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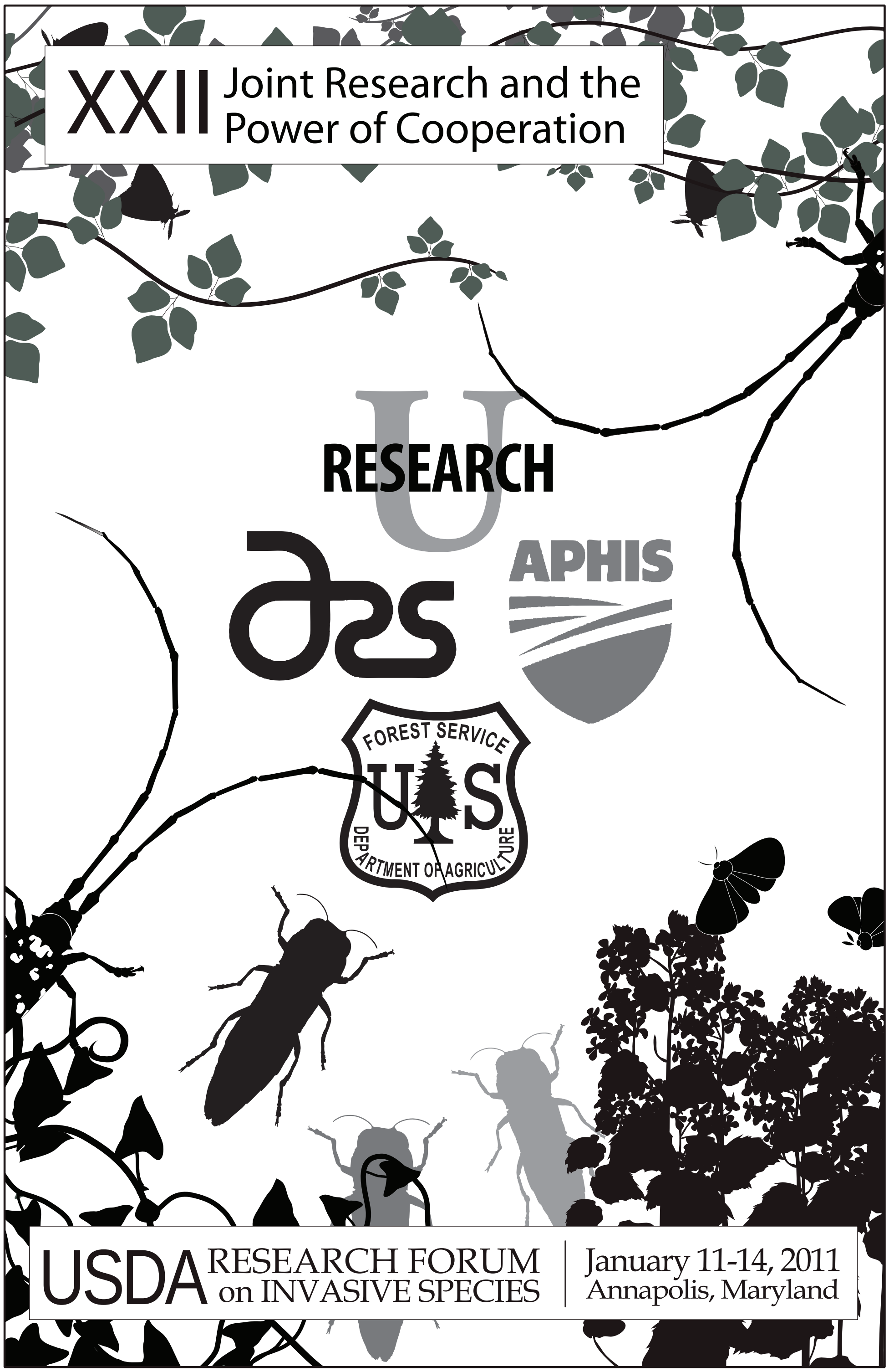

COVER ARTWORK by Vincent D'Amico, U.S. Forest Service, Northern Research Station.

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FOREWORD

This meeting was the 22nd in a series of annual USDA Interagency Research Forums that are sponsored by the Forest Service, Animal and Plant Health Inspection Service, National Institute of Food and Agriculture, and Agriculture Research Service. The group's original goal of fostering communication and providing a forum for the overview of ongoing research among the agencies and their cooperators is being realized and facilitated through this meeting.

This meeting proceedings documents the efforts of many individuals: those who organized and sponsored the meeting, those who provided oral and poster presentations, and those who compiled and edited the contributions. The proceedings illustrates the depth and breadth of studies being supported by the agencies and their many cooperators and demonstrates the benefits and accomplishments that can result through the spirit of collaboration.

Acknowledgments

The program committee would like to thank the four USDA agencies, West Virginia University for assistance with the registration process, and the management and staff of the Loews Annapolis Hotel for their continued support of this meeting.

