mortality was highest in Cool/Long treatments when compared to other environmental regimes. Though some relationship is apparent, bulk emergence from diapause does not appear to be solely dependent on length of storage. It is likely that other factors, including larval quality, wood condition, and various environmental parameters have sizable contributing effects. That being said, the data does suggest a 3-5 month storage window would likely be the ideal length of time to store these larvae in the laboratory. This will have immediate implications for future production storage and experimental procedures at the Brighton EAB rearing facility.

Reduction of photoperiod in the first generation has some positive effect on diapause propensity, but no clear effect on brood size. Light cycle management during oviposition and development does not appear to impact either variable. Temperature reduction at oviposition, meanwhile, clearly has strong effects on both brood size (decreasing) and frequency of diapause (increasing). These two effects in combination are not particularly useful when inducing diapause for production purposes since theoretical yield is reduced. This suggests the possibility of a hybrid system for ideal diapause induction; whereby exposures begin in environments that maximize brood size, and then are transported to environments with maximize diapause behavior. This strategy is currently being tested at the Brighton, MI facility. Collection of emergence data from three and six month treatments is ongoing; therefore it is currently unclear how treatment groups might impact diapause survivorship and recovery at this time. Preliminary data suggest higher survivorship may occur in those treatments in which photoperiod duration decreases precede temperature decreases.

BIOLOGY, BEHAVIOR AND ECOLOGY

AN ALTERNATIVE HOST PLANT-BASED METHOD FOR LABORATORY REARING OF EMERALD ASH BORER TO PRODUCE LARVAL PARASITOIDS FOR BIOLOGICAL CONTROL

Jian J. Duan[1], Tim Watt[2], and Craig Oppel[1]

[1]USDA ARS Beneficial Insects Introduction Research Unit, Newark, DE
jian.duan@ars.usda.gov

[2]Department of Entomology and Wildlife Ecology, University of Delaware, Newark, DE

ABSTRACT

Historically, wood-boring buprestids such as the emerald ash borer (EAB, *Agrilus planipennis*) have been extremely difficult to rear in the laboratory. To date, there have been no published artificial diets that can be practically used to rear EAB for purposes of research and natural enemy productions. Researchers have obtained various stages of EAB larvae or adults by collecting infested ash (*Fraxinus* spp.) logs from the field, storing the logs in a 2- 4°C cooler, and incubating the stored logs to obtain adult EAB emergence. Some EAB researchers have experimented with "artificial infestation", where a series of slits are cut into an ash log, EAB larvae are secured within the slits, and the EAB larvae are then allowed to develop within their natural host wood, or presented to parasitoids as need be (Ulyshen et. al. 2010). While these methods have allowed researchers to produce EAB larvae and adults, they are resource intensive, require large investments of time, personnel, and entail considerable financial investments. Here we report on an alternative host-plant based EAB rearing method which is considerably streamlined in comparison to present rearing strategies. The method involves infesting freshly cut, greenhouse-cultivated tropical ash (*F. udhei*) sticks or logs with EAB eggs. Following the egg infestation, the logs are incubated under normal rearing conditions (25 – 27 C°, 60 – 75% RH, and Photoperiod of L:D 16:8 hrs) for 5 – 6 weeks for EAB larvae to develop into late instars (3^{rd} – 4^{th} instars), and then exposed to gravid females of EAB larval parasitoids (*Spathius* spp. and *Tetrastichus planipennisi*) for parasitoid production.

Findings from our recent study suggest several important extensions of this research. First, it should now be possible to produce EAB adults in the laboratory. While our research in this area is still ongoing, this would be an exciting development. Presently, the only source of adult EAB beetles is from infested wood harvested from the field. Not only is harvesting the wood expensive, requiring travel, personnel, material, and facility funds, the wood must be cold treated for several months requiring large fiscal investments in refrigeration equipment as well as a severe time cost. The ability to produce EAB adults in the laboratory represents a huge reduction in research costs, something of vital importance in the face of the current budget austerity measures the USDA now faces.

Secondly, this method should allow us to produce greater amounts of parasitoids for use in EAB biological control projects. In the past our efforts have always been hampered by two main factors:

1) availability of EAB larvae, and 2) the quality of EAB larvae. We have found that parasitism rates are extremely low when using poor quality larvae collected from the field (≤ 30%), making it very challenging to produce the needed numbers of parasitoids. Preliminary results using logs produced using our tropical ash log-based EAB rearing protocol suggest that the parasitism rates are much higher, often approaching 95%. Since the great majority of the parasitoids we utilize are obtained from oversea sources, the ability to maintain long term colonies from the original stock material is of vital importance; it costs a great deal of money to fund the travel necessary to obtain the proper parasitoids. It is our hope that other organizations can benefit from this research and method development.

REFERENCES

Ulyshen, M.D., J. Duan, L.S. Bauer, and I. Fraser. (2010). Suitability and accessibility of immature *Agrilus planipennis*(Coleoptera:Buprestidae) stages to *Tetrastichus planipennisi* (Hymenoptera:Eulophidae). J. Econ. Entom. 103:4 1080-1085.

ASHES (OLEACEAE: FRAXINUS) IN KENTUCKY, INCLUDING THE THREE WHITE ASHES, WITH NOTES ON PLANS FOR CONSERVATION IN RESPONSE TO THE EMERALD ASH BORER

Julian Campbell

Bluegrass Woodland Restoration Center, 3525 Willowood Road, Lexington, KY 40517
julian.campbell@insightbb.com

ABSTRACT

An account of the wild and cultivated ashes in Kentucky is presented, together with maps and notes on their distribution and ecology. Geographic patterns in ash distribution, including historical data, are summarized in relation to natural regions of the state, indicating a general association between ash abundance within forests and base-rich soils. The key for identification of taxa is based partly on Nesom's (2010) recent revision of *Fraxinus americana*, sensu lato, but with additional observations on vegetative characters. In herbaria, there is a decline in the percentage of flowering or fruiting collections identified as the three segregates of traditional *americana*: from the diploid *americana* (ca. 60%), to the tetraploid *smallii* (10-20%), to the octoploid *biltmoreana* (1-5%). In recent years, green ash and blue ash appear to have produced more frequent seed than white ashes, in general, with large crops often at intervals of 2 years. Given the impending devastation from Emerald Ash Borer, a scheme is outlined for rapid collection of germplasm, including digging of seedlings. Such collection would allow organized sampling for analysis of genetic differences, for various experimental uses, and for some initial effort to conserve diversity if EAB persists to reduces the new cohort of trees after the initial epidemic. Existing institutional plans are briefly reviewed.

ATTRACTION OF INTRODUCED AND NATIVE *SPATHIUS* SPP. TO HOST-RELATED CUES

Todd D. Johnson[1], Jonathan P. Lelito[2], and Kenneth F. Raffa[1]

[1]Department of Entomology, University of Wisconsin-Madison
tjohnson23@wisc.edu

[2]USDA APHIS PPQ EAB Unit, Brighton, MI

ABSTRACT

The Asian ectoparasitoid *Spathius agrili* has been released in several states, including Wisconsin, for Emerald ash borer (*Agrilus planipennis* Fairmaire) management. A native congener, *S. floridanus*, parasitizes native *Agrilus* and has been recovered from EAB (Bauer et al. 2008). Wasps often exploit volatiles of insect (Jones et al. 1971), plant (Turlings et al. 1995), or microbial origin to locate hosts (Madden 1968; Boone et al.2009), but the chemicals affecting *Spathius* attraction are unknown. EAB is known to harbor a diverse microbial assemblage (Vasanthakumar et al. 2008).

In preliminary two-choice, Y-tube bioassays, *Spathius agrili* was attracted to green ash twigs (1-3 in. diameter) infested by 3rd-4th instar EAB larvae (n=48, χ^2 4.08, $P<0.05$), relative to blank controls.

Microbes were isolated from *Agrilus* galleries in black ash (*Fraxinus nigra*), northern red oak (*Quercus rubra*), and paper birch (*Betula papyrifera*), infested by Emerald ash borer (EAB), Twolined chestnut borer (TLCB), and Bronze birch borer (BBB), respectively, as well as healthy phloem in non-infested trees. Extracts of tissues were plated onto MEA, TSA and chitin media to sample the most representative community. NMDS analysis with the 30 most prevalent morphotypes shows that there are morphotypes unique to both tree species and tree infestation category (Table 1., Figure 1.) This suggests that the microbes may be associated with specific *Agrilus* spp.

Table 1. Preliminary results of morphotype analysis for microbes isolated from infested or non-infested trees by *Agrilus* spp. (n=5 trees/category).

Tree species	Category	Number of morphotypes	Number unique to category
Fraxinus nigra	Uninfested	13	0
	EAB-infested	21	8
Quercus rubra	Uninfested	18	5
	TLCB-infested	19	6
Betula papyrifera	Uninfested	15	4
	BBB-infested	18	7

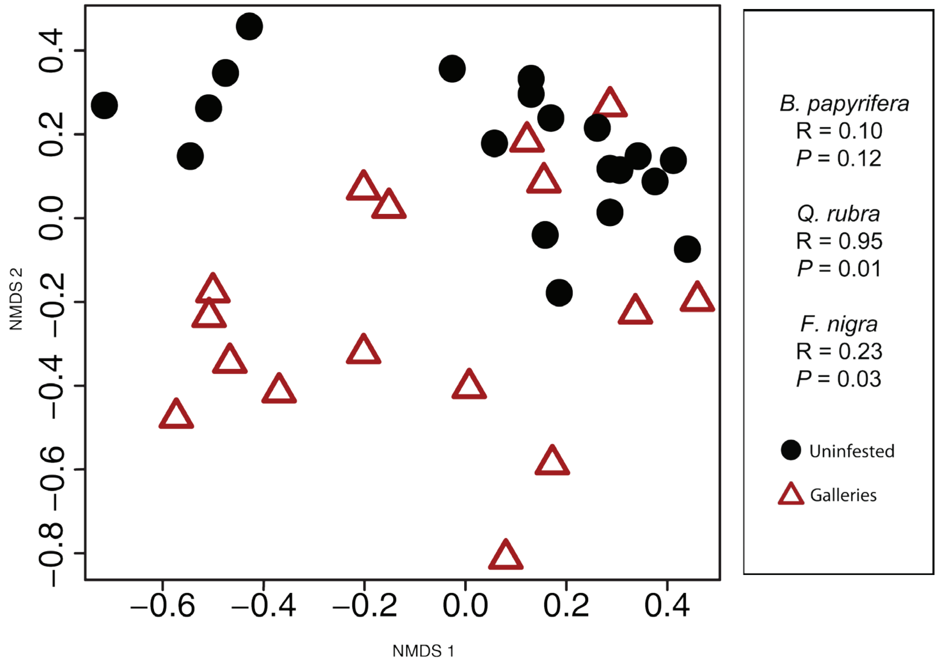


Figure 1. NMDS of microbes isolated from *Agrilus* spp. galleries in *Q. rubra*, *B. papyrifera* & *F. nigra*, and uninfested trees on MEA.

REFERENCES

Bauer, L. S.; Liu, H.; Miller, D.; Gould, J. 2008. Developing a classical biological control program for *Agrilus planipennis* (Coleoptera: Buprestidae), an invasive ash pest in North America. Newsletter of the Michigan Entomological Society. 53(3&4): 38-39.

Boone, C. K., D.L. Six, Y. Zheng,& K.F. Raffa. 2008. Exploitation of microbial symbionts of bark beetles by parasitoids and dipteran predators. Environmental Entomology 37: 150-161.

Jones, R. L., Lewis, W. J., Bowman, M. C., Beroza, M., Bieri, B. A. 1971. Host-seeking stimulant for parasite of corn earworm: isolation identification, and synthesis. Science 173:842-43

Madden, J. L. 1968. Behavioral responses of parasites to the symbiotic fungus associated with Sirex noctilio. Nature 218: 189–190.

Turliings, T.C.J., Loughrin, J.H., McCall, P.J., Rose, U.S.R., Lewis, W.J., Tumlinson, J.H. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. PNAS 92: 4169-4174.

Vasanthakumar, A. I., J. Handelsman, P. Schloss, L. Bauer & K. F Raffa. 2008. Gut microbiota of an invasive wood boring beetle, the emerald ash borer: community composition and structure across different life stages. Environmental Entomology 37: 1344-1353.

COLOR PREFERENCES OF *SPATHIUS AGRILI*, A PARASITOID OF EMERALD ASH BORER

Miriam Cooperband[1], Allard Cossé[2]
Ashley Hartness[1], and Victor C. Mastro[1]

[1]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542
miriam.f.cooperband@aphis.usda.gov

[2]USDA ARS NCAUR, Crop Bioprotection Research Unit, Peoria, IL

ABSTRACT

Spathius agrili (Hymenoptera: Braconidae), native to China, has been released in North America as a biological control agent against the emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae). Techniques to evaluate whether or not this species has established are limited because there are no effective ways to trap this species. In order to sample for *S. agrili,* trees need to be felled and logs have to be peeled or reared out in search of the developing parasitoids, which then need to be identified to distinguish them from native species in the same genus. Since the discovery of a male-produced pheromone for *S. agrili* (Cossé et al, *in prep*), species-specific trapping may provide a more feasible approach for evaluating the establishment of this species in N. America. Designing a pheromone trap is the next step, which requires an understanding of the landing preferences for this species.

Six colors (white, black, red, yellow, purple, and green) were selected for evaluation of landing preferences by virgin female *S. agrili* attracted to the male-produced pheromone in a laminar flow wind tunnel. Attempts were made to match the green and purple colors to those used in the EAB prism traps which are attractive to EAB. A paper color wheel (5 cm diam) with two colors was placed at the upwind end of the wind tunnel. The center of the color wheel held the red rubber septum containing the pheromone. Each color wheel had 14 holes punched in it to allow air to flow through it and past the rubber septum. Targets were rotated to randomize whether a color was on the left or right side. We recorded the number of wasps that flew upwind, and of those, the number that landed on the target and which color they landed on. A chi-square goodness of fit test was used to test the null hypothesis that wasps would land on both colors of a target with equal frequency.

The percent of females that flew upwind to the lures ranged from 61.4% to 97.7%. Of those that flew upwind, landing frequency ranged from 39.6% to 81.5%. The most upwind flight and highest landing frequency occurred with green, yellow, and white targets, and the lowest with red, black, or purple targets. Wasps that flew upwind and landed on a neutral part of the target, such as the septum, the mid-line, on the clip holding the target, or the back of the target, were excluded from the statistical analysis. There was no significant difference between white and green, or white and yellow. Although more wasps landed on the green when offered next to yellow, that combination resulted in more neutral choices than other color combinations. Green and yellow were chosen more than black; yellow and white were chosen more than red; and white was chosen more than purple. Based on these data, white, yellow, or green traps would be expected to perform better than red, black or purple traps.

COMPARISON OF CANOPY GAP ESTIMATION TECHNIQUES IN EMERALD ASH BORER

R. Mejia[1], W. S. Klooster[1], C. Herms[1], D. A. Herms[2], and J. Cardina[1]

[1]Department of Horticulture and Crop Science, The Ohio State University
Ohio Agricultural Research and Development Center, 1680 Madiso, Ave., Wooster, OH 44691
mejia.23@osu.edu

[2]Department of Entomology, The Ohio State University, Ohio Agricultural Research
and Development Center, 1680 Madison, Ave., Wooster, OH 44691

ABSTRACT

Since emerald ash borer was discovered in southeastern Michigan in July 2002, it has been responsible for the death of several million ash trees. As ash trees in forests die, gaps form in the canopy allowing light to reach understory vegetation, which can cause direct or indirect effects in forest ecosystems. Widespread canopy gap formation can promote the spread of invasive plant species, displacing native plants and causing changes in biodiversity. Since many organisms might be affected by these changes, it is important to measure gaps accurately and understand how gaps vary over time and space. Although estimates of canopy gaps are widely used in forest research, the techniques used to quantify gaps vary greatly. Two commonly used methods for estimating canopy gap size, hemispherical photography and spherical densiometer, were compared in 7 Michigan State Recreation Areas or Metroparks. The spherical densiometer is a convex mirror etched with a 24-square grid, enabling reflection of a large overhead area. The proportion of squares not intercepted by tree canopy provides the openness estimate. The hemispherical camera uses a fisheye lens to capture images looking upward into the canopy. Photographs are then analyzed with software (WinSCANOPY) that classifies gap fraction based on pixel color. The spherical densiometer is inexpensive and easy to use, but potentially less accurate than hemispherical photography which uses a sophisticated camera and software.

The objectives of this study were to: (1) Compare canopy gap estimates based on hemispherical photographs and spherical densiometer and determine if any relationship exists between the two methods; (2) examine the variability associated with each technique; and (3) use gap estimates to evaluate post-EAB changes in the forest canopy.