Program Committee

Michael McManus, Joseph Elkinton, David Lance, Victor Mastro, Therese Poland, Michael Smith

Local Arrangements

Katherine McManus, Kurt Gottschalk

Proceedings Publication

Katherine McManus, Kurt Gottschalk

THE INTERACTIVE ROLES OF CHEMICAL AND VISUAL STIMULI IN THE MATE-FINDING AND MATE SELECTION BEHAVIORS OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS*

T.C. Baker[1], M.J. Domingue[1], A.J. Myrick[1], and J.P. Lelito[2]

[1]Penn State University, Center for Chemical Ecology, University Park, PA 16802
[2]USDA APHIS PPQ, Brighton, MI, 48116

ABSTRACT

Emerald ash borer males and females are a beautiful iridescent green-gold color, but this species of buprestid beetles is a devastating and fast-spreading killer of ash trees in North America. Better trapping methods are needed to help detect and monitor populations to slow their spread across the midwestern and eastern portions of the United States. Males have been shown to visually locate their mates by flying over a female basking on an ash leaflet and rapidly descending onto her from ca. a 1 m height in what has been aptly termed a "paratrooper copulation" (Lelito et al. 2007). Feral males flying over an ash tree canopy can be induced to perform this behavior onto a pinned, dead female on an ash leaflet, but this only works if the dead beetle model is placed in direct sunlight. We used a super-continuum (white) laser to capture the reflected light scattered from these beetles' elytra, and now have shown that the greenish reflections form a complex series of "strands" of colored light interspersed with bands of lower intensity emissions that will create motion across a flying male's ommatidia; the male stops this motion by hovering and then pouncing straight down onto the female. Odor cues do not directly guide the male onto the female, as can be seen clearly from the behavior of a male dropping directly down onto the female from above when wind is blowing horizontally 1 m below the male. However, evidence shows that plant volatiles emitted from strong point source dispensers, not necessarily from the trap itself, can significantly increase the trap catch of males when they are emitted from a tree in which visual-lure traps are deployed (Lelito 2009). When the male lands on the female, two contact sex pheromone components extractable from the cuticle contribute to mate selection and copulatory behavior (Lelito et al. 2009, Silk et al. 2009).

We acknowledge the generous support for this work by grants from the USDA APHIS PPQ.

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SAMPLING METHODS FOR RECOVERY OF EXOTIC EMERALD ASH BORER PARASITOIDS AFTER ENVIRONMENTAL RELEASE

Leah Bauer[1,2], Juli Gould[3], Jian Duan[4], Jason Hansen[2], Allard Cossé[5], Deborah Miller[1], Kris Abell[2,6], Roy Van Driesche[6], Jonathan Lelito[7], and Therese Poland[1,2]

[1]U.S. Forest Service, Northern Research Station, East Lansing, MI 48823
[2]Michigan State University, Department of Entomology, East Lansing, MI 48824
[3]USDA APHIS, Otis Center for Plant Health Science and Technology, Buzzards Bay, MA 02542
[4]USDA ARS, Beneficial Insects Introduction Research Unit, Newark, DE 45433
[5]USDA ARS, National Center for Agricultural Utilization Research, Peoria, IL 61064
[6] University of Massachusetts, Department of Plant Science, Amherst, MA 01003
[7]USDA APHIS PPQ, EAB Biocontrol Facility, Brighton, MI 48116

ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive phloem-feeding beetle from Asia that attacks and kills ash (*Fraxinus* spp.) trees. EAB presumably arrived in North America via infested ash lumber shipped from China to southeast Lower Michigan in the early 1990s and became established in the abundant ash resources throughout the area. In 2002, EAB was identified as the cause of ash mortality in Michigan and nearby Ontario. Despite efforts to eradicate EAB in North America, this destructive beetle is now known in 15 states and 2 Canadian provinces, resulting in the death of tens of millions of ash trees.

Brief Background on EAB Biological Control

Soon after the discovery of EAB in North America, researchers began studying EAB field populations for parasitoids in both the United States and Asia. Although parasitoids were scarce in the United States, three species of Hymenoptera were found parasitizing EAB in northeast China: 1) *Tetrastichus planipennisi* (Eulophidae), a gregarious larval endoparasoid; 2) *Spathius agrili* (Braconidae), a gregarious larval ectoparasitoid; and 3) *Oobius agrili* (Encyrtidae), a solitary parthenogenic egg parasitoid. After research on the biology and host range of these three parasitoid species was completed, an environmental assessment that proposed their use as EAB biocontrol agents was posted in the Federal Register. Following public comment and a risk-benefit analysis in 2007, USDA APHIS and the State of Michigan approved the release in the field of these three species for biological control of EAB. *O. agrili* and *T. planipennisi* reared in East Lansing, MI and *S. agrili* reared in Otis, MA were first released in Lower Michigan by U.S. Forest Service and APHIS scientists. Additional release sites were established in Ohio and Indiana in 2008, and in Illinois and Maryland in 2009. After efforts to eradicate EAB were abandoned, these three parasitoids became the basis of the USDA EAB Biological Control Program. A parasitoid-rearing facility was established in Brighton, MI to produce the EAB parasitoids for release by land managers and researchers. By 2010, more release sites were set up in Lower Michigan, in addition to new sites in Upper Michigan, Maryland, Illinois, Indiana, Ohio, West Virginia, Kentucky, and Minnesota. Michigan State University, the U.S. Forest Service, and APHIS also started a web-based database for parasitoid requests, and for tracking and mapping data on parasitoid release and establishment.

Our standard method of determining parasitoid overwintering or establishment involves destructive sampling of EAB-infested ash trees at release sites. Using these methods, we recovered one or more parasitoid species from release sites in Michigan, Ohio, and Maryland to confirm parasitoid overwintering or establishment. This method, however, becomes problematic due to the increasing scarcity of ash trees at our field sites. To track the establishment and spread of *O. agrili*, *T. planipennisi*, and *S. agrili* at release sites, we are developing alternative methods of trapping and detecting the introduced parasitoids.

Methods for Recovery of the Egg Parasitoid *O. agrili*

Using destructive sampling to determine overwintering or establishment of *O. agrili* in the field, two to four ash trees were felled in late winter or early spring at or near the original release epicenter. The logs or bark samples from each tree were placed in dark cardboard rearing tubes at room temperature for 6 to 8 weeks, and emerging insects were collected every few days from a clear plastic emergence cup attached to each tube. Insects were placed in labeled vials with ~85 percent ethanol, and identified. Logs and bark samples stored in paper bags could be kept in a walk-in refrigerator (4 °C) awaiting available rearing tube space for up to 3 months. Although *O. agrili* successfully emerged from ash log and bark samples, this method requires considerable space and does not provide data on parasitoid prevalence.

The following methods are useful for estimating percent parasitism of EAB eggs by *O. agrili* in the field: 1) sampling naturally occurring EAB eggs from the bark of ash trees; or 2) hanging egg sentinel logs (ESLs) made in the laboratory on the trunks of ash trees. Sampling naturally occurring EAB eggs can result in either an underestimate or overestimate of egg parasitism. Underestimates occur because finding EAB eggs requires considerable time as well as experience to locate the small, cryptically located eggs between bark layers and in bark crevices. Overestimates occur because EAB eggs from previous years are persistent on the bark of ash trees, and can lead to over counts. The use of ESLs, therefore, is the preferred method.

ESLs were made in the laboratory by exposing small ash logs (~5 cm diameter x 25 cm long) to gravid EAB females for 2 to 3 days. Prior to exposure, the log ends were dipped in paraffin and each log was wrapped with a spiral of curling ribbon to stimulate egg deposition on the log beneath the ribbon. After the eggs were counted, marked, and the ribbon was placed over the eggs to reduce egg predation, ESLs were hung on ash trees in the field for 1 to 2 weeks. The ESLs were collected and returned to the laboratory, and each egg was scored for parasitism by *O. agrili*. Although labor intensive to prepare in the laboratory, ESLs appear to be useful in detecting changes in *O. agrili* parasitism over time and space. Using five ESLs at one of our Michigan study sites, we found parasitism increased at the release plot from 3.9 percent (599 eggs deployed) to 6.1 percent (132 eggs deployed) between 2009 and 2010. None were recovered at the control plot, which was located ~800 m away.

Methods Used to Recover the Larval Parasitoids *T. planipennisi and S. agrili*

To determine overwintering or establishment of the introduced larval parasitoids at field sites using destructive sampling, ash trees were felled and log sections were: 1) placed in cardboard rearing tubes to rear out parasitoids (as described above); or 2) debarked and EAB and parasitoids collected and reared to the adult stage for identification. Mature larvae or the naked pupae of the endoparasitoid *T. planipennisi* collected from the host galleries readily matured to the adult stage after transfer to a Petri dish (BD-Falcon #351006; 50 × 9 mm with tight-fit lid). Larvae and/or pupae (in cocoons) of exotic and native ectoparasitoids attacking EAB (*Spathius* spp., *Atanycolus* spp., *Balcha indica*, *Leluthia astigma*, and others) were reared to the adult stage by placing them in small ventilated Petri dishes with their host larvae. Collections of mature larvae or cocoons from ectoparasitoids made during the fall, however, usually required several weeks of refrigerated chill (4 °C) for adult eclosion to succeed. Both of these methods were successful at recovering *T. planipennisi* and *S. agrili* at release sites in Michigan, Indiana, Ohio, Maryland, and Illinois.

In stands where ash trees are scarce, we found larval-sentinel logs (LSLs) were attractive to parasitoids. LSLs were made by cutting small ash logs (~5 cm dia × 10 cm long), inserting five 3rd- or 4th-instar EAB larvae in chambers cut under bark flaps, and sealing the ends with Parafilm. After a 1-week exposure period, LSLs were returned to the laboratory, and parasitoids were removed and reared to the adult stage for identification. LSLs deployed at three biocontrol study sites in late July and early August 2010 exhibited *T. planipennisi* parasitism ranging from 7 to 45 percent, and an unknown *Spathius* spp. (no emergence from cocoons) exhibited a 4 percent larval parasitism at one of the sites.

We also tested the use of yellow pan traps (YPTs), which are attractive to certain hymenopterans including parasitoids. YPTs were made of yellow plastic disposable bowls (~12 cm dia) containing water and detergent and mounted on ash trees using shelf brackets. Traps were changed at least once a week to avoid decomposition of adult insects attracted to and trapped in the soapy water. After collection in the field, YPT samples were returned to the laboratory, strained, stored in ethanol, and EAB parasitoids were removed using a dissecting microscope. Of the introduced EAB biocontrol agents, *T. planipennisi* was more prevalent than *S. agrili*; however, no *O. agrili* were recovered using this method. YPTs proved to be a useful and simple method of determining the establishment of larval parasitoids at release sites.

Semiochemicals May be the Future of Parasitoid Recovery with Field Traps

Considerable progress has been made on the use of semiochemical attractants for recovery of *S. agrili*, the largest of the three introduced parasitoid species. Using GC-FID (Gas Chromatography - Flame Ionization Detector), seven male- and two female-specific compounds were identified from body washes of *S. agrili*. In a wind tunnel, female wasps, and males to a lesser extent, were highly attracted to plumes of both natural and synthetic blends from male wasps. Female responses to males and a male synthetic blend pheromone tested in a release-recapture study in a field tent were similar. Preliminary results of male and female *T. planipennisi* body washes with GC-FID detected a female-produced volatile. Behavioral response studies in a wind tunnel demonstrated males were highly attracted to this female pheromone. The response of females to this compound has not yet been tested.