The study was conducted in 129 circular plots (0.1 ha each) within 7 Michigan State Recreation Areas or Metroparks from 2008 to 2011. However, for these analyses, we selected 52 plots representing a range of canopy conditions, and focused only on gap data from the initial year (2008) and final year (2011). Plots were classified according to soil moisture condition on a scale of 1 to 5, with 1 representing dry (xeric) plots and 5 wet (hydric) plots. Once per summer after full leaf expansion, hemispherical photographs and densiometer readings were taken at 8 m from the center of the plot in each cardinal direction, and gap fraction values calculated from each. We used regression analyses in SAS to describe the relationship between gap estimates from hemispherical photographs and densiometer. Variability in gap estimates among parks and within and among plots for each technique was examined using Proc Mixed.

The relationship between gap estimates from hemispherical photographs and spherical densiometer was described in the initial year by an exponential growth function, and in the final year by a linear function. Gap estimates based on these two techniques were significantly correlated in hydric, mesic, and xeric plots, (Pearson's correlation, $\alpha = 0.1$). Variation in gap estimates among parks and among plots within a park was similar for both techniques. However, variation within plots was higher for densiometer estimates than for those from hemispherical photographs, possibly reflecting the difference in angle of view, which is smaller for the densiometer vs. the fisheye lens. A single gap would take up a larger proportion of the view in the densiometer than in the hemispherical photograph. Average gap estimates from both techniques for initial and final years generally indicate that canopy gaps may be starting to close, except for densiometer estimates in hydric plots, which showed larger gaps in the final year.

EAB INDUCED TREE MORTALITY IMPACTS ECOSYSTEM RESPIRATION AND TREE WATER USE IN AN EXPERIMENTAL FOREST

Charles E. Flower[1], Douglas J. Lynch[1]
Kathleen S. Knight[2], and Miquel A. Gonzalez-Meler[1]

[1] Department of Ecology and Evolution, University of Illinois at Chicago
SES 3223 M/C 066
845 W. Taylor St., Chicago, IL 60607
cflowe3@uic.edu

[2] USDA Forest Service Northern Research Station
359 Main Road, Delaware, OH 43105

ABSTRACT

The invasive emerald ash borer (*Agrilus planipennis* Fairmaire, EAB) has been spreading across the forest landscape of the Midwest resulting in the rapid decline of ash trees (*Fraxinus* spp.). Ash trees represent a dominant riparian species in temperate deciduous forests of the Eastern United States (USDA FIA Database). Prior research suggests that the mechanism by which EAB impacts ash trees is through extensive larval gallery formation which alters the transport of water and nutrients (Flower et al. 2010). Such disturbances have been shown to impact photosynthetic uptake and thereby fundamental ecosystem processes such as hydrology and soil respiration (Edwards and Ross-Todd 1979; Hogberg et al. 2001). These processes are integral to forest nutrient cycling and successional dynamics. Despite the fact that ash is an integral component of forests in the Great Lakes Region, the impacts of EAB induced ash decline on forest ecosystems remain largely uninvestigated. The objectives of this study were to assess EAB larval feeding on tree-level water relations and investigate the impacts of EAB induced ash mortality on soil respiration in an ash dominated experimental forest.

Measurements were conducted during 2009 in an EAB infested ash dominated forest located at the USDA Forest Service Lab near Delaware, OH. During the spring, eleven 12 m x 12 m plots were assigned to the following treatments: girdle, insecticide and control. In the girdling treatment, phloem tissue was removed from a 5 in band at breast height to replicate heavy feeding of EAB larvae. For the insecticide treatment, the arbor jet system was used to inject emamectin benzoate (Tree-äge) in dosages recommended by the manufacturer. Control plots were left to senesce naturally. Soil respiration (R_{Soil}) was measured at 22 PVC collars (10 cm x 5 cm) inserted 2.5 cm into the soil (2 collars per plot). Herbaceous plants were excluded from within the collars. Measurements of R_{Soil} were conducted weekly at each PVC collar using an infrared gas analyzer and a soil chamber. To minimize diurnal variability, measurements were conducted between 1100 and 1300 hours. Additionally, Garnier (1985) type thermal dissipation probes (TDP) were deployed to measure sap flow in 9 ash trees along a gradient of EAB infestation. Each TDP system consists of a pair of thermocouples inserted radially into the xylem tissue ~ 10 cm apart, the upper probe was heated and the lower an unheated reference. Two TDP systems were deployed at breast height per tree; one on the north-facing side and one on

the south facing-side. Temperature differentials between the heated and reference thermocouple on each probe, which are correlated with sap flux rates, were measured continuously and 30 min means recorded.

The experimental forest treatments resulted in significant shifts in rates of soil respiration. Specifically the insecticide and control treatments exhibited significantly greater R_{Soil} than the girdled treatment (RMANOVA; $P<0.05$). The insecticide and control treatments exhibited no differences in R_{Soil}. The girdling treatment resulted in ~30% reduction in R_{soil} compared to the non-girdled treatments.

Results suggest that ash trees exhibited significant diurnal variability in sap flux density (RMANOVA; $P<0.01$). Specifically, trees exhibited high daytime sapflux rates compared to night-time driven by transpiration during photosynthesis (Figure 1). Furthermore, heavily infested trees (AC4) exhibited significantly lower sap flux densities compared to lightly infested trees (AC1, Figure 1; see Flower et al. 2010 for a description of how larval gallery cover correlates with AC condition classes). These altered sapflux rates led to significant reduced quantities of daily water use between lightly and heavily infested trees within this forest stand (ANOVA; $P<0.01$).

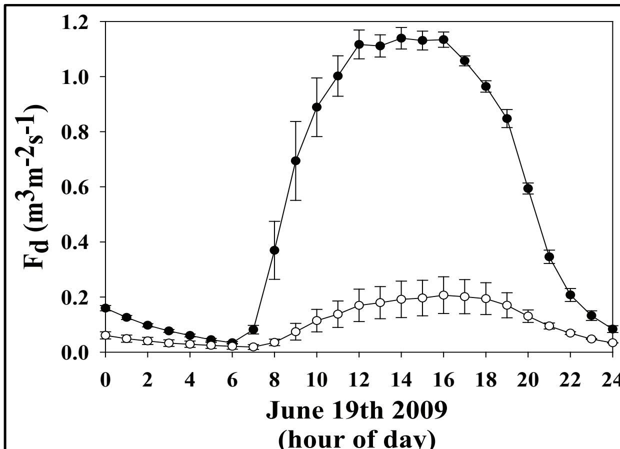


Figure 1: Diurnal sapflux from ash trees lightly and heavily infested with EAB (closed and open circles respectively, F_d= m^3 water per m^2 sapwood area per second).

Despite the widespread nature of the EAB disturbance, the potential ecosystem impacts of EAB are uncharacterized to date. Here we demonstrate how the girdling behavior of EAB may impact ecosystem carbon budgets through reductions in soil respiration. Furthermore, EAB induced tree declines can reduce sapflux rates and alter forest water use which can lead to shifts in local hydrology.

REFERENCES

Edwards, N.T., and B.M. Ross-Todd. 1979. The effects of stem girdling on biogeochemical cycles within a mixed deciduous forest in eastern Tennessee: 1. Soil solution chemistry, soil respiration, litterfall and root biomass studies. *Oecologia* 40:247-257.

Flower C.E., K.S. Knight, M.A. Gonzalez-Meler. 2010. Using stable isotopes as a tool to investigate the impacts of EAB on tree physiology and EAB spread. In: Proceedings, Emerald ash borer research and technology development meeting. pp. 53-54; 2009 October 21-22; Pittsburgh PA.

Granier, A. 1985. Une nouvelle method pour la measure du flux de sève brute dans le tronic des arbres. *Ann. For. Sci.* 42: 193-200.

Högberg, P., A. Nordgren, N. Buchmann, A.F.S. Taylor, A. Ekblad, M.N. Högberg, G. Nyberg, M. Ottosson-Löfvenius, and D.J. Read. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411: 789-792.

EFFECTS OF EMERALD ASH BORER INDUCED ASH MORTALITY ON SOIL INVERTEBRATES

Kayla I. Perry[1] and Daniel A. Herms[1]

[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison, Ave., Wooster, OH 44691
perry.1864@osu.edu

ABSTRACT

Emerald ash borer has caused wide-scale simultaneous mortality of ash in invaded forests, increasing coarse woody debris (CWD) on the forest floor, and light gaps in forest canopies. Ash mortality is predicted to have a variety of direct and indirect effects on forest ecosystems. For example, ash mortality may change forest microhabitats through increasing the amount of light reaching the forest floor, altering abiotic soil conditions such as moisture and temperature, and increasing habitat heterogeneity. Higher densities of CWD on the forest floor may be beneficial for the diversity, abundance, and distribution of soil invertebrate species in the short-term by stabilizing environmental conditions as well as providing resources and habitat.

The objectives of this study are to determine the effects of EAB-induced ash mortality on the diversity and abundance of the forest floor invertebrate community as well as the indirect effects of ash CWD on litter decomposition rates. Forest floor invertebrates were surveyed in 2011 in forest plots established in the Upper Huron River Watershed in SE Michigan to document natural patterns associated with canopy gaps and decaying ash woody debris. Abiotic soil characteristics, canopy gaps, and CWD decay stage were measured to determine changes in forest microhabitats. Additionally, a manipulative experiment was conducted at the NASA Plum Brook Station in N Ohio to understand the mechanistic basis for patterns detected in the observational survey, and to isolate the effects of light gaps, coarse woody debris, and their interaction on the forest floor. Four experimental plots were created: light gap with woody debris (+/+), light gap without woody debris (+/-), light gap absent with woody debris (-/+), and light gap absent without woody debris (-/-). Sections of CWD approximately the same size and decay stage (e.g. one meter in length, 15-20 cm diam, and decay stage I-II) were used for the experiment.

Soil invertebrate activity was assessed through litter decomposition rates. Mesh bags containing leaf litter were placed on the forest floor at each experimental site. Forest floor invertebrates were sampled with pitfall traps and Berlese funnels, and will be categorized based on feeding guild to construct invertebrate food webs. Soil temperature and moisture were measured at each site, and canopy gaps were assessed with a densiometer to determine the percentage of canopy openness.

INFLUENCE OF EMERALD ASH BORER INVASION ON FEEDING HABITS OF BARK-FORAGING BIRDS

Lawrence C. Long[1], Kathleen S. Knight[2], and Daniel A. Herms[1]

[1]Dept. of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave, Wooster, OH 44691
long.643@osu.edu

[2]USDA Forest Service, Northern Research Station, 359 Main Rd., Delaware, OH 43015

ABSTRACT

Ash is common in the fragmented forests of Ohio and other Midwestern states, and members of the genus *Fraxinus* make up nearly 10 percent of Ohio's forested landscape. This creates the potential for emerald ash borer (EAB) to seriously impact the ecology of communities in these forests. The rapid generation of canopy gaps, snags and coarse woody debris that result from EAB-induced ash mortality may create new niches and narrow or close existing ones, resulting in a cascade of direct and indirect ecological effects that may reverberate across trophic levels. Hence, EAB could have substantial impacts on forest structure and insect communities, ultimately altering habitat and food availability for native insectivores.

Insectivorous birds that forage primarily on bark may be ecologically primed to benefit from EAB infestations. For example, EAB predation during winter by species such as red-bellied woodpecker (*Melanerpes carolinus*), hairy woodpecker (*Picoides villosus*), and downy woodpecker (*Picoides pubescens*) can be substantial (Lindell et al. 2008). However, we are unsure if there exists a similar foraging response by bark-gleaning birds during spring and summer. Dietary evidence suggests that both picid and non-picid bark foragers consume large numbers of beetles throughout the year. In several species; wood-borers comprised a large proportion of beetles consumed (Beal 1911). White-breasted nuthatch has been observed to routinely forage on buprestids (Bent 1948; Anderson 1976). Birds that forage on bark during spring and summer, such as white-breasted nuthatch (*Sitta carolinensis*), brown creeper (*Certhia americana*), and summer migrants like the black-and-white warbler (*Mniotilta varia*) may capitalize on the late spring and summer emergence of EAB adults, which generally coincides with the nesting cycle of these birds.

The primary objective of this study, initiated in spring 2011, is to determine the effects of EAB-induced ash decline and resulting resource pulses on the bark-foraging bird community throughout the season. To do this, we are documenting the effects of EAB-induced ash mortality on communities of breeding birds in 34 fragmented forest sites, representing a gradient of ash decline, across western and central Ohio. Specifically, our aim is to understand the impact of EAB infestation, which may offer a distinct resource pulse for utilization by bark foraging birds.

REFERENCES

Anderson, S. H. 1976. Comparative food habits of Oregon nuthatches. Northwest Sci. 50:213-221.

Beal, F.E.L. 1911. Food of the woodpeckers of the United States. USDA Biol. Surv. Bull. 37:1-64.

Bent, A. C. 1948. Life histories of North America nuthatches, wrens, thrushes and their allies. U.S. Natl. Mus. Bull. 195.

Lindell, CA, DG McCullough, D Cappeart, NM Apostolou, and MB Roth. 2008. Factors influencing woodpecker predation on emerald ash borer. Am. Midl. Nat. 159:434-444.

RESPONSES OF NATIVE ARTHROPODS TO EMERALD ASH BORER-INDUCED DECLINE AND MORTALITY OF ASH TREES

Kamal J.K. Gandhi[1], Annemarie Smith[2]
Diane M. hartzler[3], and Daniel A. Herms[3]

[1]Daniel B. Warnell School of Forestry and Natural Resources
University of Georgia, Athens, GA 30602
kgandhi@warnell.uga.edu

[2]Ohio Department of Natural Resources, Division of Forestry
2045 Morse Road, Building H-1, Columbus, OH 43229

[3]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison, Ave., Wooster, OH 44691

ABSTRACT

Invasion by emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB), an introduced buprestid beetle from Asia, is causing large-scale decline and mortality of ash (*Fraxinus* spp.) trees in eastern North America. For example, from 2006 to 2007, mean mortality of ash trees increased from 76% to 93% in southeastern Michigan. Few studies have assessed the direct and indirect effects of the EAB invasion on forest fauna, especially on native arthropods. In terms of direct effects, we assessed which arthropod species that are associated with ash for some part of their life-cycle, may become endangered and co-extinct with the loss of ash trees. In terms of indirect effects, we determined the short-term impacts of ash mortality on ground beetles (Coleoptera: Carabidae) in hydric, mesic, and xeric habitats in which black (*F. nigra* Marsh.), green (*F. pennsylvanica* Marsh.), and white (*F. americana* L.) ash were the most common ash species, respectively.

To identify arthropods associated with ash trees, exhaustive literature searches were conducted on 16 North American ash species. To each arthropod species, we assigned an endangerment risk rating: 1) high risk- monophagous; 2) high moderate risk- biphagous; 3) moderate risk- triphagous; and 4) low risk- polyphagous arthropod species. Survey revealed that a total of 282 native and exotic arthropod species in eight orders are associated with ash species. There were 44, 17, 13, and 208 arthropods, respectively in the high, high-moderate, moderate, and low endangerment risk rankings. Most monophagous ash arthropods are gall-formers followed by folivores, subcortical phloem/xylem feeders, sap feeders, and seed predators. Biphagous and triphagous ash arthropods (30 species) may show an increased association with other host plants as ash trees continue to decline on these invaded landscapes.

In 2006 and 2007, pitfall traps were used to assess ground beetle abundance, diversity, and assemblage composition in EAB-invaded stands in southeastern Michigan. Results indicated that there was a negative relationship between ground beetle abundance and percent ash mortality in 2006.

Hence, beetle populations appear to be adversely affected by ash mortality for at least one year. This relationship was absent in 2007 when the ash mortality was ~93%. Slightly lower species diversity was found in mesic and xeric sites in 2007 than in 2006. Beetle assemblages in hydric and mesic stands diverged in their composition from 2006 to 2007, suggesting alterations in beetle species composition due to ash mortality. Overall, our results on native arthropods indicate that EAB invasion and therefore, decline of North American ash species may lead to cascading ecological impacts in these forested landscapes.

RETROSPECTIVE ANALYSIS OF EAB SPREAD: UNDERSTANDING THE EFFECT OF LANDSCAPE PATTERN ON INVASION AND PROPAGATION

Susan J. Crocker, Greg C. Liknes, and Dacia M. Meneguzzo

[1]USDA Forest Service, Northern Research Station, 1992 Folwell Ave, Saint Paul, MN 55108
scrocker@fs.fed.us

ABSTRACT

Spread of emerald ash borer (*Agrilus planipennis* Fairmaire, EAB) in the United States has been rapid and extensive. Nearly 10 years after its initial detection near Detroit, Michigan, EAB is now found in 15 states spanning a radius greater than 500 miles. While human activity has been central to facilitating the spread of EAB over long distances, the role of landscape pattern is not well understood. The purpose of this study was to gain a better understanding of how landscape pattern influences the spread of EAB at a large scale. Specifically, we wanted to: (1) determine how well landscape metrics predict the presence or absence of EAB, (2) provide a means to model the spatial distribution of future infestations, and (3) use spatial statistics to assess the directional trend of EAB occurrence.