CHEMICAL SIGNALS AFFECTING *SIREX NOCTILIO* BEHAVIOR

K. Böröczky[1] and J.H. Tumlinson[2]

[1]North Carolina State University, Department of Entomology, Raleigh, NC 27695-7613
[2]The Pennsylvania State University, Center for Chemical Ecology, University Park, PA 16802

ABSTRACT

The woodwasp *Sirex noctilio* (Hymenoptera: Siricidae) is an invasive pest of pine species in North America (Hurley et al. 2007). Females inject a mucous material and the symbiotic fungus, *Amylostereum aerolatum*, in the tree whenever they lay eggs or just probe the wood. These two stressors can overcome the defenses of a healthy tree and eventually kill it. To date, herbicide injected trees have been the most efficient lures for trapping the woodwasp (Zylstra et al. 2010). While females are known to be attracted to stem sections of stressed pines (Madden 1968, 1988) and are responsive to typical terpene components of pine resin (Simpson 1976), using blends of these terpenes in lures has been only moderately successful for trapping. Studying the chemicals that govern the host seeking and mating behavior of the woodwasp can provide candidate compounds to be used in lures for trapping. Here we summarize the results of the research that has been done thus far in this field.

In a joint study with Penn State University and USDA APHIS in 2008-2009, we conducted trapping experiments in the field using Lindgren multifunnel traps hung on herbicide treated pines as lures. Untreated trees were our negative controls. Parallel to trapping, we analyzed the volatile profile of the trees throughout the trapping season. In 2008, we compared two host species, Scots pine (*Pinus sylvestris*) and eastern white pine (*P. strobus*). The following year we included the two chemotypes of Scots pine (high-carene and low-carene producer) as two separate groups. Both years, Scots pine lure trees captured significantly more female woodwasps than white pine lures (Böröczky et al., unpublished). In 2009, the high-carene producer Scots pines caught more woodwasps than the low-carene producers. The volatile emission rates were significantly higher for the Scots pine chemotypes than the white pine. We also identified terpenes such as δ-3-carene, thujene, sabinene, γ-terpinene, and terpinolene that are more abundant in the high-carene producer Scots pine compared to the other hosts in the study. We conclude that both chemical composition and emission rate are important factors in developing an effective lure. As a next step, it will be essential to test blends of candidate compounds in bioassay.

The mating behavior of *S. noctilio* was described in early papers as a complex behavior: first males and females get into the vicinity of each other in the canopy, and then males follow females upon antennal contact, trying to mate (Morgan and Stewart 1966). Our group identified a contact sex pheromone blend of (*Z*)-7-heptacosene, (*Z*)-7-nonacosene, and (*Z*)-9-nonacosene from the hexane body wash of females that elicit copulation attempts in males (Böröczky et al.2009). However, these compounds are probably not suitable for lures due to their low volatility. Current research is focusing on testing volatile compounds emitted by males and females of *S. noctilio* in behavioral experiments.

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UPDATES AND FUTURE DIRECTIONS OF RESEARCH ON MECHANISMS OF ASH RESISTANCE TO EMERALD ASH BORER

Sourav Chakraborty[1], Pierluigi Bonello[1], Don F. Cipollini[2], Daniel A. Herms[3], and Omprakash Mittapalli[3]

[1]The Ohio State University, Department of Plant Pathology, Columbus, OH 43210
[2]Wright State University, Department of Biological Sciences, Dayton, OH 45435
[3]The Ohio State University, Department of Entomology, Ohio Agricultural Research and Development Center, Wooster, OH 44691

ABSTRACT

Manchurian ash (*Fraxinus mandschurica* Ruprecht), an Asiatic species, has been shown to be resistant to the invasive insect pest emerald ash borer (EAB), *Agrilus planipennis* Fairmaire Coleoptera: Buprestidae. Such resistance, compared to the high susceptibility exhibited by North American ash species, has been hypothesized to be based on the coevolutionary history shared by EAB and Asisan species of ash. Phylogenetically, black ash (*Fraxinus nigra* Marshall), one of the most susceptible North American species, is closely related to Manchurian ash, whereas green ash (*Fraxinus pennsylvanica* Marshall) and white ash (*Fraxinus americana* L.), two other susceptible North American species, are more distantly related to Manchurian ash.

Trees manifest two different types of defense against insects and pathogens: constitutive (pre-attack) and induced (post-attack). Constitutive defenses include the physical barrier represented by the outer bark and defensive proteins and secondary metabolites (mostly phenolics) toxic to the insect. Induced defenses include the formation of necrophylactic periderm and the induced accumulation of phenolics, lignin and defensive proteins.

We have employed multiple approaches over the last few years to understand the nature of resistance of Manchurian ash, including identification of biochemical mechanisms and associated genes and markers.

Analysis of ash phloem via constitutive defenses based on comparative proteomics and metabolomics has revealed four putative resistance genes: a major allergen (pathogenesis related (PR)-10 protein), an aspartic protease, a phenylcoumaran benzylic ether reductase, and a thylakoid-bound ascorbate peroxidase. Analysis and identification of phenolics from metabolic profiles of all four ash species is currently under investigation, but significant variation in metabolic profiles among the species is apparent.

Actively feeding EAB larvae exude an oral secretion when they are excised from the host. This secretion might have critical biochemical components for eliciting host defense responses. An LC-MS/MS-based proteomics and metabolomics approach of the secretion revealed a mix of proteins of plant, bacterial, and insect origin broadly classified into categories such as reactive oxygen species (ROS)-related enzymes, PR proteins, and plant degrading enzymes, as well as several host phenolic compounds. The interaction between actively feeding larvae and host trees is clearly extremely complex and is under active investigation.

The phytohormone methyl jasmonate (MeJA), synthesized from the octadecanoid pathway, plays important roles in plant-insect interactions by inducing direct and indirect defense responses in trees. MeJA application to outer bark of ash elicited elevated concentrations of phenolic compounds in phloem tissue.

A primary objective is to develop a phloem-free artificial diet for EAB in which to incorporate putative resistance compounds isolated from ash phloem. A comparison of ash phloem composition with the existing EAB diet revealed significant departures in key components including nine-fold higher protein levels in the artificial diet, as well as higher pH.

We have also applied the newer generation sequencing (Roche-454) strategy to decipher the transcriptomes of the tree phloem and insect-specific tissues. A total of 58,673 high quality expressed sequence tags (ESTs) were obtained from the pooled sample of the tree phloem. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis assigned 4,667 sequences to 142 pathways of interest. ESTs were related to various pathways such as biosynthesis of secondary metabolites, e.g. phenylpropanoids. Extensive gene mining revealed candidate genes like key transcription factors, proteases, cytochrome P450s, lipases, and lipoxygenases. Tissue-specific transcriptomics of EAB larvae resulted in 25,173 and 37,661 high quality ESTs from larval midgut and fat body, respectively. Candidate genes of interest revealed high levels of ROS-related proteins and enzymes in the derived databases. ROS-related proteins play important roles in detoxification and antioxidant defense. It appears that cytochrome P450s (particularly CYP6s) may act as detoxifying agents in the insect midgut, and thus might participate in overcoming ash defenses. Furthermore, the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase(GPX) likely help prevent oxidative damage to the larvae and adults. Some of the CYPs assayed showed high mRNA levels in the midgut, fatbody and cuticle tissues, which suggests probable involvement of CYPs in degradation of allelochemicals during digestion and other physiological roles. Similar observations were made in relation to CAT, which is responsible for hydrogen peroxide decomposition.

In summary, some unique proteins and metabolites have been identified in Manchurian ash that may be associated with its resistance to EAB. However, the mechanisms of action of those proteins and metabolites remain uncharacterized. A simple yet robust method was developed and implemented to quantify MeJA in ash phloem tissue, and a phloem free diet is under development based on quantification of ash phloem nutrient composition. A transcriptomics approach revealed some candidate genes from ash and EAB that suggest important roles for ROS in ash-EAB interactions.

FOREST DISTURBANCE MONITORING USING MODIS DATA

Robert A. Chastain

Remote Sensing Applications Center, Salt Lake City, UT 84119

ABSTRACT

The overarching objective of this project is to provide timely information derived from MODIS satellite image data relating to changes in forest conditions to the digital aerial sketch mapping (DASM) community to assist in their flight mission planning. To this end, a digital change detection methodology has been developed to discern forest disturbances in near real time (updated every 8 days). Although the spatial resolution of its visible and infrared bands is rather coarse at 250 and 500 meters, MODIS Aqua and Terra satellite image data provide daily repeat coverage for any given location, permitting frequent observations that can follow developing disturbances, and capturing forest disturbances that have a very finite temporal window. Additionally, MODIS data contains a relatively high spectral grain, capturing data in the visible, NIR, and MIR wavelengths, and permitting the calculation of spectral indices of vegetation greenness and vigor (e.g., NDVI, NDMI, etc). Both MODIS Aqua and Terra satellite data have been synergistically employed in this project, such that data obtained from one sensor can be used if cloud cover exists in the other. (Terra collects imagery in the morning and Aqua collects data during the mid-afternoon.)

MODIS-based forest disturbance detection analysis has been performed within a series of 8-day composite periods over the conterminous 48 U.S. states (CONUS) for the 2008 and 2009 growing seasons (April 15 to October 31). The baseline composites used for disturbance detection across the CONUS during the 2008 and 2009 growing seasons were produced by combining data from 2003 through 2007. Similarly, an updated baseline was produced for disturbance detection during the 2010 growing season using imagery from 2005 through 2009. Methodological alterations applied for the 2010 growing season include moving from 8-day compositing periods to staggered 16-day composites, with an 8 day overlap between adjacent compositing periods so that a new forest disturbance product can be made available every 8 days. In the eastern deciduous biome, this forest disturbance detection approach has been successful in tracking forest tent caterpillar (*Malacosoma disstria*) defoliation outbreaks in Michigan and Pennsylvania (2009 and 2010), as well as in Louisiana (2010). Additionally, gypsy moth (*Lymantria dispar*) outbreaks in Virginia and Pennsylvania (2009) have been identified and tracked using this method. In the western coniferous biome, this approach has been used to track red attack and mortality associated with mountain pine beetle (*Dendroctonus ponderosae)* activity in Colorado during the 2008 to 2010 growing seasons, as well as mountain pine beetle and spruce beetle *(Dendroctonus rufipennis)* activity on the north slope of the Uinta Mountains.