A classification regression tree model was created to represent an objective, data-driven analysis of EAB presence/absence in Illinois, Indiana, Kentucky, and Ohio between 2002 to 2011 (as of August 2011). Counties were used as analysis units to match the accuracy of available data and because they are well-suited to regional or statewide planning and mitigation efforts. For each of the 743 counties, data describing landscape-scale factors that could potentially influence the spread of EAB were assembled (see Figure 1 for a list of variables). Metrics for host density (e.g. trees per acre), forest distribution (e.g. proportion of forest edge), and landscape features (e.g. population density) were aggregated by county for model development. Because infestations began to occur more frequently in counties with relatively higher proportions of forest in 2008, detection year was used to split the data into 2 models: period of initial EAB invasion or introduction (INTRO, 2003-2007) and subsequent EAB propagation (PROP, 2008-2011). The Random Forests statistical classification algorithm (Breiman 2001) was used to relate EAB presence/absence to landscape variables for each of the two periods. The models were then used to predict the progression of EAB presence through counties in Iowa, Minnesota, and Wisconsin. Additionally, a standard deviational ellipse was used to determine the directional trend of EAB presence over time across all seven states (IL, IN, KY, OH, IA, MN, and WI).

Landscape variables had a high success rate when predicting EAB presence/absence. In the 4-state study area, accuracy of the classification model was 88% and 83% for the INTRO and PROP time periods, respectively. Human population density and forest pattern metrics, including percentage of core and edge forest, were the most important variables in predicting the presence or absence of EAB (Fig. 1). Urbanized/ highly fragmented landscapes with a low proportion of forest had an initially higher likelihood of EAB presence and relative importance of the explanatory variables varied

from one time to the next. Additional trials were conducted in which spatial information was included in the model, and preliminary results indicate accuracy can be increased by 3-4% by including distance to infestation epicenter as an explanatory variable. Projections of EAB presence for Iowa, Minnesota, and Wisconsin cannot be validated. However, the forecast for the INTRO period resembles the current distribution of EAB presence and provides an indication of future risk and EAB distribution. The directional trend of EAB distribution across the 7-state area shows that the spatial pattern of infestation changed from nearly isotropic during the INTRO period to a SE/NW directional trend in the PROP period (Fig. 2). As human population density has a large influence on EAB presence, the change in directional trend could be a result of movement through the metropolitan area of Chicago, IL and north to Minneapolis/St. Paul, MN. This is an indication that future projections of EAB should not assume simple transmission from one county to the next.

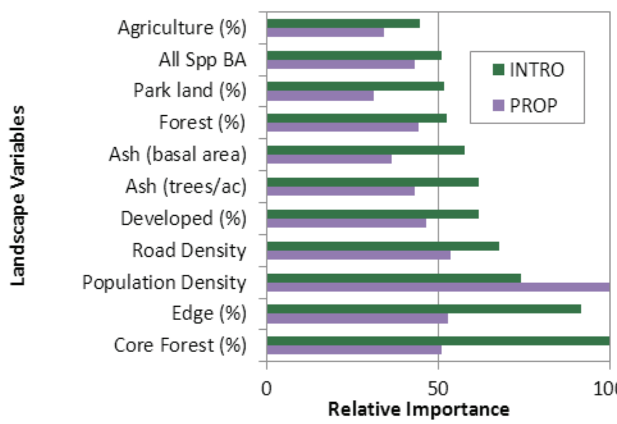


Figure 1. Relative importance of explanatory landscape variables used to model the presence/absence of EAB for the INTRO and PROP time periods. (Note: Importance does not necessarily indicate a positive relationship between the variable and EAB presence.)

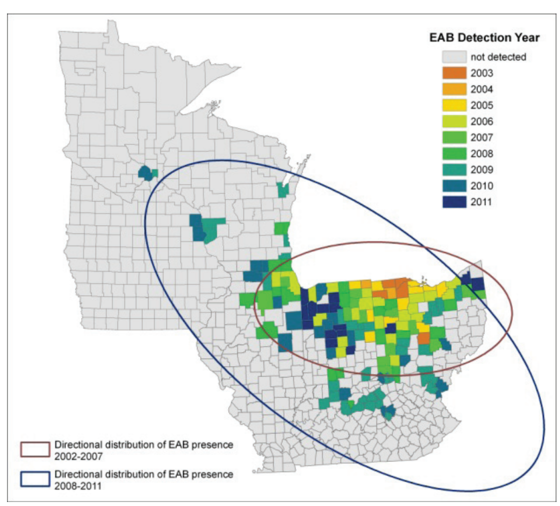


Figure 2. Directional trend (standard deviational ellipse) of EAB occurrence for the INTRO and PROP time periods, MN, WI, IA.

Future work will focus on model enhancements with the aim of accounting for spatial autocorrelation and improving prediction accuracy through better use of spatial or neighborhood information. Climate information will be added to the model to account for the effect of extreme cold weather on EAB propagation. Finally, we will examine strategies for incorporating non-detection probability.

REFERENCES

Breiman, L. 2001 Random forests. Machine Learn .45:5-32.

UNDERSTORY VEGETATION CHANGES AFTER EMERALD ASH BORER INVASION

Delmy V. Sanchez[1], Wendy S. Klooster[1], Catherine P. Herms[1] Daniel A. Herms[2], and John Cardina[1]

[1]Department of Horticulture and Crop Science, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691
Sanchez.892@osu.edu

[2]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691

ABSTRACT

Emerald ash borer (EAB; *Agrilus planipennis*) is a major threat to ash (*Fraxinus*) species in North American forests. As ash trees die, gaps form in the forest canopy, allowing light to reach understory vegetation. Invasion by non-native plant species may be facilitated by the increased light levels, enabling plants to colonize new areas, grow rapidly, and reproduce, resulting in changes in diversity. Invasive plants can have significant impacts on forest communities by disrupting ecological processes, degrading wildlife habitat and hindering forest use and management activities. Forest managers need to know how plant species composition and community structure are changing in response to EAB, and how to interpret quantitative data measured at different scales of sampling.

The objectives of this study were to: 1) measure abundance and diversity of native and invasive woody understory plants in response to disturbance caused by EAB; and 2) compare estimates of vegetative growth response using three methods of sampling.

From 2008 to 2011, we monitored the diversity of native and invasive woody plants in 129 circular plots located in seven Michigan State Recreation Areas or Metroparks. Plots were classified according to soil moisture condition, on a scale from 1 (xeric) to 5 (hydric). Each 18-m radius main plot contained an 8-m radius subplot and four microplots (each 4-m^2) 8 m from the center in the four cardinal directions.

We used three sampling methods, each at a different scale of measurement and detail, to quantify the response of vegetation to EAB-induced ash mortality: a) detailed inventory of woody understory plants in microplots, b) tree sapling counts & shrub cover in the subplot and c) FIA (Forest Inventory and Analysis) total cover in the subplot. In microplots, all woody understory plants were counted and categorized into four size classes: a) newly germinated (with cotyledons), b) < 1 ft tall, c) > 1 ft but < breast height (1.37 m) tall, and d) > breast height tall but < 2.5 cm diameter at breast height (DBH). Within the subplot, we counted tree saplings (> breast height tall but < 2.5 cm DBH), and measured the area (length x width) of shrubs > 1 m tall.

A Forest Inventory and Analysis (FIA) was also conducted in the subplot. This involved estimating the percent cover of native and invasive vegetation in four different height classes: 0 - 2, 2 - 6, 6 - 16, and > 16 ft.

For these analyses, we selected a subsample of 52 plots representing a range of canopy conditions and soil moisture levels, and focused only on data from the initial year (2008) and final year (2011). Data analyzed included plant abundance and diversity in microplots, and sapling diversity, shrub richness (number of species) and total vegetative cover in subplots. Diversity was estimated using Hill's N_1 Index. Differences between initial and final measurements were determined using repeated measures in SAS Proc Mixed.

Data from microplots showed that more native and invasive species were found in xeric than mesic and hydric plots for both sampling periods. For all hydroclasses, the number of invasive species increased from the initial to final sampling. However, diversity in microplots did not change significantly between sampling periods for any hydroclass Tree sapling diversity in subplots decreased significantly in xeric plots, but did not change in mesic and hydric plots. In contrast, shrub richness did not change from the initial to final year in xeric plots, but increased significantly in mesic and hydric plots. Total FIA % cover (sum of all 4 height levels) did not change from the initial to final year in the three hydroclasses; however, as with the microplots, the number of invasive species increased over that time for all hydroclasses. Overall, composition of the vegetation in EAB-impacted plots did not change markedly from the initial to final sampling period based on any of the methods used. This might be due to the short period of time between initial and final measurements.

USING A CELLULAR MODEL TO EXPLORE HUMAN-FACILITATED SPREAD OF RISK OF EAB IN MINNESOTA

Anantha Prasad[1], Louis Iverson[1]
Matthew Peters[1], and Steve Matthews[1,2]

[1]Northern Research Station, USDA Forest Service, Delaware, OH
aprasad@fs.fed.us

[2]School of Natural Resources, The Ohio State University, Columbus, OH

ABSTRACT

Introduction: The Emerald Ash Borer has made inroads to Minnesota in the past two years, killing ash trees. We use our spatially explicit cell based model called EAB-SHIFT to calculate the risk of infestation owing to flight characteristics and short distance movement of the insect (insect flight model, IFM), and the human facilitated agents like roads, campgrounds etc. (insect ride model, IRM). We combine the IFM and IRM to realize the potential risk of infestation in the near term.

Modelling approach: EAB-SHIFT calculates the probability of infestation of a currently unoccupied cell ($P_{i,t}$) based the habitat availability of ash (HQ_i), the abundance of EAB in the occupied cells ($F_{j,t}$), and the distance between occupied and unoccupied cells ($D_{i,j}$). C is calibrated to achieve a dispersal rate of 20 km/year and the value of x determines the rate of decline with distance. The infestation probability (0-1) for each unoccupied cell is summed across all occupied cells within

$$P_{i,t} = HQ_i\left(\sum_{j=1}^{n}\left(HQ_j \times F_{j,t} \times \left(\frac{C}{D_{i,j}^{x}}\right)\right)\right)$$

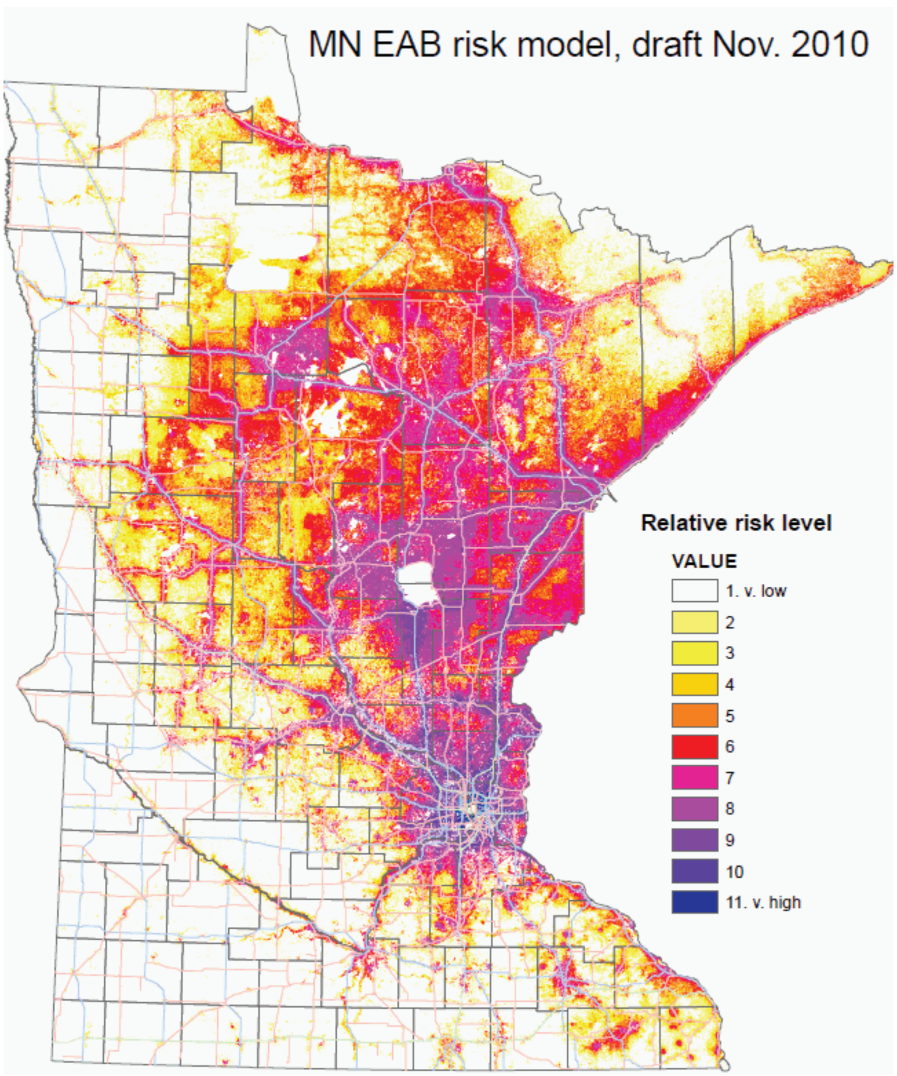
the search window at each generation. If cell sum is greater than one, it is considered infested. For cells with less than one, a random number between 0-1 is chosen and the cell is declared infested if that number is greater than the cell sum, adding an element of stochasticity. These newly infested cells contribute to the infestation probability of unoccupied cells in the next generation, assuming a 10-year generation time between initial infestation and complete death of ash.

Because Minnesota has recently been infested by EAB the core area of infestation is quite small - the IFM did not contribute much to the spread. With IRM, we boost the ash content of each cell by weighting the human facilitated factors in a GIS, contributing substantially to the risk of spread. The road network (weighted by average traffic density) contributes 40% to the overall weight; the roads most likely to be EAB transport routes (LETR) contribute 26% to the overall weight. LETRs were picked by assuming that people making campground reservations from eastern zip codes (likely carriers of EAB) would primarily travel via I-90 or I-94. The likely EAB transport routes were then chosen by visually selecting intersecting major roads leading to the campgrounds. The other human

facilitators included campgrounds (13%), sawmills (7%), firewood vendors (7%) and population density (7%).

Results: The preliminary map from the merger of IFM and IRM shows the risk of spread (see map). The areas near the Twin Cities are highly vulnerable as are the popular tourist attractions around Lake Mille Lacs. The northern areas of MN have large black ash resources in the swampy areas and are sites of high risk.

Conclusions: The modelled map can be used to prioritize monitoring locations with greatest risk of EAB infestation and delineate vulnerable areas. We are working closely with the Minnesota Department of Natural Resources to fine tune our model with better weights and model parameters. We also plan to rerun the model with newer core infestations when discovered to make the model more responsive to newer realities.



Chemical and Microbial Control

LABORATORY BIOASSAY OF EMERALD ASH BORER ADULTS WITH A *BACILLUS THURINGIENSIS* FORMULATION SPRAYED ON ASH LEAVES

Leah S. Bauer[1,2], Deborah L. Miller[1], and Diana Londoño[2]

[1] USDA Forest Service, Northern Research Station, East Lansing, MI 48823
lbauer@fs.fed.us

[2] Department of Entomology, Michigan State University, East Lansing, MI 48824

ABSTRACT

The emerald ash borer (EAB) (*Agrilus planipennis*), a buprestid native to Asia that feeds on ash trees (*Fraxinus* spp.), was discovered in southeast Michigan and nearby Ontario in 2002. It apparently arrived in the 1990's via infested solid-wood packing materials from China. As of 2011, areas considered generally infested with EAB in North America include Michigan, Ohio, Indiana, and Ontario, Canada. Additionally, infestations are known in Illinois, Iowa, Kentucky, Maryland, Minnesota, Missouri, New York, Pennsylvania, Tennessee, Virginia, West Virginia, Wisconsin, and Quebec, Canada (www.emeraldashborer.info). Since 2002, we have been studying biological and microbial controls for management of EAB in forested ecosystems of North America.

Select strains of *Bacillus thuringiensis* (Bt), a group of insect-pathogenic bacteria, are active ingredients of host-specific bioinsecticides, several of which are sprayed aerially to control certain forest defoliators. Bt-based insecticides have been used for over 50 years and have an excellent safety record with respect to human health and the environment, a high public acceptance, and good compatibility with other management strategies such as classical biological control. Thousands of Bt strains, each containing different crystalline protein toxins and host ranges, have been isolated from diseased insects, soil, and leaves.

Background of Bt toxicity in EAB adults. We are evaluating the potential of Bt SDS-502 for use as a microbial insecticide to suppress EAB adult populations by aerial application. Bt SDS-502 expresses the Cry8Da insecticidal crystalline protein, which is toxic to some scarab species but not Lepidopotera (Asano et al. 2003). Previously, we reported on results of our research including 1) the toxicity in EAB adults to the native crystal-spore complex, purified Cry8Da protoxin (130 kDa), and the activated Cry8Da toxin (65 kDa); 2) Bt mode of action as observed in electron micrographs of midgut epithelial cells taken from adults during intoxication; 3) lack of toxicity in adult hymenopteran parasitoids important in EAB biocontrol (*Oobius agrili*, *Tetrastichus planipennisi*, *Spathius agrili*, *Atanycolus* spp.); and, 4) EAB adult toxicity following exposure to ash leaves sprayed with an oil-based formulation (Bauer et al. 2009; Bauer and Londoño 2011). Below, we summarized the methods and results of a recent spray trial of a newly developed water-based formulation.

Mortality of EAB adults fed ash leaves sprayed with formulated Bt suspensions. A batch of Bt SDS-502 was grown in a 40,000 L fermentor, concentrated, spray-dried into a technical

powder (TP), and formulated into water-dispersible granules (Bt-WDG). A sample of the Bt-WDG containing 50% TP (Lot #PHY-3-11) was provided by John Libs of Phyllom LLC, the licensor of the Bt SDS-502 (Patent US6962977) for a laboratory-spray trial against EAB adults. The Bt-WDG was suspended in a 10% sucrose solution, vortexed, and a 1-mL aliquot of suspension was pipetted into a sprayer reservoir; the sprayer was a rotary atomizer (Micronair ULVA+), which was used to simulate aerial spray deposits (Dimond 1989). The sucrose functioned as a feeding stimulant. The spray was applied 1-m above 6 to 8 ash leaves (greenhouse-grown *F. uhdei* leaves trimmed to 3 to 4 leaflets/leaf), which were laid horizontally over one-square meter. To keep the leaves fresh, the petiole of each leaf was inserted in a hole punched into the lid of a water-filled 1-ml plastic vial. Water- and oil-sensitive spray paper (Teejet®, Spraying Systems Co., Wheaton, IL) was placed at regular intervals among the leaves to assess spray deposition. After spray, each leaf was placed inside a clear plastic box with three EAB adults (3- to 4-d-old) and held at 24ºC for a 4-d-exposure to the sprayed leaves, at which time the beetles were given fresh, untreated leaves. We used 30 EAB adults for each of the following treatments: three concentrations of Bt-WDG (25, 50 or 100 mg Bt-WDG/ml sucrose solution) and two controls (10% sucrose and WDG-blank formulation). Daily mortality of the EAB adults was monitored for a total of 7 d.

The mortality of EAB adults after 7 d was: 20% at 25-mg Bt-WDG/ml spray; 43% at 50-mg Bt-WDG/ml spray; 50% at 100-mg Bt-WDG/ml spray. Control mortality was 3% for the WDG-blank formulation and 0% for the 10% sucrose. Time to death averaged 4.3 d, and was similar at the three Bt-WDG concentrations. Droplet density and diameter were determined from the spray paper using a dissecting microscope with a micrometer. The droplet density was positively correlated with WDG concentration and highly variable between leaves; e.g. a range of 12 to 265 droplets/cm^2 was determined at the highest Bt-WDG concentration. We categorized the droplet diameters as small (<100 μm, medium (101-200 μm), or large (>201μm). We found the medium-sized droplets were by far the most abundant, averaging 32.1 droplets/cm^2. Droplet density for the small- and large-sized droplets was similar, averaging 3.5 and 6.4 droplets/cm^2, respectively.

Conclusion. After almost a decade of research on EAB and ash in North America, we are getting closer to developing an IPM program to suppress populations of this invasive beetle in forested ecosystems. An essential part of such a program is an environmentally friendly, narrow-spectrum bioinsecticide that can be applied aerially. Previously, we reported on the toxicity of Bt SDS-502 in EAB adults, which feed in the forest canopy on the leaves of ash trees. Laboratory studies have confirmed that Bt SDS-502 is compatible with exotic and native EAB parasitoids, Lepidoptera, and select nontarget insects. EPA registration for Bt SDS-502 is in progress. We are continuing to optimize the efficacy of Bt-test formulations in the laboratory and anticipate the first field trials in 2012.

REFERENCES

Asano, S., Yamashita, C., Iizuka, T., Takeuchi, K., Yamanaka, S., Cerf, D., and Yamamoto, T. 2003. A strain of *Bacillus thuringiensis* subsp. *galleriae* SDS-502 containing a cry8Da gene highly toxic to *Anomala cuprea* (Coleoptera: Scarabaeidae). *Biol. Contr.* 28: 191-196.

Bauer, L. S., Londoño, D., and Libs, J. 2009. Toxicity of *Bacillus thuringiensis* var. SDS-502 in emerald ash borer adults, *Agrilus planipennisi* (Coleoptera: Buprestidae). pp. 39-40. *In*: Proceedings of the 42nd Annual Meeting of the Society for Invertebrate Pathology 2009. http://www.sipweb.org/Meeting_Abstracts/Meeting%20abstracts09.pdf

Bauer, L. S. and Londoño, D. K. 2011. Effects of *Bacillus thuringiensis* var. SDS-502 on adult emerald ash borer. pp. 74-75. *In*: Proceedings of the 21st U.S. Department of Agriculture Interagency Research Forum on Invasive Species 2010. Gen. Tech. Rep. USDA FS NRS-P-75. http://nrs.fs.fed.us/pubs/37564

Dimond, J. B. 1989. Inexpensive system for simulation of aerial spray deposits. J. Econ. Entomol. 82: 665-667.

LETHAL TRAP TREES: A POTENTIAL TOOL FOR MANAGING EMERALD ASH BORER

Jacob Bournay[1,2], Deborah G. McCullough[1,2], Nicholas J. Gooch[1]
Andrea Anulewicz[1] and Phillip Lewis[3]

[1]Dept. of Entomology, Michigan State University, East Lansing, MI 48824
bournayj@msu.edu

[2]Dept. of Forestry, Michigan State University, East Lansing, MI 48824

[3]USDA Animal and Plant Health Inspection Service, Otis ANGB, MA 02542

ABSTRACT

Tactics to help slow emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) population growth could delay the onset and progression of ash mortality, particularly in localized outlier sites. Previous studies have shown adult EAB, including ovipositing females, are highly attracted to *Fraxinus* spp. trees that have been intentionally girdled. Girdled ash trees can be used for EAB detection and can function as population "sinks" if larvae in the tree are destroyed before they complete development. Removal or destruction of infested, girdled trees, however, can be costly and labor intensive.

A relatively new insecticide product sold as TREE-äge™ with the active ingredient emamectin benzoate provided nearly 100% control of EAB for two years in a large-scale field study. We hypothesized that injecting trees with emamectin benzoate (EB), then girdling trees 2-3 weeks later, could effectively create "Lethal Trap Trees." Volatiles emitted by the girdled trees would presumably attract adult EAB, but the insecticide would control leaf-feeding adults, larval EAB, or both life stages.

In 2009 and again in 2010, we used a block design replicated at 3 sites to compare densities of EAB larvae on similarly-sized ash trees. Each block consisted of 4 trees that were randomly assigned to be left as untreated controls (C), girdled (G),injected with TREE-äge, (EB) or injected and subsequently girdled (EB+G). Trees were felled and debarked in autumn of each year to quantify larval density.

Results from both 2009 and 2010 showed there were almost no live larvae in any of the trees injected with TREE-äge, even those that were girdled 3 weeks after injection (EB+G). Larval density was significantly & substantially higher on girdled trees and untreated controls than on the EB and EB+G trees in both years. In 2010, residues of emamectin benzoate averaged (± SE) 5.8 ±1.40 and 5.6 ±1.23 ppm, respectively, in leaves from EB and EB+G trees. This shows the product was effectively translocated to the canopy, despite the girdling that was applied three weeks after the insecticide was injected. In bioassays with adult EAB, 90-100% of beetles died by Day 3 when caged with leaves from the EB or EB+G trees, compared to <10% EAB mortality on C and G trees. Analysis of foliar residues and adult EAB bioassay data from 2011 is underway. Overall results indicate that lethal trap trees are effective and could be useful as an option in integrated management programs to slow EA-population growth and ash mortality.

SUCCESS IN AERIAL APPLICATION OF SPINSAD FOR EMERALD ASH BORER

Phillip A. Lewis[1] and David M. Smitley[2]

[1]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542
Phillip.A.Lewis@aphis.usda.gov

[2]Department of Entomology, Michigan St. University, East Lansing, MI 48824

ABSTRACT

This study was initiated to determine if aerial application of spinosad could be an effective means to reduce EAB populations and sustain the health of ash trees within wooded areas. Spinosad is an aerobic fermentation product of the soil microbe, *Saccharopolyspora spinosa*. It is currently labeled for use on a wide variety of crops and on stored grain. The manufacturer, Dow AgroScience, considers it safe for forest use and supports the concept of using aerial applications of spinosad for EAB. Due to its low toxicity and low impact on the environment, EPA registered spinosad as a reduced-risk pesticide.

In 2009 we initiated testing the aerial application of spinosad to a number of isolated woodlots in Clinton County, Michigan for control of EAB. After three years of applying the pesticide and two years of evaluation, we report here that the applications are implicated in the continued health of the ash trees within the sprayed woodlots, as opposed to the definitive decline and death of ash within the unsprayed woodlots.

Methods: Twenty privately-owned woodlots (10 - 40 acres) in an intense farming area in Clinton County, Michigan were left untreated, or were sprayed twice per year for three years with spinosad. This area was recently infested with emerald ash borer, and woodpecker attacks and dieback were scarce in the test blocks at the initiation of the project. Two applications were timed to target peak adult emergence. The first application, made in mid to late June depending on spring weather conditions, was followed with a second application two weeks later. Spinosad was applied by a fixed-wing aircraft as the GF-976 formulation (44.2% spinosyns A & D) mixed with (2009) or without (2010, 2011) a spreader/sticker (Kinetic 0.025%) in a spray volume of 2 quarts per acre (7.2 oz spinosad per acre). Applications were made with an Air Tractor 502B fitted with Micronair AU5000 nozzles set at a 55 degree pitch (Figure 2). 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. All of the woodlots selected had evidence of a trace to low level infestation of EAB. Twenty suitable ash woodlots were ranked for the intensity of EAB infestation, and then assigned to a spray treatment or control treatment so that all levels of infestation were represented equally in both treatments.

Residue assessment and EAB adult bioassays: Upper canopy leaf samples were collected from a number of trees in each plot post-application, using a shotgun to dislodge small branches. A portion of the foliage was used to monitor the persistence of spinosad on the foliage. A second portion was used for EAB adult bioassays in the laboratory. In the assay, 10 recently collected EAB adults were placed in 1.0 liter plastic containers. Adults fed freely and mortality assessed at 8 days post application demonstrated good control (control, 11%; sprayed plots, 72%). Residue analysis confirms the rapid decline and degradation of spinosads from the leaf foliage (75% remaining after 2 days, 5% after 1 week). Adult bioassays demonstrated that the product is effective in killing adult EAB when sprayed over a woodlot.

Canopy health and exit hole data: During the late summer, 30 ash trees from each plot were assessed for canopy decline and the lower 2 m of the tree trunks were also checked for adult EAB emergence holes. The location on the trunk of the exit holes was recorded in order to maintain consistency in subsequent years. Emergence hole data for 2011 suggests that the spinosad treatments slow population growth to 1/5 (81% reduction) of the level observed in control woodlots (average of 1.3 emergence holes per tree for spinosad compared with the 6.8 exit holes per tree for control plots).

Results: Canopy thinning and dieback were very similar in control and spinosad-sprayed woodlots at the beginning of our test in 2009 ((38.4% and 40.9%), Figure 1). These values reflect mostly healthy ash trees in a dry year. Frequent rain in 2010 and 2011 resulted in better canopy ratings in 2010 compared with 2009, and allowed the control woodlots to remain outwardly healthy even though the level of EAB infestation had increased. In 2011 spinosad-treated woodlots had very good ratings; however, even in the spinosad-treated woodlots EAB has killed a few trees. Unprotected ash trees in the control woodlots began to decline and die rapidly in 2011 (average dieback rating = 53%, high plot average was 92.2%).

Overall, our data suggests that two applications of spinosad to woodlots in June or early July for two consecutive years will slow population growth. We will have more data next summer that will reflect three years of spinosad application. At this point it appears that spinosad sprays delay population growth of EAB by at least 3 years, in comparison with control woodlots.

From this data we anticipate that aerial sprays will greatly reduce the natural spread of EAB (by flight) out of outlier infestations, and therefore could be a very useful tool for states that would like to delay the spread of EAB in the first few years after it is discovered.

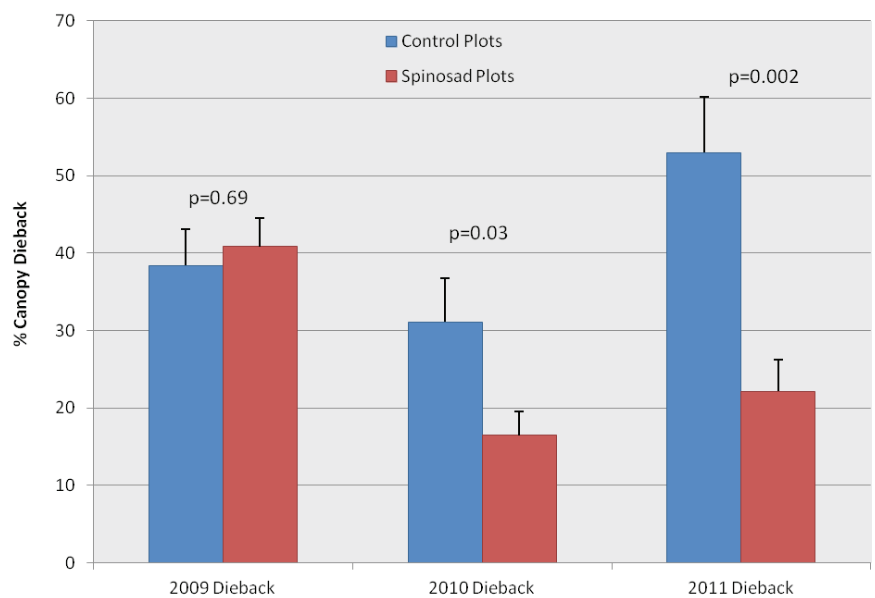


Figure 1. Canopy ratings of treated and untreated woodlots over time.

Host Interactions

ADVENTITIOUS SHOOT REGENERATION AND ROOTING OF *FRAXINUS PROFUNDA*

Micah E. Stevens[1] and Paula M. Pijut[2]

[1]Purdue University, Department of Forestry and Natural Resources
Hardwood Tree Improvement and Regeneration Center (HTIRC)
715 West State St., West Lafayette, IN 47907

[2]USDA Forest Service, Northern Research Station, HTIRC, West Lafayette, IN 47907
ppijut@purdue.edu

ABSTRACT

Pumpkin ash (*Fraxinus profunda)*, along with other members of its genus, is at risk for extirpation by an exotic insect, the emerald ash borer (EAB). The range of pumpkin ash is limited to wetland areas of the Eastern U.S. and it is listed as endangered in two states and threatened in one other. Pumpkin ash provides many benefits to the ecosystem, and its wood is used in the manufacturing industry. In vitro regeneration provides an integral tool for the mass propagation and genetic transformation of desired plant material to combat this threat. Although much work has been done developing regeneration systems for many other ash species, an in vitro protocol for pumpkin ash has yet to be determined. The objective of this research was the development of a successful in vitro regeneration protocol for pumpkin ash. This protocol would provide a means for genetic transformation for EAB resistance and mass propagation for conservation. Aseptically extracted hypocotyls successfully formed adventitious shoots following 4 wk on Murashige and Skoog (MS) medium supplemented with 13.3 mM 6-benzyladenine (BA) and 4.5 mM thidiazuron (TDZ), then transferred for another 4 wk on MS medium with Gamborg B5 vitamins and 0.2 g L^{-1} glycine (MSB5G) plus 6.7 mM BA, 1 mM indole-3-butryic acid (IBA), and 0.29 mM gibberellic acid (GA_3). As adventitious shoots developed, shoots were transferred to MSB5G medium with 13.3 mM BA, 1 mM IBA, and 0.29 mM GA_3 for shoot elongation. Elongated shoots were successfully micropropagated using MSB5G medium with 10 mM BA and 10 mM TDZ. Adventitious root formation was as high as 94% using a woody plant medium supplemented with 4.9 mM IBA and 0 mM IAA cultured for 10 days in the dark followed by culture in the light. Acclimatization to the greenhouse was successful and normal growth was observed.