A Web-based mapping application was launched in June 2010 wherein users (currently limited to local and state forestry personnel) can view disturbance detection analytical results as continuous raster data. This application permits timely delivery of these data to members of the DASM community, enabling the manipulation of raster change data products to identify thresholds in the data based on local knowledge of historical and current forest conditions as well as disease and pest activity.

IMPACTS OF NONNATIVE PLANTS ON HABITAT OCCUPANCY OF SONGBIRDS IN SUBURBAN FOREST FRAGMENTS

Amanda M. Conover[1], Vincent D'Amico[2], and Christopher K. Williams[1]

[1]University of Delaware, Department of Entomology and Wildlife Ecology, Newark, DE 19716
[2]U.S. Forest Service, Northern Research Station, Newark, DE 19716

ABSTRACT

Development into forested areas is occurring rapidly across the eastern United States, and many of the remnant forests within suburban landscapes are being fragmented into smaller patches. One ecological effect linked to forest fragmentation is the invasion of nonnative plants into the ecosystem. Nonnative plants have been proposed as a factor in the population declines of many bird species that nest in our suburban landscapes. However, few studies have explicitly examined the link between the density of native plants and avian communities and habitat use.

The objective of this project was to estimate occupancy of six songbird species common to suburban forest fragments as a function of nonnative plant density, forest structure, and associated invertebrate abundance. I conducted 98 avian point counts three times between 15 May 2009 and 15 August 2010 in Delaware and Pennsylvania. Vegetative structure and composition were analyzed within the plots by measuring understory coverage, canopy coverage, and proportion of native vegetation. Invertebrate biomass was measured within each point by vacuum sampling to estimate the avian food supply. Insect biomass values and vegetation characteristics were used as covariates in an occupancy model to explain the presence of candidate bird species observed during our surveys. We used the program PRESENCE to build models of the detection and presence of each species and evaluated the models using Akaike Information Criterion (AIC) to determine the most parsimonious model.

The proportion of native plants at a site was the most important variable in predicting whether wood thrush (*Hylocichla mustelina*) would be present at the site. The structure of the forest, rather than its composition of native and nonnative species, was identified in the models as the strongest predictor of presence for other species of birds. These species include American robin (*Turdus migratorius*), grey catbird (*Dumetella carolinensis*), and Acadian flycatcher (*Empidonax virescens*). For the remaining species, Carolina wren (*Thryothorus ludovicanus*) and Carolina chickadee (*Poecile carolinensis*), the variables important to occupancy remain unclear, as either the null or global model was included in the highest ranked models. Although our research supports that, for wood thrush, the proportion of native plants within a forest fragment affects the use of this habitat, and further research is needed to fully understand how these impacts occur.

BALANCING THE USE OF PHEROMONES, ASH VOLATILES AND COLORED TRAPS TO DEVELOP EFFECTIVE TRAPPING METHODS FOR *AGRILUS PLANIPENNIS*

Damon Crook[1], Peter Silk[2], Krista Ryall[3], Allard Cossé[4], Ashot Khrimian[5], Ivich Fraser[6], Joseph Francese[1], and Victor Mastro[1]

[1]USDA APHIS PPQ CPHST, Otis Lab, Buzzards Bay, MA 02542
[2]Natural Resources Canada, Atlantic Forestry Centre, Fredericton, NB E3B 5P7 Canada
[3]Natural Resources Canada, Great Lakes Forestry Centre, Sault Ste. Marie, ON P6A 2E5 Canda
[4]USDA ARS Crop Bioprotection Unit, Peoria, IL 61604
[5]USDA ARS, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705
[6]USDA APHIS PPQ CPHST, Brighton, MI 48116

ABSTRACT

The main aim of our 2010 research was to better understand trap color and lure combinations so that monitoring tools for detecting emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, populations could be improved. Several field studies were setup throughout western Michigan and Ontario, Canada during the summer of 2010.

The first test monitored EAB attraction to six trap and lure combinations. All traps in this study were a new "prototype" dark green prism trap (hung below canopy height, n=15). The treatments were: 1) 80/20 Manuka/ Phoebe + (3*Z*)-hexenol; 2) Manuka oil + (3*Z*)-hexenol; 3)(3*Z*)-hexenol; 4) 80/20 Manuka/Phoebe; 5) Manuka oil; and 6) unbaited control. All trap catches were mainly skewed towards males over females. Based on previous unbaited color studies, we believe that green is more attractive to males than purple. Contrast paired analysis showed that (3*Z*) -hexenol caught significantly more males than unbaited green traps. Manuka oil or phoebe oil treatments did not catch significantly more insects of either sex versus unbaited green traps. (3*Z*)-hexenol therefore works better (for males) when used with green traps placed in the sub-canopy. Manuka oil + (3*Z*)-hexenol caught significantly more male and total count insects than Manuka oil alone. Manuka oil + (3*Z*)-hexenol did not catch more males, females or total insects when compared to (3*Z*)-hexenol alone. Manuka oil does not appear to enhance (3*Z*)-hexenol adult catch on green traps placed in the sub-canopy.

Two dosage test studies (n=10 each) were done for (3*Z*)-hexenol and phoebe oil using the new dark green prism traps. A 0.5 mg release rate of (3*Z*)-hexenol was as effective as 5 mg, 50 mg and 500 mg per day. A release rate of 5 mg per day of Phoebe oil seemed adequate for monitoring purposes (50 mg per day has previously been used in field tests) when compared to 0.5 mg, 50 mg and 500 mg trap catches.