DELIMITATION AND MANAGEMENT OF A TIER II INFESTATION IN SOUTHWETERN NEW YORK STATE

Christopher Foelker[1], Melissa Fierke[1], John Welsh[1], John Vandenberg[2] Mark Whitmore[3], and Jerry Carlson[4]

[1] College of Environmental Science and Forestry, State University of New York
1 Forestry Drive, Syracuse, NY 13210
cjfoelke@syr.edu

[2]USDA ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853

[3]Natural Resources Department, Cornell University, Ithaca, NY 14853

[4]New York Department of Environmental Conservation, Albany, NY 12201

ABSTRACT

An outlier infestation of emerald ash borer (*Agrilus planipennis* Fairmaire) was discovered in Cattaraugus County, New York in June 2009. This was the first confirmed identification of EAB in New York State and the New York Department of Conservation (NYDEC) subsequently removed and destroyed >200 proximate ash trees over the following six weeks. Thirty-nine trees were confirmed infested and provided an initial infestation area for delimitation, management and research. In the past two years, efforts have been underway to limit the spread of EAB, identify high-risk areas, and educate landowners on management strategies. In spring 2010, we deployed 106 girdled trap trees, green and white ash (*Fraxinus pennsylvanica* and *F. americana*), within a 10 km radius of the initial infestation. We monitored purple prism traps (PPTs) in girdled trap trees, or "sentinels," throughout the summer for EAB presence. In early 2011, we felled and debarked sentinels to delimit the infested area to just over 5 km. Eighteen clusters or "sinks" of 3-8 girdled trees within the area of highest EAB presence were also established in 2010 to lower EAB population densities and minimize dispersal of adults away from the infestation zone. Debarking of girdled sink trees revealed they harbored significantly higher densities of EAB than proximate control trees. In summer 2011, 29 sentinel trees were girdled and PPTs hung in them to further delimit the infestation and 10 sinks were deployed to manage EAB densities and dispersal. Asian EAB parasitoids, *Tetrastichus planipennis* and *Spathius agrili*, were released in August 2011 and *Oobius agrili* will be released in June 2012. Future work includes continued evaluation of EAB population suppression efforts and establishment and efficacy of parasitoid releases.

DEVELOPMENT OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) IN NOVEL ASH (*FRAXINUS* SPP.) HOSTS

Andrea C. Anulewicz[1] and Deborah G. McCullough[1,2]

[1]Department of Entomology, Michigan State University,
243 Natural Science, East Lansing, MI 48824
andreaa@msu.edu

[2]Department of Forestry, Michigan State University,
243 Natural Science, East Lansing, MI 48824

ABSTRACT

The range of ash (*Fraxinus* spp.) spans the continent of North America and widespread devastation by the emerald ash borer (*Agrilus planipennis* Fairmaire), similar to that in southern Michigan and northern Ohio, could potentially occur. Emerald ash borer 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 11 other ash species native to North America, along with several non-native species used in landscapes. Susceptibility of these species to EAB is unknown. If one or more of these species proves to be resistant, then we may be able to identify and enhance resistance mechanisms. In addition, information about 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.

In spring 2008 and 2009, we established a plantation with three European ash species, including European ash (*F. excelsior*), flowering ash (*F. ornus*) and Raywood ash (*F. oxycarpa*), the Asian species Manchurian ash (*F. mandshurica*), the tropical species *F. uhdei*, and four North American ash species, including green ash, blue ash, Oregon ash (*F. latifolia*) and velvet ash (*F. velutina*), along with privet (*Ligustrum vulgare*), a close relative of ash. Two of the North American species are native to western states and EAB populations have not yet encountered these species. In addition to the plantation, in spring 2010, we acquired five species of Asian ash seedlings propagated from seed harvested in China and Japan (*F. insularis, F. lanuginose, F. mandshurica, F. paxiana*, and *F. stylosa*) by colleagues at the Morton Arboretum. These seedlings were maintained in pots in a lath house. Our objectives were to determine (1) if adult EAB will feed on foliage of novel ash species and, if so, evaluate their longevity and (2) whether female EAB will oviposit on novel ash species and, if so, evaluate larval survival and development.

To assess adult leaf feeding, we constructed cages that enabled EAB adults to feed on intact ash leaves. Two male and two female EAB were placed into each cage and allowed to feed for four days. Each leaf was removed and scanned to determine the area consumed by beetles. To assess adult mortality, beetles used in the intact leaf-feeding bioassays were moved to new leaves on the same tree and allowed to feed for an additional 10 days (14 days total). Cages and beetles were moved to new leaves twice per week and beetle mortality was recorded each time.

Trees in the plantation were left undisturbed and exposed to wild EAB populations. In summer 2011, we carefully inspected each tree in the plantation to assess EAB infestation rates prior to 2011. We surveyed the entire tree for EAB emergence holes and woodpecker attacks on late instar larvae. (Additional plant health parameters were also measured, but are not presented here). Adult EAB emergence holes and woodpecker attacks were stapled, enabling us to track EAB infestation rates from year to year.

Adult EAB fed to some degree on foliage from all species tested. Beetles consumed twice as much leaf area on velvet ash and four of the Asian ash species as they did on the other ash species, but few beetles survived for 14 days on velvet ash and Asian ashes. Larval EAB developed successfully on all species tested, except privet. We recorded EAB emergence holes on five previously undocumented host species, including Oregon ash (*F. latifolia*) and velvet ash (*F. velutina),* native to the west coast and southwestern U.S., respectively, and flowering ash (*F. ornus*) and Raywood ash (*F. oxycarpa*), both native to Europe, and tropical ash (*F. uhdei*), native to Mexico.

EVALUATION OF ASIAN AND EUROPEAN ASH (*FRAXINUS SPP.*) BIOTYPES FOR PREFERENCE AND SUSCEPTIBILITY FOR THE EMERALD ASH BORER (*AGRILUS PLANIPENNIS*)

Fredric Miller[1] and Devin Krafka[2]

[1]Research Associate-Entomology, The Morton Arboretum, 4100 Lincoln Ave., Lisle, IL
fmiller@jjc.edu

[2]Research Assistant, The Morton Arboretum, 4100 Lincoln Ave., Lisle, IL

ABSTRACT

Emerald ash borer (*Agrilus planipennis* Fairmaire), an Asian species was discovered attacking ash trees (*Fraxinus* spp.) in 2002. EAB is established throughout much of the Midwestern and eastern United States. In North America, the emerald ash borer (EAB) attacks only native ash species. To date, none have been observed to be resistant.

The objectives of this study were to determine:

- the relative suitability and preference of Asian and European ash (*Fraxinus* spp.)biotypes for the emerald ash borer, *Agrilus planipennis.*
- which Asian and European ash (*Fraxinus* spp.) biotpyes are suitable for future ash breeding programs.

Beginning in the 2009 field season, a study was initiated to evaluate a selection of Asian and European ash (*Fraxinus* spp.) biotypes for preference and suitability for the emerald ash borer (EAB). Laboratory no-choice adult feeding studies were used to test for feeding preference and suitability. Adult beetles were reared from infested logs and two to three adult female beetles were placed in clear plastic cylinders with candidate ash foliage and allowed to feed. Feeding cylinders were held in a rearing cage at approximately 75^{o}F and 60-70 relative humidity, with a 16:8 photoperiod. The beetles were monitored daily for evidence of feeding and mortality and records kept.

No-choice feeding studies in 2009 on Asian and European ash biotypes indicate that adult EAB beetles lived the longest (mean=14 days) on *F. pennslyvanica* (preferred host) compared to the Asian and European species tested. Several beetles lived several months on *F. pennsylvanica*. Adult beetles lived the second longest time (mean=8 days) on *Fraxinus angustifolia* var. *australis* and consumed approximately 22% of the leaf tissue. The mean # of fecal pellets (275) was second only to *F. pennsylvanica* with a mean of 326 fecal pellets. Beetles feeding on the remaining Asian and European ash biotypes (*F. angustifolia* var. *pannonica*, *F. excelsior*, *F. ornus*) all removed an average of 3%.of the leave tissue, lived less than six days, and had a mean of <100 fecal pellets.

Asian No-Choice Laboratory Feeding Bioassay for 2010 revealed adult beetles feeding on *F. chinensis* ssp. *rhynchophylla,* and *F. longicuspis* var. *siebolidiana* removed <8% of leaf tissue. Biotypes with

medium susceptibility included *F. bungeana*, *F. chinensis*, *F. platypoda* and *F. mandshurica* var. *japonica* with 10-15% of the foliage consumed. Highly susceptible species (24-32% foliage consumed) included *F. apertisquamifera*, *F. insularis, F. paxiana*, *F. stylosa* and *F. pennsylvanica* (highly preferred species)

Asian and European No-Choice Laboratory Feeding Bioassays for 2011 resulted in consistent EAB feeding and percent beetle mortality. Mortality rate were higher on less suitable hosts and lower on more suitable hosts. Biotypes with low susceptibility consisted of *F. angustifolia* var. *pannonica*, *F. apertisquamifera, F. chinensis, F. chinensis* ssp. *rhychophylla, F. mandshurica, F. mandshurica* var. *japonica* and *F. syriaca*. Highly susceptibility biotypes included *F. angustifolia* var. *austalis* and *F. longicuspis* var. *siebolidiana*.

These preliminary results indicate:

- *F. bungeana*, *F. chinensis*, *F. angustifolia* var. *austalis* appear to be more suitable for adult EAB feeding.
- *F. apertisquamifera*, *F. insularis*, *F. languinos*, *F. oxycarpa* var. *tamariscifolia*, *F. pallisae* and *F. syriaca* appear to be less suitable for adult EAB feeding.
- EAB feeding and percent beetle mortality were fairly consistent over the three year study period. Mortality rates were higher on less suitable hosts and lower on more suitable hosts.

GENETIC TRANSFORMATION OF *FRAXINUS AMERICANA*

Kaitlin J. Palla[1] and Paula M. Pijut[2]

[1]Purdue University, Department of Forestry and Natural Resources
Hardwood Tree Improvement and Regeneration Center (HTIRC)
715 West State St., West Lafayette, IN 47907

[2]USDA Forest Service, Northern Research Station
HTIRC, West Lafayette, IN 47907
ppijut@purdue.edu

ABSTRACT

White ash trees provide both an economic and ecological benefit. Ash is a valuable hardwood for wood products, and provides food and shelter for wildlife. The emerald ash borer (EAB) is an invasive pest that poses substantial risk to the ash resource in North America. There are no means of complete eradication and no innate resistance in our native ash. Therefore, the development of white ash with resistance to the EAB is an urgent goal. White ash hypocotyls were transformed using *Agrobacterium tumefaciens* strain EHA105 harboring the binary vector pq35GR containing the neomycin phosphotransferase (*nptII*) and β-glucuronidase (GUS) genes. Mature embryos were cultured on Murashige and Skoog (MS) medium containing 10 μM 6-benzyladenine (BA) and 10 μM thidiazuron (TDZ) for 5 d, before hypocotyls were excised and pre-cultured on MS medium containing 22.2 μM BA, 0.5 μM TDZ, 50 mg L^{-1} adenine sulfate, and 10% coconut water (CW). Pre-cultured hypocotyls were transformed in the presence of 100 μM acetosyringone using 90 s sonication plus 10 min vacuum-infiltration. Hypocotyls were then co-cultured for 2 d in the dark. Kanamycin-resistant shoots were selected on MS medium containing 22.2 μM BA, 0.5 μM TDZ, 50 mg L^{-1} adenine sulfate, 300 mg L^{-1} timentin, 20 mg L^{-1} kanamycin, and 10% CW, then transferred to shoot elongation medium. Shoot elongation was optimized on MS medium with Gamborg B5 vitamins and 0.2 g L^{-1} glycine (MSB5G), containing 0-20 μM BA plus 0-10 μM TDZ, 300 mg L^{-1} timentin, and 20 mg L^{-1} kanamycin. The GUS and *nptII* genes were confirmed in hypocotyls, and three kanamycin-resistant shoots are in the process of being confirmed.

QUANTITATION OF REACTIVE OXYGEN SPECIES IN ASH PHLOEM TISSUE

Sourav Chakraborty, Amy Hill, and Pierluigi Bonello

Department of Plant Pathology, The Ohio State University
2021 Coffey Road, 201 Kottman Hall, Columbus, OH 43210
chakraborty.27@osu.edu

ABSTRACT

Reactive oxygen species (ROS) are by-products of photosynthesis and respiration in plant tissues. Previous research has shown that ROS can act as secondary messengers/chemical mediators in plant defense signaling. H_2O_2 is a key molecule among the ROS of plant and/or animal origin. It is produced in cells by enzymatic reduction and/or dismutation of superoxide. Attacks by both herbivores and pathogens induce the production and transient accumulation of H_2O_2 and other ROS in plants.

The emerald ash borer (EAB, *Agrilus planipennis* Fairmare, Coleoptera, Buprestidae) is an invasive insect pest that has been killing millions of susceptible North American ash trees since its discovery in Michigan in 2002. However, Manchurian ash (*Fraxinus mandschurica* Rupr.), a native species from China, is resistant to EAB, most likely due to its co-evolutionary history with the pest. We hypothesize that ROS, including H_2O_2, play a critical role in the biochemical defense of Manchurian ash, and predict that they can be induced by EAB attack in this species at higher levels than in susceptible species. However, no practical methods are available to quantitatively measure low levels of ROS in plants. We are currently developing fluorescence-based methods using DCFH and Amplex red dye for detection and quantification of ROS in ash tissues. Preliminary results show sub-micromolar detection of H_2O_2 and method optimization is currently in progress.

Regulatory, Management and Outreach

EFFICACY OF RADIOFREQUENCY (RF) ON EAB INFESTED ROUNDWOOD IN MICHIGAN

Ron Mack

[1]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542
Ron.mack@aphis.usda.gov

ABSTRACT

The use of radiofrequency (RF) was explored as a methyl bromide alternative treatment for solid wood packing material (SWPM) per ISPM-15. A 15 kW RF chamber with a 19 MHz operating frequency was custom built for USDA-CPHST researchers for feasibility study. EAB-infested ash roundwood bolts were field collected in Michigan for use in the experiment. Study objectives included a determination of lethal temperature for EAB using a "brute force" statistical approach that considers sample size and reliability, characterization of specific heating patterns in wood, and development of power density equations for overall economic analysis of the RF treatment for use on wood. Results indicate that RF has clear potential for treating ash to acceptable levels in a fraction of the time currently required by ISPM-15 standard. Future work planned includes additional pests of global concern as part of a comprehensive RF submission for ISPM-15 inclusion.

EVALUATION OF LOG SUBMERGENCE TO CONTROL EAB AND PRESERVE BLACK ASH FOR NATIVE AMERICAN BASKETRY

Therese M. Poland,[1,2] Damon J. Crook,[3] and Tina M. Ciaramitaro[1]

[1] USDA Forest Service, Northern Research Station, East Lansing, MI
tpoland@fs.fed.us

[2] Department of Entomology, Michigan State University, East Lansing, MI

[3] USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542

ABSTRACT

Many Native American cultures use black ash, *Fraxinus nigra*, for basket-making because its ring-porous wood allows the annual layers of xylem to be easily separated. The emerald ash borer (EAB, *Agrilus planipennis*) is threatening North America's ash resource including black ash, and a centuries-old native art form. Native tribes are gravely concerned about the availability of large black ash trees for basket making and about movement of black ash from areas where it is cut to tribal lands where it is pounded and split to make baskets. We evaluated the practice of storing black ash logs submerged in water to maintain their moisture as a possible method for simultaneously killing EAB. In 2010, we submerged EAB infested black ash logs in running water in the spring and evaluated EAB mortality as well as the color and quality of wood splints from the submerged ash logs at different time periods after submergence. In 2011, we evaluated the efficacy of submergence for different time periods in winter and in spring.

In 2010, five black ash trees infested with overwintering EAB larvae were felled in southern Michigan in late April 2010 and cut into 60-cm bolts. Bolts were randomly divided among 5 treatments with 8 logs per treatment: a) unsubmerged control bolts; or bolts submerged for b) 1 week; c) 1 month; d) 2 months; or e) 3 months. Logs were submerged on 10 May in the Red Cedar River, Okemos, Ingham County, MI. After treatment, half of the logs were dissected within 24 h to determine larval mortality then pounded and peeled into splints to assess splint color and quality. Larvae from dissected logs were held at room temperature for 24 h and observed periodically for movement. The remaining logs were placed into rearing tubes to determine survival and adult emergence. After 1 week of submergence, there was very little mortality of EAB larvae. Mortality was higher after 1 month of submergence; however, there were still several live EAB larvae and a few pupae. While some larvae had died, it appeared that others had fed and continued to develop. By 2 months and 3 months of submergence, all of the larvae had died. Similarly, the number of live EAB adults that emerged from logs decreased with length of submergence time and no adults emerged from logs that were submerged for 2 or 3 months. Water in the river was about 1.5 m deep with a flow rate of 0.88 to 1.05 m/s and average temperature of 13.5 ºC during the first month of the study. The temperature increased to 21.1 ºC while flow rate and depth decreased to 0.33 m/s and 36 cm, respectively, over the following 2 months. The spectral reflectance patterns for black ash splints were similar after submergence for different periods of time. Quality and pliability of splints did not decrease after 3 months of submergence.

In 2011, we conducted two experiments (winter submergence and spring submergence) to evaluate efficacy of log submergence at different times of the year with different water temperature and to refine the period of time required for complete mortality. Infested black ash trees were felled in February 2011 and cut into 60-cm bolts. For the winter submergence experiment, infested logs were submerged in the Red Cedar River during the first week of March when water temperature was freezing (0 ºC). We hypothesized that longer periods of submergence may be required at colder temperatures because larvae would remain dormant and would not attempt to feed and be affected by the saturated conditions. Therefore, we selected longer time periods for the treatments of this experiment. Logs were randomly divided among 5 submergence time treatments with 6-8 replicates per treatment: a) 2 months, b) 4 months, c) 6 months, d) 12 months (on-going), and e) 18 months (on-going). All logs were dissected within 24 h of removal from the river then pounded and peeled into splints in order to assess the impact of prolonged submergence on splint quality. Larvae dissected from logs were held for 48 h at room temperature and observed periodically for movement. Very few larvae died after 2 months of submergence at which time water temperatures had reached 5.6 ºC. All larvae had died after 4 or 6 months of submergence, when water temperatures had reached 21.5 ºC and 25.6 ºC, respectively. Splint pliability and quality did not deteriorate after 2-4 months of submergence; however, by 6 months the outer 1 or 2 rings of sapwood began to darken and decay and crumbled from the log during dissection. Interior rings of sapwood remained intact and pliable.

For the spring submergence experiment, infested logs were submerged in the Red Cedar River on 12 May 2011 when water temperature was 14.4 ºC. We wanted to refine our estimate based on 2010 results indicating that some larvae were alive at 1 month but all were dead after 2 months of submergence. Therefore, we selected weekly time periods between 1 and 2 months. Initially, we assigned 8 logs per treatment with 4 logs designated for dissection and 4 logs for rearing. However, by week 6 when we still found little mortality of larvae during log dissection, we decided to prolong the experiment by pulling only 4 logs for dissection each week and foregoing rearing, allowing those logs to remain submerged for a longer period. As a result the experiment had 9 treatments: a) unsubmerged control, or submerged for b) 4 weeks, c) 5 weeks, d) 6 weeks, e) 7 weeks, f) 8 weeks, g) 9 weeks, h) 13 weeks, or i) 14 weeks. For submergence periods up to 6 weeks, 4 logs were dissected to determine larval mortality and 4 logs were placed in rearing tubes to determine survival and adult emergence. For logs submerged 7, 8, 9, or 13 weeks, only 4 logs were dissected and no logs were placed in rearing tubes. After 14 weeks of submergence, the final logs were removed from the river and placed in rearing tubes to determine survival and adult emergence. After 6 weeks of submergence some live larvae were found in dissected logs and adults emerged from rearing logs. Some live larvae were found in logs submerged for 7 or 8 weeks, but by 9 and 13 weeks of submergence all larvae had died. No adults emerged from logs submerged for 14 weeks.

Although all EAB larvae had died and no adults emerged from logs submerged for 2 months in 2010, some larvae survived and adults emerged from logs submerged during winter for 2 months and during spring for up to 9 weeks, in 2011. It is possible that greater larval survival was found in 2011 because larvae were observed over a 48 h period after dissection rather than only 24 h as in 2010, allowing them more time to adjust after removal from the saturated logs. Different temperature conditions during the different experiments may also have influenced EAB survival. Based on our two years of data, 4 months of submergence during winter or spring should ensure complete mortality of EAB larvae and no emergence of adults while still preserving wood quality for basket making.

INVASION ALONG "AMERICA'S RHINE": DETECTION, DELIMITATION AND MANAGEMENT OF A LARGE SATELLITE INFESTATION OF EMERALD ASH BORER IN THE HUDSON RIVER VALLEY

Nathan W. Siegert[1], Jeffrey A. Rider[2], Michael J. Callan[2] Jeffrey A. Wiegert[2], and Michael J. Bohne[1]

[1]USDA Forest Service, Northeastern Area State & Private Forestry
Forest Health Protection, 271 Mast Road, Durham, NH 03824
nwsiegert@fs.fed.us

[2]New York State Department of Environmental Conservation
Division of Lands & Forests, 21 South Putt Corners Road, New Paltz, NY 12561

ABSTRACT

The establishment of new, isolated emerald ash borer populations and the expansion of existing satellite infestations rapidly increase the rate of spread of this pest across the landscape. Satellite infestations distant from the primary contiguous emerald ash borer populations and near large urban areas are predicted to most significantly affect spread and associated economic damages (Kovacs et al. 2011). Here, we provide information on the detection, delimitation and preliminary management of the easternmost known infestation of emerald ash borer in the United States to-date.

In July 2010, an established emerald ash borer population was detected in eastern New York State in the Hudson River Valley near the city of Kingston in Ulster and Greene counties. Its proximity to the Catskills, several economically high-risk urban areas (i.e., Albany, Poughkeepsie, New York City), ecologically sensitive watersheds that supply water to major metropolitan areas (i.e., New York City), and the six New England states caused considerable concern in the Northeast. Following the detection, a multi-agency delimitation survey and regional forest insect pest training opportunity was developed in cooperation with the USDA Forest Service, Northeastern Area State and Private Forestry and the New York State Department of Environmental Conservation (NYS-DEC) at the request of the state foresters in the Northeast.

The goal was to rigorously delimit the extent and distribution of the emerald ash borer infestation in the Hudson River Valley in winter 2010-11, so that science-based management options to reduce emerald ash borer population density and slow local expansion of the infestation in New York and southern New England could be developed, prescribed and implemented by spring 2011.

In late winter 2011, a 0.5 mile sampling grid over the approximately 225 sq. mile survey area was used to delimit the emerald ash borer infestation in the greater Kingston area. More than 500 plots were sampled. The survey area was expanded as necessary until no evidence of infestation was detected for ≥2 miles along the periphery of the survey area. Up to three trees were sampled per plot. Three bolts, each a meter long, were collected from the base of the canopy and from prominent branches

within the canopy of each tree. Felling of trees, stockpiling of bolts, and collection of plot-specific information was conducted from January through March 2011. Debarking of bolts and training of forest health personnel from 30+ agencies in New York and New England occurred in March 2010. More than 1438 m^2 of phloem area was examined for emerald ash borer on approximately 4500 ash bolts. The multi-agency delimitation survey identified several small isolated emerald ash borer infestations beyond the area known to be generally infested, including critical detections on the New York City watershed properties. No infestations were detected east of the Hudson River during this survey, suggesting that dispersing adults were more likely to spread along areas with continuous ash stands than fly across the river which is about a mile wide in the survey area.

Despite NYS-DEC having limited resources for implementing management in 2011, they nonetheless established detection trees along the eastside of the Hudson River (n = 25 trees) and created clusters of girdled trees to serve as population sinks (n = 125 trees). These trees will be evaluated in winter 2011-12. In addition, parasitoid releases were conducted in the generally-infested area in co-operation with APHIS and ARS researchers. Funding to support management efforts at this site over the next 2-3 years has been secured and it is anticipated that management strategies will be developed annually to most effectively reduce local emerald ash borer population growth and possibly slow the progression of ash mortality at this large, satellite infestation.

In addition to producing results needed to most effectively distribute limited resources, NYS-DEC's delimitation survey has strengthened working relationships with numerous other agencies in New York and New England and, in turn, created several opportunities for forest health personnel, foresters and arborists from across the region to develop the skills necessary to detect, delimit and survey for low-density emerald ash borer populations in their respective states.

REFERENCES

Kovacs, K.F., R.J. Mercader, R.G. Haight, N.W. Siegert, D.G. McCullough and A.M Liebhold. 2011. The influence of satellite populations of emerald ash borer on projected economic costs in U.S. communities, 2010-2020. *Journal of Environmental Management*. 92(9): 2170-2181. DOI: 10.1016/j.jenvman.2011.03.043.

MANAGING EMERALD ASH BORER IN WOODLOTS: AN INTEGRATED APPROACH

Houping Liu, Timothy M. Frontz, and Donald A. Eggen

[1]Pennsylvania Department of Conservation and Natural Resources
208 Airport Dr., Middletown, PA 17057
hliu@pa.gov

ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an exotic wood-borer from northeast Asia, was first discovered attacking ash trees in Michigan in 2002. Since then, it has been found in 15 U. S. states and two Canadian provinces across the Great Lakes region and beyond. Larval feeding in the cambial region disrupts water and nutrient transportation inside the tree, resulting in tree mortality within 4-5 years. An estimated 20 to 55 million ash trees have been killed by this pest in the infested areas. The potential economic damage may exceed $10 billion in 25 states expected to be affected within in the next 10 years (Kovacs et al. 2010). Managing this pest in North America has been confounded by difficulties in early detection, limitations in control options, and scarcity in available resources.

A total of 22 counties in Pennsylvania are currently infested by EAB and the entire state is under federal quarantine. Many communities will be forced to face the economic and ecological challenges brought along by this pest in the years to come. We are putting together an integrated project that includes limited tree removal, chemical treatment, and biological control to serve as a model for EAB management in Pennsylvania.

A 120-ha study site was selected from North Park in Allegheny County where EAB was first recorded in 2009. Green ash is concentrated in 15 0.1-ha plots (**A**-**N**) within the site. Ash resource and its conditions were documented at the beginning of the project through inventory. The basal area for each plot (except one) was measured at three spots using a wedge prism (BAF 10). Ash species, GPS coordinates, and diameter-at-breast-height (DBH) were recorded for each tree. Crown condition was rated based on dieback rate (0-100%) at an increment of 5% after full flush. Only trees > 10 cm were included. EAB population at the study site was estimated through tree dissection (2010) and adult trapping (2011). For tree dissection, a total of four 30-cm sections were sampled from the main trunk of 9 trees at 2, 4, 6, and 8 m above the ground. Each trunk section was then dissected for EAB life stages (larvae, prepupae, pupae, and emerging adults). For adult trapping, a total of 10 purple panel traps baited with Manuka oil were deployed in 10 selected plots (one trap/plot) in early June. Traps were monitored and EAB adults removed and recorded every 2 weeks until early September. Limited tree removal was carried out in combination with normal salvage and sanitation operations in the park to reduce EAB populations. Heavily infested trees along major roads and popular trails within the center were removed first. Chemical insecticide Tree-*äge*® (emamectin benzoate) is highly effective against EAB larvae when applied through trunk injection (Herms et al. 2009). This insecticide was

used to protect high-value ash trees with ecological, historic, recreational, or aesthetic significance throughout the center. Only trees with a crown dieback rate <30% were treated. Biological control has the potential to provide long-term management of EAB across different habitats and landscapes (Gould et al. 2010). Three hymenopterans, including one egg parasitoid, *Oobius agrili* Zhang and Huang (Encyrtidae), and two larval parasitoids, *Tetrastichus planipennisi* Yang (Eulophidae) and *Spathius agrili* Yang (Braconidae) were introduced to one plot between June and August in 2011.

Results showed that the average basal area ranged from 17.7 to 33.8 m^2/ha for the study plots, with green ash accounting for 11.1% (**L**) to 79.6% (**H**). Other major tree species include black cherry, yellow poplar, black walnut, red maple, red and white oak. A total of 713 trees were marked, with the DBH ranging from 10.4 to 94.0 cm. The number of ash trees in each plot ranged from 5 (**N**) to 152 (**O**). Most of them (>70%) are in the size class of 12.8-38.1 cm (5 to 15 inches). Crown dieback varied in ash trees among different plots in 2010, ranging from 0.9% (**L**) to 59.7% (**H**). Health conditions of ash trees continued to deteriorate from 2010 to 2011, with a higher crown dieback rate observed in all plots except **M** and **N** where crowns were not rated in 2010. All trees were infested based on log dissection, with the density ranging from 4.4 to 140.6 insects/m^2. The highest density was found at 6 m along the trunk, followed by 8, 4, and 2 m. A total of 115 adults were caught on the traps, with 69.6% recorded by the week of June 23 in 2011. Population declined toward the end of July, with the last adult recovered in the week of August 31. Traps placed in plot **E** caught the most beetles through the season, followed by those in plot **O**, **H**(**I**), **J**, and **A**(**D,N**). No adults were recorded from plot **F**(**M**). A total of 17 infested trees were removed. A total of 249 ash trees (7,283 cm total DBH) were treated with Tree-*äge*® through trunk injection in 2011, including 99 small (< 25.4 cm), 146 medium (25.5 – 63.5 cm), and 4 large (>63.5 cm) trees. The percentage of trees treated in each plot ranged from 0 (**I**) to 100% (**M** and **N**). A total of 60 trees were treated in plot **D**, followed by plot **A** (37) and **L** (34). Treatment efficacy will be evaluated in 2012. A total of 5,429 parasitoids were released in the study site, including 2,696 *T. planipennisi*, 1,758 *S. agrili*, and 975 *O. agrili* through multiple releases between June and August. The treatment efficacy of Tree-*äge*® and the establishment of parasitoids at the study site will be monitored in 2012.

REFERENCES

Gould, J.S., Bauer, L.S., Duan, J., and Buck, J.H. 2010. Emerald ash borer, *Agrilus planipennis* (Fairmaire), biological control release guidelines. USDA-APHIS-ARS-FS. Riverdale, MD.

Herms, D.A., McCullough, D.G., Smitley, D.R., Sadof, C.S., Williamson, R.C., and Nixon, P.L. 2009. Insecticide options for protecting ash trees from emerald ash borer. North Central IPM Center Bulletin. 12 pp.

Kovacs, K.F., Height, R.G., McCullough, D.G., Mercader, R.J., Siegert, N.W., and Liebhold, A.M. 2010. Cost of potential emerald ash borer damage in U. S. communities, 2009-2019. Ecological Economics 69: 569-578.

MAPPING ASH IN HIGH PUBLIC USE AREAS: A CITIZEN SCIENCE APPROACH

Constance E. Hausman and Terry L. Robison

Division of Natural Resources, Cleveland Metroparks, Cleveland, OH 44126
ceh@clevelandmetroparks.com

ABSTRACT

Public land management agencies are among those coping with the effects of emerald ash borer (EAB) and the need to remove ash trees. Knowing the location, quantity, size, and condition of ash trees can help prioritize areas for tree removal. However, staff time to conduct ash tree surveys is limited because of budget restrictions and available resources. To assist with tree inventory, we designed and implemented a volunteer driven ash tree survey of Cleveland Metroparks all-purpose trail (APT).

The APT is an 85 mile network of paved trails through Cleveland Metroparks 16 reservations. It is used by walkers, runners, bicyclists, roller bladders, and strollers, and is the most used feature in the Park District. The trail primarily borders parkway roads passing along streams and rivers and through natural areas including forests and meadows.

Park District volunteers known as Trail Monitors regularly walk the APT assisting park visitors and reporting trail conditions to park management staff. Because of their enthusiasm and dedication, several Trail Monitors were recruited to serve on the ash mapping team. These volunteers participated in training to learn project goals, EAB biology, tree species identification, survey design, data collection techniques, and use of hand-held global positioning system (GPS) receivers. A field training component focused on winter tree identification, estimating tree size, and recognizing woodpecker feeding damage.

The survey design includes sample points at 50 meter intervals along the APT. At each point, observers surveyed both left and right sides of the trail. The sample area consisted of a 90º observation window where ash trees were tallied in each of 3 DBH size classes: 4-12 inches, 13 to 20 inches, and >20 inches. Signs of woodpecker feeding were recorded as affecting 0, 1-50, or 51-100 percent of ash trees in the sample area.

Four survey teams consisting of two volunteers each surveyed 4 of the Park Districts 16 reservations during this pilot year. Data were entered into a geographic information system to summarize and display survey information by ash tree location, size, and woodpecker feeding activity. Maps were produced for each reservation and are being used by Cleveland Metroparks Divisions of Natural Resources, Forestry, and Park Operations to prioritize management and restoration areas.