An important component of Phoebe oil (7-epi-sesquithujene) was tested on the new dark green traps with and without (3*Z*)-hexenol (n=5). Trap catch was not significantly improved when 7-epi-sesquithujene was tested by itself or with (3*Z*)-hexenol.

The final field test was done in Ontario, Canada and Michigan (n=12 each, using light green traps). The aim of this field test was to see if the insect produced lactone (3Z and 3E isomers, on septa at low mg release rate) would improve trap catch when used with (and without) Z3-hexenol lures on light green traps. The following treatments were tested: 1) (3Z)-lactone plus (3*Z*) -hexenol; 2) (3E)-lactone plus (3*Z*)-hexenol; 3) (3*Z*)-hexenol; 4) (3Z)-lactone,;5) (3E)-lactone; and 6) unbaited control trap.

In the Canadian half of the study, the (3*Z*)-lactone significantly increased capture of male *A. planipennis* when traps were deployed in the mid canopy. Captures of males on traps with both (3*E*)-lactone and (3*Z*)-

hexenol or with (3*Z*)-lactone and (3*Z*)-hexenol were increased by 45-100 percent, respectively, as compared to traps baited with just (3*Z*)-hexenol. The lactones did not improve trap catch when used by themselves. (3*Z*)-hexenol, therefore, appears to work synergistically with either isomer of the lactone. The North American half of the study showed the same trends as the Canadian study, but differences were not significant. These data are the first to demonstrate synergy in attraction of a sex pheromone and green leaf volatile in a Buprestid species.

MATE FINDING BEHAVIOR OF THREE EUROPEAN OAK BUPRESTID BEETLES IN HUNGARY

Michael J. Domingue[1], Miklós Tóth[2], Victor Mastro[3], György Csóka[4], and Thomas C. Baker[1]

[1]The Pennsylvania State University, Department of Entomology, University Park, PA 16802
[2]Hungarian Academy of Sciences, Plant Protection Institute, Hungary
[3]USDA APHIS PPQ, Otis PSDEL Buzzards Bay, MA 02542
[4]Forest Research Institute, Department of Forest Protection, Hungary

ABSTRACT

The buprestid beetle species *Agrilus biguttatus* Fabricius and *Agrilus sulcicollis* Lacordaire are oak (*Quercus*) feeding exotic pests from Europe that threaten the forest and urban landscapes of North America. Females lay eggs in bark crevices, and larvae that hatch feed on the bark of the trunk and larger branches. In Europe, such activity can result in substantial tree mortality, often among stressed trees when populations of the insects are high. A third smaller European oak buprestid species, *Agrilus angustulus*, Illiger is sympatric with these larger species, more often attacking the bark of smaller branches. All three species have been observed maturation feeding on the foliage of oaks. With substantial evidence that other *Agrilus* species, such as the emerald ash borer (*Agrilus planipennis* Fairmaire) use visual cues for mate finding, it is of interest whether similar behavior is exhibited in these three European species.

In Hungary, we observed each of these oak buprestid species at a field site that consisted of log piles of freshly cut oak placed below the foliage of other standing oak trees. Dead, pinned female models of five *Agrilus* species (*biguttatus*, *sulcicollis, angustulus, planipennis,* and *cyanescins*) were placed in the foliage where the native species could be observed maturation feeding and mating.

A. biguttatus males approached and landed on the models by means of direct visually mediated flight, similar to that described previously for emerald ash borers. There was substantial cross-attraction to models of other species. Attraction was correlated with the size of the model, with *A. planipennis* and *A. angustulus* respectively being approached most and least frequently. After such visual approaches, *A. biguttatus* males would often attempt to copulate with the two largest models, *A. biguttatus* and *A. planipennis.* Associated with copulation were extended periods of guarding by the males, sometimes for periods greater than 10 minutes. On average, the durations of such pair formations were greater when *A. biguttatus* males landed on *A. planipennis* models rather than the conspecific models.

Males of *A. sulcicollis* landed on the pinned models of all of the species with the exception of *A. angustulus*, which was never approached. Neither copulation nor prolonged contact was ever observed between *A. sulcicollis* males and any of the pinned female models. *A. angustulus* were not observed landing directly on models, but rather 1 or 2 cm away, often walking closer to the model before flying off. Such behavior was observed toward all the models, but most rarely to *A. sulcicollis* models.

The observed levels of cross-attraction suggest the possibility of a common behavioral template for visual mate finding among buprestids. However, additional factors such as female size, or the recognition of sympatric competitors, may mediate such interactions. Further elucidation of the mechanisms contributing to these behavioral interactions will assist in the development of visual trapping approaches.