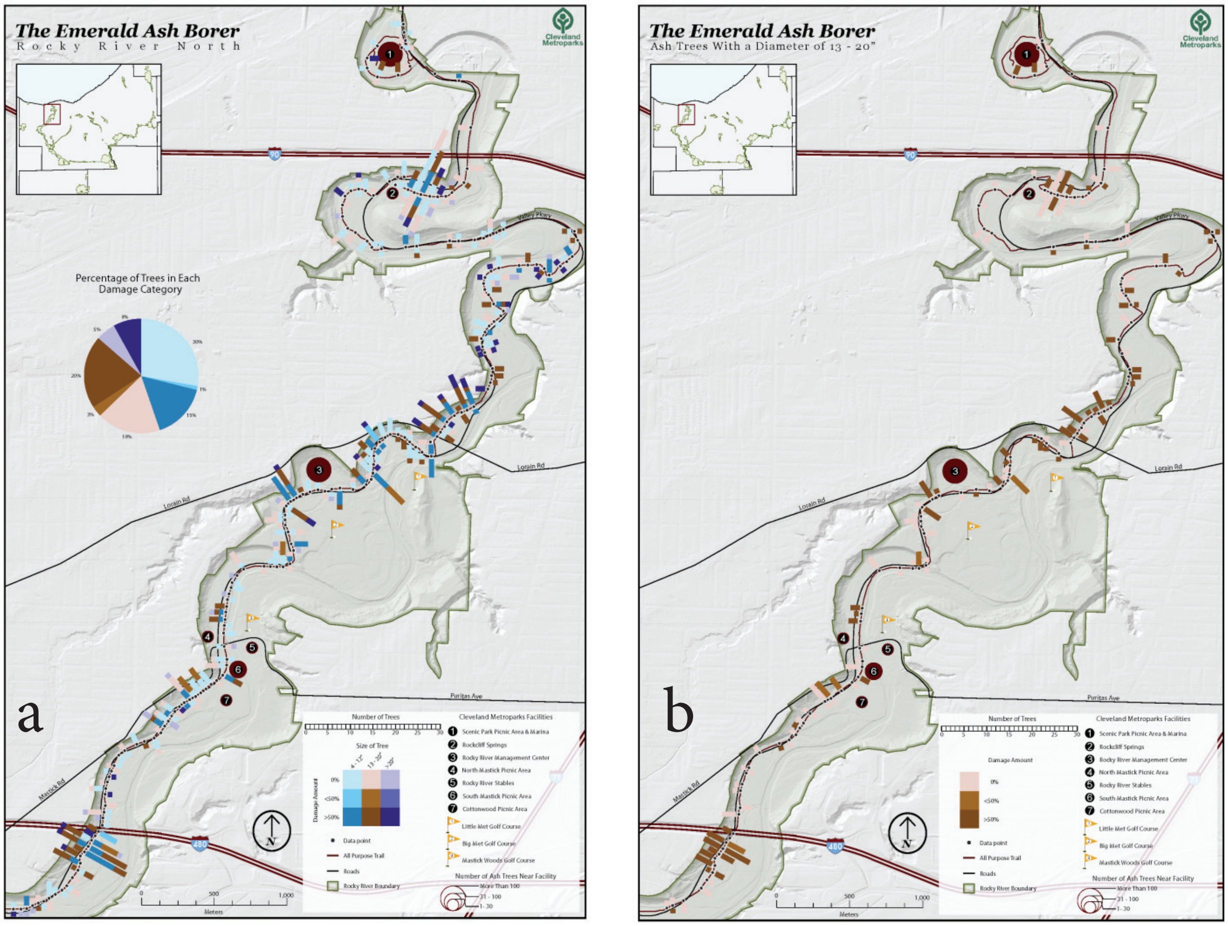


Figure 1. Sample ash tree location maps created using data collected by volunteer "ash mappers". The left map (a) illustrates the northern section of Rocky River Reservation, which contains the highest density of ash trees in the Park District. Blue shades represent small trees (4-12"), orange shades represent mid-size trees (13-20"), and purple shades indicate large trees (>20"). Increasing darkness of bars corresponds to greater woodpecker feeding activity. On the right (b), only mid-sized trees (13-20") at each sample point are represented by bar length with increasing length indicating more trees. Darker shaded bars indicate greater levels of woodpecker feeding activity.

SURVEY AND TRAPPING

LATEST DEVELOPMENTS ON EMERALD ASH BORER MONITORING USING SEMIOCHEMICAL-BAITED FUNNEL TRAPS

D. J. Crook[1], K. Ryall[2], P. J. Silk[3], and V. C. Mastro[1]

[1]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542
Damon.J.Crook@aphis.usda.gov

[2]Natural Resources Canada, Great Lakes Forestry Centre
1219 Queen Street East, Sault Ste Marie, ON, Canada

[3]Natural Resources Canada, Atlantic Forestry Centre
1350 Regent Street, Fredericton, NB, Canada

ABSTRACT

In 2010 the standard survey trap used nation-wide by the USDA-APHIS-PPQ-EAB Cooperative Program was a glued, purple, prism trap, baited with a semiochemical lure (manuka oil, 50mg /d), hung at 6m in the sub-canopy of an ash tree. A more user-friendly 'non glued' trap would be desirable because tens of thousands of glued traps are currently deployed and discarded at the end of each year. For program use, lure release devices should ideally last 3 months without being changed.

Recent electroretinographic assays and trapping work have shown that light green traps catch significantly more emerald ash borer adults (especially males) than traps of other colors (Crook et al, 2009). Light green traps typically catch more adults only when deployed high in the tree canopy. Thus, trap placement, as well as color and lure combination, must be considered when evaluating traps for a monitoring program. Francese et al. (2010) recently improved the attractancy of light green (540nm) prism traps by adjusting the reflectance of the green to 49% (i.e. creating a darker green). When this dark green color was incorporated into funnel traps and then field tested alongside standard purple prism traps in an unbaited study, dark green funnel traps caught significantly more beetles when hung at 5~8m (Francese et al. 2011). Funnel trap catch was improved further by the addition of Rain-X. No lures have yet been tested on the new dark green funnel traps. Two types of host volatiles have been demonstrated to be attractive to *A. planipennis*, bark sesquiterpenes (found in Manuka and Phoebe oil) and leaf volatiles (particularly (3*Z*)-hexenol). The first putative long-range pheromone for *A. planipennis* was identified as (3*Z*)-dodecen-12-olide [(3*Z*)-lactone] by Bartelt et al. (2007) although no behavioral activity was reported. Silk et al. (2011) demonstrated that (3*Z*)-lactone significantly increased male trap catch when combined with the green leaf volatile, (3*Z*)-hexenol, in light green prism traps deployed in the canopy. Captures of males with the (3*Z*)-lactone + (3*Z*)-hexenol were at least 50-100% greater compared to the (3*Z*)-hexenol. It appears that two cue modalities are required by *A. planipennis* in the host & mate-finding process: a visual cue (green) and a two-component olfactory cue: a foliage volatile (kairomone), (3*Z*)-hexenol, and the sex pheromone, (3*Z*)-lactone. By fine-tuning each of these three components it should be possible to improve trap effectivity even further.

The main aim of our 2011 research was to test the latest dark green funnel traps with the most promising available lures. The lure treatments were:

1. unbaited green funnel control
2. green funnel trap baited with Manuka oil (50mg/d)+ (3Z)-hexenol (50mg/d)
3. green funnel trap baited with (3Z)-hexenol (50mg/d)
4. green funnel trap baited with (3Z)-hexenol (50mg/d) + (3Z)-lactone (80ug/d)

Field tests on dark green funnel traps were carried out along the edges of infested white and green ash wood lots in Michigan, USA (n=15), as well as Ontario, Canada (n=17). USA field sites contained ash trees with moderate to severe levels of decline. Canadian field sites contained ash trees with low levels of decline. Rain-X coated dark green funnel traps were set within 2 m of tree stands, spaced 20-30 m apart in a randomized complete block design. Traps were checked thru June and July 2011 every week in the USA and every 2 weeks in Canada. All *A. planipennis* were collected, sexed and summed for the entire field season. Catches of males, females and total catch (males plus females) were analyzed separately for each experiment. Data from all experiments were transformed by log (x + 0.5) before being analyzed by randomized complete block design ANOVA. Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$) was used to compare differences in catch for each sex between treatments.

At the low infested sites in Canada and the high level infestated sites in USA, highest male catch was seen on green funnel traps baited with (3*Z*)-hexenol + (3*Z*)-lactone. No significant differences were seen in trap catch for males, females or total (male + female) in either study.

Our results show that in low and medium/high infestation areas dark green funnel traps (coated with Rain-X) caught high numbers of *Agrilus planipennis* with or without the presence of manuka, (3*Z*)-hexenol or (3*Z*)-lactone lures. At the release rate used (3*Z*)-Hexenol did appear to help increase male trap catch but the differences were not significant. At the release rate used, the (3*Z*)-lactone lure improved male catch slightly (not significantly) in both regions. Further testing of the (3*Z*)-lactone and (3*Z*)-hexenol at difference release rates on these traps is ongoing, along with further trap placement studies. It is hoped that this will optimize trap catch and further improve the detection rate of the current monitoring program effort for this insect.

REFERENCES

Bartelt, R. J., Cossé, A. A., Zilkowski, B. W., and Fraser, I. (2007). Antennally active macrolide from the emerald ash borer, *Agrilus planipennis* emitted predominantly by females. J. Chem. Ecol. 33:1299–1302.

Crook, D. J,. Francese, J. A. Zylstra, K. E. Fraser, I., Sawyer, A.J ., Bartels, D. W,. Lance, D. R., and Mastro, V.C. (2009). Laboratory and field response of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), to selected regions of the electromagnetic spectrum. J. Econ. Entomol. 102:2160–2169.

Francese, J.A., Crook, D.J., Fraser, I., Lance, D. R., Sawyer, A. J., and Mastro, V. C. (2010). Optimization of trap color for emerald ash borer (Coleoptera: Buprestidae). J. Econ. Entomol 103(4): 1235-1241.

Francese, J.A., Fraser, I., Lance, D. R., and Mastro, V. C. (2011). Efficacy of multifunnel traps for capturing emerald ash borer (Coleoptera: Buprestidae): Effect of color, glue and other coatings. J. Econ. Entomol. 104 (3): 901-908.

Silk, P. J., Ryall, K., Mayo, P., Lemay, M. A., Grant, G., Crook, D. J., Cossé, A., Fraser, I., Sweeney, J. D., Lyons, D. B., Pitt, D., Scarr, T., and Magee, D. (2011). Evidence for a Volatile Pheromone in *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) that Increases Attraction to a Host Foliar Volatile. Environ. Entomol. 40(4): 904-916.

NEW PRISM TRAP COLORS FOR IMPROVING SURVEY OF EMERALD ASH BORER

Joseph A. Francese[1], Michael L. Rietz[2], David R. Lance[1]
Ivich Fraser[2], and Victor C. Mastro[1]

[1]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542
joe.francese@aphis.usda.gov

[2]USDA APHIS PPQ CPHST, Brighton Laboratory, Brighton, MI 48116

ABSTRACT

The current emerald ash borer survey trap used in the US is a prism trap constructed from a stock purple corrugated plastic. Recent electroretinogram assays have demonstrated that male and female EAB are sensitive to light in the UV, violet and green (420 – 430, 460 nm and 530 – 560, respectively) ranges of the visible spectrum, while mated females are also sensitive to light in the red (640 – 670 nm) range (Crook et al. 2009). Trapping studies have shown that green traps painted in the mid-range (22%-67%) of reflectance (brightness), and purple traps painted with a color originally shown to be attractive to buprestids, were most attractive to EAB (Francese et al. 2010). Several plastics have been produced from these colors, and our goal was to determine if these plastics can improve on and serve as a new alternative to plastics already in use for EAB survey. In 2010, six colors were tested at ~5-8 m in the lower canopy of heavily infested ash woodlots.

1. Coroplast Purple plastic – the standard color used for EAB survey in the US since 2008
2. TSU Purple paint – a color found by Oliver et al. (2002) to be attractive to at least 31 species of buprestids including some *Agrilus* spp.; painted on translucent prism traps
3. A New Purple plastic (A) – a newly manufactured plastic based on the TSU purple paint
4. An Old Green plastic – manufactured from a paint described by Crook et al. (2009) as being attractive to EAB; 540 nm, 67% reflectance; sometimes referred to as light green
5. A New Green paint – found by Francese et al. (2010) to also be attractive to EAB; 530 nm, 49% reflectance; sometimes referred to as medium or dark green
6. A New Green plastic – a newly manufactured plastic based on the New Green paint

In 2011, treatments 1, 3, 4 and 6 were tested along with two other colors.

1. New Purple plastic B – a purple plastic based on TSU purple, manufactured by a different company than A
2. Purple Card-stock – a wax-coated card-stock prism designed to be more "user-friendly"; folds to a small size and is lighter than corrugated plastic

New purple and green plastic traps showed promise as alternatives to the standard purple corrugated plastic, but there was greater degree of variability in catch on green traps than on purple traps in 2010 and among all traps in 2011. Several of the purple card-stock traps fell apart during the field season so these may need work if they are to be an alternative to the current corrugated plastic.

REFERENCES

Crook, D. J., J. A. Francese, K. E. Zylstra, I. Fraser, A. J. Sawyer, D. W. Bartels, D. R. Lance, and V. C. Mastro. 2009. Laboratory and field response of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) to selected wavelength regions of the visible spectrum. J. Econ. Entomol. 102: 2160 - 2169.

Francese, J. A., D. J. Crook, I. Fraser, D. R. Lance, A. J. Sawyer, and V. C. Mastro. 2010 . Optimization of trap color for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). J. Econ. Entomol. 103: 1235 - 1241.

Oliver J. B., N. Youssef, D. Fare, M. Halcomb, S. Scholl, W. Klingeman, and P. Flanagan. 2002. Monitoring buprestid borers in production nursery areas. pp. 17-23. In G. Haun (ed.), Proceedings of the 29th Annual Meeting of the Tennessee Entomological Society. 10-11 October 2002. Nashville, TN. 17: 12-13.

THE SCENT OF A BUPRESTID: HOW THE SMOKY-WINGED BEETLE BANDIT RECOGNIZES HER PREY

Claire E. Rutledge[1] and Peter Silk[2]

[1]The Connecticut Agricultural Experiment Station, New Haven, CT
Claire.Rutledge@ct.gov

[2]Natural Resources Canada-Canadian Forest Service, Fredericton, NB, Canada

ABSTRACT

Cerceris fumipennis uses a wide range of adult buprestid beetles to provision her nest. Wasps seldom make 'mistakes' and generally ignore beetles in other families. Bio-assays showed that the wasps use contact chemical cues in the beetles' epicuticle to discriminate between buprestids and other beetles. Buprestid beetles washed in hexanes were not accepted by the wasps, but 68% of wasps accepted the washed beetles when re-coated with the extracted cuticular hydrocarbons. About 40% of wasps tested accepted washed beetles coated with just the female contact sex pheromone, 9Me-C25 of *Agrilus planipennis*. Washed beetles coated with extracts of non-buprestid beetles were not accepted. We analyzed the hydrocarbon profile of 10 common *C. fumipennis* prey items, which comprised 6 genera and 3 subfamilies. The hydrocarbon profiles are relatively simple, using only a few classes of hydrocarbons, namely straight-chain, odd numbered hydrocarbons ranging from nC21-nC29, monomethyl-alkanes with odd-numbered chain lengths from C23-C29 and methyl groups at the 9^{th}, 11^{th} and 13^{th} carbon, and single alkenes. Straight chain alkanes were found in all sub-families, whereas monomethyl alkanes were found only in the Agrilinae and Buprestinae, and alkenes were found only in the Buprestinae and the Chrysochroinae. Comparisons with published literature showed that buprestids were distinct from other arboreal beetle families, which had more complex hydrocarbon profiles. Experiments adding a crude extract of buprestid hydrocarbons to unwashed Japanese beetles, which have dimethyl alkanes, suggest that they may be deterred by the presence of hydrocarbon groups not present in Buprestidae. Future work will examine more members of the Buprestidae to see if these patterns hold up across the family.

UTILIZING GIRDLED ASH TREES FOR OPTIMAL DETECTION, DELIMITATION AND SURVEY OF LOW-DENSITY EMERALD ASH BORER POPULATIONS

Nathan W. Siegert[1], Nicholas J. Gooch[2], Deborah G. McCullough[2]
Therese M. Poland[3], and Robert L. Heyd[4]

[1]USDA Forest Service, Northeastern Area State & Private Forestry, Forest Health Protection,
271 Mast Road, Durham, NH 03824
nwsiegert@fs.fed.us

[2]Department of Entomology, Michigan State University
243 Natural Sciences Building, East Lansing, MI 48824

[3]USDA Forest Service, Northern Research Station, Stephen S. Nisbet Building,
1407 S. Harrison Road, Room 220, East Lansing, MI 48823

[4]Michigan Department of Natural Resources
1990 US-41 South, Marquette, MI 49855

ABSTRACT

Early detection of emerald ash borer increases the likelihood that management tactics will effectively reduce population growth of infestations and slow the progression of ash mortality. Consequently, monitoring for emerald ash borer remains a high priority in many states. Stressed ash trees are highly attractive to dispersing emerald ash borer adults and girdled trees are an efficacious tool that may be used for detection of low-density populations. We present here results from three large-scale field studies that examined various aspects of using girdled trees as detection tools in the field. Specifically, we evaluated: (1) the detection ability of four girdled trees per 4 ha (10 ac) compared to 16 girdled trees per 4 ha (10 ac); (2) the efficacy of 3-tree versus 12-tree clusters of girdled trees; and (3) adult beetle emergence from girdled trees the summer after being used as trap trees.

Girdled trees were significantly more attractive to dispersing adult beetles and colonized at higher rates than non-girdled trees in these three studies. Results suggest that low-density emerald ash borer populations in forested areas can be detected as readily with lower densities and smaller clusters of girdled trees. Specifically, establishing girdled ash trees at four trees per 4 ha (10 ac) was as effective at detecting low densities of emerald ash borer as establishing girdled ash trees at 16 trees per 4 ha (10 ac). Also, establishing 3-tree clusters of girdled ash trees was as effective at detecting low densities of emerald ash borer as establishing 12-tree clusters of girdled ash trees. Results additionally suggest that adult emerald ash borer emergence from girdled trees may be negligible if not debarked or removed after being used as trap trees, thereby greatly improving the economics of utilizing girdled trees as a management tool. Density of emerald ash borer adults that emerged from girdled trees one year after being used as trap trees was very low on trees that were bucked into meter lengths (0.7 ± 0.2 per m2) and was positively correlated with both prepupal emerald ash borer density (R2 = 0.81) and

overall emerald ash borer density (R2 = 0.62). Girdled trees that were bucked and left on the ground produced an average of 2.3 ± 0.8 emerald ash borer adult beetles per tree and incurred no additional predation by woodpeckers. Girdled trees that were felled but not bucked into meter lengths produced a similar number of emerald ash borer adult beetles (2.4 ± 1.6 per tree) due to increased predation by woodpeckers (3.0 ± 2.3 emerald ash borer prepupae consumed by woodpeckers per tree). Variability of emerald ash borer adult beetle emergence, however, was considerably greater on whole trees (range: 0 – 8.3) than bucked trees (range: 0.3 – 4.3). These results suggest that girdled detection trees left on-site should be bucked to facilitate drying, thereby increasing the likelihood of only negligible emerald ash borer adult emergence the following summer. Overall, preliminary results from these three field studies provide useful guidelines for using girdled ash trees for optimal detection, delimitation and survey of low-density emerald ash borer populations.

Attendees

Last	First	Organization	Location	E-mail Address
Abell	Kristopher	Michigan State University	MI	abellk@msu.edu
Abrahamson	Mark	Minn. Dept. of Agriculture	MN	Mark.Abrahamson@state.mn.us
Adkins	Dave	Ohio Dept. of Agriculture	OH	adkins@agri.ohio.gov
Aiken	Joe	Arborjet	MA	joeaiken@arborjet.com
Ambe	Shu			
Anulewicz	Andrea	Michigan State University	MI	andreaa@msu.edu
Appleton	Erin			
Aukema	Brian	University of Minnesota	MN	BrianAukema@umn.edu
Bauer	Leah	USDA FS Northern Research Station	MI	lbauer@fs.fed.us
Bean	Dick	Maryland Dept. of Agriculture	MD	dick.bean@ maryland.gov
Bernick	Shawn	Rainbow Tree Care Scientific	MA	
Bienemann	David S.	City of Bowling Green	OH	DBienemann@bgohio.org
Bolan	Paul	BioForest Technologies Inc.	CANADA	pbolan@bioforest.ca
Bonello	Pierluigi	Ohio State University Plant Pathology	OHIO	bonello.2@osu.edu
Bonham	J Bradford			
Bournay	Jacob	Michigan State University	MI	bournayj@msu.edu
Buck	Jim	APHIS USDA		James.H.Buck@aphis.usda.gov
Burks	Susan	Minnesota DNR Forestry	MN	susan.burks@dnr.state.mn.us
Burlett	Edward			
Burns	Barbara		VT	
Burr	Stephen	Michigan State University	MI	burrstep@msu.edu
Campbell	Julian	Bluegrass Woodland Restoration Center	KY	julian.campbell@insightbb.com
Carballo	Gabriel	Michigan State University	MI	carball1@msu.edu
Cardina	John	Ohio Agric. Res. & Dev. Center, Entomology	OH	cardina.2@osu.edu
Carey	Dave	Ohio Agric. Res. & Dev. Center, Entomology	OH	dcarey@fs.fed.us
Chakraborty	Sourav	Ohio State University Plant Pathology	OH	chakraborty.27@osu.edu
Chaloux	Paul	APHIS USDA	FL	Paul.Chaloux@aphis.usda.gov
Chamberlin	Joe	Valent USA		jcham@valent.com
Chambers	Bryant	Ohio Agric. Res. & Dev. Center, Entomology	OH	chambers.94@osu.edu
Chiriboga	Alejandro	Ohio Agric. Res. & Dev. Center, Entomology	OH	chiriboga.3@osu. edu
Cinnamon	Mark	Illinois Dept. of Agriculture	IL	mark.cinnamon@illinois.gov
Cipollini	Don	Wright State University	IN	don.cipollini@wright.edu
Clanton	David			
Collins	Anne H.	Michigan Technical University	MI	ahcollin@mtu.edu

Last	First	Organization	Location	E-mail Address
Cosky	Steve	Syngenta Crop Protection PPC		steve.cosky@syngenta.com
Costilow	Kyle	Ohio Agric. Res. & Dev. Center, Entomology	OH	costilow.5@osu.edu
Crook	Damon	USDA, APHIS	MA	Damon.J.Crook@aphis.usda.gov
Darnell	Stephanie	Bayer CropScience		stephanie.darnell@bayercropscience.us
Davis	Samantha	Wright State University	OH	davis.598@wright.edu
Dawson	Nevin	College of Agric. & Nat. Res., University of Maryland	MD	ndawson@umd.edu
Dean	Kim	State University of New York College of Env. Sci. & For.	NY	kmdean01@syr.edu
Dillner	Dan			
Discua	Samuel	Ohio Agric. Res. & Dev. Center, Entomology	OH	discua.1@osu.edu
Domingue	Michael	Penn State University	PA	mjd29@psu.edu
Duan	Jian	USDA ARS B11R	DE	Jian.Duan@ars.usda.gov
Eitam	Avi	APHIS USDA	OH	Avraham.Eitam@aphis.usda.gov
Esden	Jim			
Fahrner	Samuel	University of Minnesota	MN	fahr0051@umn.edu
Fausey	Jason	Valent USA		Jason.Fausey@valent.com
Fierke	Melissa	State University of NY College of Env. Sci. & For	NY	mkfierke@esf.edu
Flower	Charles	University of Illinois	IL	cflowe3@uic.edu
Foelker	Chris	State University of NY College of Env. Sci. & For	NY	cjfoelke@syr.edu
Fraedrich	Bruce	Bartlett Tree Expert Co.		bfraedrich@bartlettlab.com
Francese	Joe	USDA, APHIS	MI	Joe.Francese@aphis.usda.gov
Fraser	Ivich	USDA Forest Service	MI	ifraser@fs.fed.us
Fuester	Roger	USDA-ARS-BIIR	DE	Roger.Fuester@ars.usda.gov
Gagne	Lucie			
Gandhi	Kamal JK	Forestry & Natural Res. University of Georgia	GA	kgandhi@warnell.uga.edu
Gertin	Claude	INRS - Institut Armand-Frappier	CANADA	claude.guertin@iaf.inrs.ca
Ginzel	Matthew	Purdue University	IN	mginzel@purdue.edu
Goetsch	Warren	Illinois Dept. of Agric.	IL	warren.goetsch@illinois.gov
Gottschalk	Kurt W.	USDA Forest Service Northern Research Station	WV	kgottschalk@fs.fed.us
Gould	Juli	USDA, APHIS	MA	Juli.R.Gould@aphis.usda.gov
Grant	Jerome	University of Tennessee Knoxville	TN	jgrant@utk.edu
Hahn	Jeff	University of Minnesota Dept. of Entomology	MN	hahnx002@umn.edu

Last	First	Organization	Location	E-mail Address
Hanks	Larry	University of Illinois	IL	hanks@life.illinois.edu
Hansel	James	Great Lakes IPM	MI	
Hanson	Trish			
Hartzler	Diane	Ohio Agric. Res. & Dev. Center, Entomology	OH	hartzler.1@osu.edu
Haun	Walker G.	Tennessee Dept. of Agriculture	TN	Walker.Haun@tn.gov
Hausman	Constance	Cleveland Metroparks	OH	ceh@clevelandmetroparks.com
Herms	Catherine	Ohio Agric. Res. & Dev. Center, Entomology	OH	herms.3@osu.edu
Hill	Amy	USDA Forest Service	WV	amyhill@fs.fed.us
Hyslop	Mike	School of Forestry Res. & Environ. Sci., Michigan Technical University	M I	mdhyslop@mtu.edu
Iverson	Louis	Northern Research Station, USDA Forest Service		liverson@fs.fed.us
Johnson	Todd D.	University of Wisconsin - Madison	WI	todd.johnson@ces.uwex.edu
Jones	Grant	Davey Tree		gjones@davey.com
Katovich	Steven	USDA Forest Service, NA S&PF	MN	skatovich@fs.fed.us
Keena	Melody	USDA Forest Service, ARS	CT	mkeena@fs.fed.us
Klooster	Wendy	Ohio Agric. Res. & Dev. Center, Entomology	OH	klooster.2@osu.edu
Knight	Kathleen	USDA Forest Service Northern Research Station		ksknight@fs.fed.us
Koch	Jennifer	Ohio Agric. Res. & Dev. Center, Entomology	OH	jkoch@fs.fed.us
Krafka	Devin	Joliet Junior College	IL	
Lance	Dave	USDA, APHIS	MA	David.R.Lance@aphis.usda.gov
Lavallee	Robert	Candian Forest Service Natural Resources Canada	CANADA	Raymond.Pigati@state.mn.us
LeDoux	Douglas	Missouri Dept. of Agriculture	MO	Douglas.LeDoux@mda.mo.gov
Lelito	Jonathan	APHIS	PA	Jonathan.Lelito@aphis.usda.gov
Lewis	Phil	USDA, APHIS	MA	Phillip.A.Lewis@aphis.usda.gov
Liu	Houping	Forest Pest Management	PA	hliu@state.pa.us
Lloyd	John	Urban Forestry Institute	FL	
Long	Beth	University of Tennessee	TN	ealong@utk.edu
Long	Larry	Ohio Agric. Res. & Dev. Center, Entomology	OH	long.643@osu.edu
Lyons	Barry	Natural Resources Canada	CANADA	Barry.Lyons@NRCan-RNCan.gc.ca
Malinoski	Mary Kay	University of Maryland Extension	MD	mkmal@umd.edu

Last	First	Organization	Location	E-mail Address
Mamidala	Praveen	Ohio Agric. Res. & Dev. Center, Entomology	OH	mamidala.2@osu.edu
Marcotte	Mireille	Canadian Food Inspection Agency	CANADA	mireille.marcotte@inspection.gc.ca
Marshall	Phil	Indiana Dept. of Natural Resources	IN	PMarshall@dnr.IN.gov
Martinson	Holly	University of Maryland Dept. of Entomology	MD	hmartins@umd.edu
Mason	Mary	Ohio Agric. Res. & Dev. Center, Entomology	OH	mason.33@osu.edu
Mastro	Vic	USDA, APHIS		Vic.Mastro@aphis.usda.gov
McCullough	Deb	Michigan State University	MI	mccullo6@msu.edu
Mejia	Rina	Ohio Agric. Res. & Dev. Center, Entomology	OH	
Miller	Fredric	Joliet Junior College	IL	fmiller@jjc.edu
Mittapalli	Omprakash	Ohio Agric. Res. & Dev. Center, Entomology	OH	mittapalli.1@osu.edu
Muilenburg	Vanessa	Ohio Agric. Res. & Dev. Center, Entomology	OH	muilenburg.1@osu.edu
Nagle	Annemarie	Exotic Forest Pest Educator Purdue University	IN	naglea@purdue.edu
Nielsen	Dave	Ohio Agric. Res. & Dev. Center, Entomology	OH	nielsen.2@osu.edu
Nieto-Saenz	Sebastian	Ohio Agric. Res. & Dev. Center, Entomology	OH	nieto-saenz.1@osu.edu
Nixon	Phil	University of Illinois	IL	pnixon@illinois.edu.
O'Brien	Erin			
Orr	Mary	Toronto, Ontario, CA	CANADA	mary.orr@inspection.gc.ca
Owen	Brenda	SLAM-EAB Pilot Project	MI	brenda.owen@macd.org
Parra	Greg	APHIS USDA	NC	greg.r.parra@aphis.usda.gov
Perry	Kayla	Ohio Agric. Res. & Dev. Center, Entomology	OH	perry.1864@osu.edu
Pigati	Ray	Minnesota Dept. of Agriculture	MN	Raymond.Pigati@state.mn.us
Pijut	Paula	USDA Forest Service Hardwood Tree Improvement	IN	ppijut@fs.fed.us
Poland	Therese	USDA Forest Services, NRS	MI	tpoland@fs.fed.us
Prasad	Anantha	Northern Research Station USDA Forest Service	OH	aprasad@fs.fed.us
Rabaglia	Bob	USDA Forest Service, FHP	VA	brabaglia@fs.fed.us
Rajarapu	Priya	Ohio Agric. Res. & Dev. Center, Entomology	OH	rajarapu.1@osu.edu
Rao	Bal	Davey Tree		bal.rao@davey.com
Reardon	Richard	USDA Forest Service	WV	rreardon@fs.fed.us

Last	First	Organization	Location	E-mail Address
Rhodes	Dana	Pennsylvania Dept. of Agriculture	PA	danrhodes@pa.gov
Rice	Kevin	OSU, Entomology	OH	rice.467@osu.edu
Rietz	Mike	USDA, APHIS, PPQ, EAB Project		Michael.L.Rietz@aphis.usda.gov
Rigsby	Chad	Wright State University	IN	rigsby.7@wright.edu
Rivera-Vega	Loren	Ohio Agric. Res. & Dev. Center, Entomology	OH	rivera-vega.2@osu.edu
Robison	Terry	Cleveland Metroparks	OH	tlr@clevelandmetroparks.com
Roscoe	Lucas Edward	University of Toronto	CANADA	l.roscoe @utoronto.ca
Rudawsky	Sarah	Ohio Agric. Res. & Dev. Center, Entomology	OH	rudawsky.6@osu.edu
Rutledge	Claire	Conn. Agric. Experiment Station	CT	Claire.Rutledge@ct.gov
Ryall	Krista			
Sadof	Clifford	Dept. of Entomology Purdue University	IN	csadof@purdue.edu
Sanchez	Delmy	Ohio Agric. Res. & Dev. Center, Entomology	OH	
Sapio	Frank	USDA Forestry Service	CO	fsapio@fs.fed.us
Schaffer	Elliott	Environmental Horticultural Svcs.		eschaffe@columbus.rr.com
Schirmer	Scott	Illinois Dept. of Agriculture	IL	
Schneeberger	Noel F.	USDA Forest Service	PA	nschneeberger@fs.fed.us
Showalter	David	OSU	OH	showalter.53@osu.edu
Siegert	Nathan	USDA Forest Service	MI	nwsiegert@fs.fed.us
Silk	Peter	Natural Resources Canada	CANADA	Silk@NRCan-RNCan.gc.ca
Spichiger	Sven-Erik	Pennsylvania Dept. of Agriculture	PA	sspichiger@state.pa.us
Stefan	Mike	APHIS USDA	MD	Michael.B.Stefan@aphis.usda.gov
Stone	Amy	OSU Extension	OH	stone.91@osu.edu
Storer	Andrew	Mich. Tech. University Forest Resource, Env. Science	MI	storer@mtu.edu
Sydnor	T. Davis	The Ohio State University	OH	sydnor.1@osu.edu
Tanis	Sara	Michigan State University	MI	tanissar@msu.edu
Taylor	Philip	USDA-ARS-BIIR	DE	Philip.Taylor@ars.usda.gov
Taylor	Robin	Ohio Agric. Res. & Dev. Center, Entomology	OH	taylor.69@osu.edu
Thairu	Margaret	University of Wisconsin - Madison	WI	thairu@wisc.edu
Thompson	Jody			
Threadgill	Chris	Valent USA		chris.threadgill@valent.com
Tinkel	Chad	City of Fort Wayne	IN	chad.tinkel@cityoffortwayne.org
Tluczek	Andrews	Michigan State University	MI	tluczek@msu.edu

Last	First	Organization	Location	E-mail Address
Tomon	Tim	West Virginia Dept. of Agriculture	WV	ttomon@ag.state.wv.us
Trickel	Robert	North Carolina Forest Service	NC	rob.trickel@ncagr.gov
Turcotte	Rick	USDA Forest Service	ME	rturcotte@fs.fed.us
Usborne	Robin	Michigan State University	MI	robinu1@msu.edu
Vandenberg	John	USDA ARS, Cornell	NY	John.Vandenberg@ars.usda.gov
Verdesoto	Patricia	Ohio Agric. Res. & Dev. Center, Entomology	OH	verdesoto.1@osu.edu
Walker	Melody	Wisconsin Dept. of Agric. Trade & Cons. Prot.	WI	melody.walker@wisconsin.gov.
Walker	Rosemary	NASA Plum Brook Station		
Warner	Brenda			
Whitmore	Mark	Dept. of Natural Resources Cornell University	NY	mcw42@cornell.edu
Williamson	R. Chris			
Ziegler	Amos	Michigan State University	MI	ziegler2@msu.edu