THE IMPACT OF BIOTIC FACTORS ON POPULATIONS OF THE EMERALD ASH BORER: A COMPARISON BETWEEN ITS NATIVE NORTHEAST ASIAN AND NEWLY INVADED NORTH AMERICAN RANGES

Jian J. Duan[1], Galina Yurchenko[2], Juli Gould[3], Xiao-Yi Wang[4], Zhong-Qi Yang[4], Leah Bauer[5], Kristopher J. Abell[6], and Roy Van Driesche[6]

[1]USDA ARS Beneficial Insects Introduction Research Unit, Newark, DE 19713
[2]Far East Forest Research Institute of Russian Federation, Khabarovsk, Russia
[3]USDA APHIS PPQ, Buzzards Bay, Otis, MA 02542
[4]Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing, China
[5]U.S. Forest Service, Northern Research Station, East Lansing, MI 48823
[6]University of Massachusetts, Department of Plant, Soil and Insect Science, Amherst, MA 01003

ABSTRACT

Between its discovery in Michigan and Ontario in 2002 and 2010, the emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), spread to 15 U.S. states and two provinces in Canada. Understanding population dynamics in both the pest's native range of northeast Asia and the newly invaded region in North America is critically important to the development of effective manage strategies for suppression of emerald ash borer. It is known that populations of EAB are affected by a variety of biotic factors in part of its native range such as northeast China and the Russian Far East (Liu et al. 2003, 2007; Yang et al. 2010), as well as in some newly invaded areas in North America (Cappaert and McCullough 2009; Duan et al. 2009; Duan et al. 2010a, 2010b; Kula et al. 2010). These factors include host tree defense, woodpecker predation, disease, and both endemic and introduced hymenopteran parasitoids. To date, however, there have been no systematic studies on the comparative impact of these different biotic factors on population dynamics of EAB in northeast Asia and North America.

To compare the impact of different biotic factors on population dynamics of EAB, we sampled eggs and larval stages of EAB populations and measured mortality from parasitoids, predators, host plant resistance, and microbial diseases in both northeast Asia (Russian Far East and northeast China) and North America (Michigan). Results from our studies in northeast Asia showed that two braconid parasitoids (*Spathius* sp. and *Antanycolus picipes* Telenga) were the dominant mortality factors of larval EAB infesting planted North American ash (*Fraxinus pennsylvanica*), causing 65 percent larval mortality. In EAB populations infesting oriental species of ash (*F. ryhchophylla* or *F. manschurica*), however, the dominant mortality factor was host-plant resistance, which killed >75 percent of feeding EAB larvae by forming callus around young larvae and/or the dieback of cambium tissues surrounding EAB larvae. Predation by woodpeckers and microbial diseases caused less than 5 percent mortality to larvae. In contrast, the results of three years of field studies in North America (Michigan) showed that several species of North American woodpeckers and one group of North American braconid parasitoids (primarily *Antanycolus cappaerti*) have become the primary biotic factors killing EAB larvae. Mortality of EAB larvae from parasitism and predation in Michigan increased nearly five-fold from 2009 to 2010, suggesting that the role of natural enemies in North American EAB populations is changing rapidly.

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WINTER MOTH: BIOLOGICAL CONTROL AND SPREAD IN NEW ENGLAND

Joseph Elkinton[1], George Boettner[1], Marinko Sremac[1], and Andrew Liebhold[2]

[1]University of Massachusetts, Department of Plant, Soil and Insect Sciences, Amherst, MA 01003
[2]U.S. Forest Service, Northern Research Station, Morgantown, WV 26505

ABSTRACT

The winter moth, *Operophtera brumata*: a leaffeeding geometrid native to Europe, has recently invaded eastern New England and is causing widespread defoliation. Previous invasions by this species in Nova Scotia and British Columbia have been suppressed by the introduction of two parasitoids from Europe, the tachinid *Cyzenis albicans* and the ichneumonid, *Agrypon flaveolatum*. As a result of these introductions, low-density populations of winter moth now persist indefinitely in these regions similar to those that exist in Europe. Over the past 6 years, we have introduced *C. albicans* at six locations in Massachusetts, and in 2010, for the first time we have concrete evidence of establishment of this parasitoid. We recovered parasitized winter moth larvae at four of our six release sites. At three of those sites, we did not release *C. albicans* in 2010, so the parasitoids must have survived and reproduced from previous years. In Nova Scotia in the 1950s, *C. albicans* was first recovered in 1959, five years after first release, but then quickly suppressed winter moth permanently beginning in 1961. We hope *C. albicans* parasitism and impact follows this same trajectory in New England.

In December 2009, we continued our pheromone trap survey for winter moth to document the spread of the population in the Northeast. We focused mainly on traps deployed along an east-west transect in central Massachusetts, along with other traps in New Hampshire and on Long Island. We were surprised to recover winter moths in Westminster, MA, about 35 km west of where we thought the invasion front was in 2007. We were also surprised to recover winter moths in Ringe, NH and on Staten Island in New York. It appears that winter moth may be moving south and west a lot faster than we had thought. We plan to continue these surveys and to use the data to estimate rates of spread.

We have now collected data on density and survival of different winter moth life stages at long-term population monitoring sites in eastern Massachusetts for the past seven years. We document two periods of high density (2004-2005 and 2009-2010). In 2010, there was a dramatic decline in larval densities. Preliminary analyses suggest that we have density dependent mortality occurring in all life stages, as well as density-related changes in fecundity. We hope to assemble these findings into a comprehensive account of the dynamics of outbreak populations of winter moth prior to the establishment of *C. albicans* in New England.

REARING AND SPLITTING TO KNOW: NEW INSIGHTS INTO THE SIRICID-PARASITOID COMPLEX IN NEW YORK

Melissa Fierke, Patrick Eager, Christopher Standley, Dylan Parry, and Douglas Allen

State University of New York, College of Environmental Science and Forestry, Syracuse NY 13210

ABSTRACT

We assessed within tree distributions of the European woodwasp, *Sirex noctilio* F. (Hymenoptera: Siricidae), and its parasitoid complex, and developed a more cost effective approach for sampling and monitoring. To do this, 18 infested pines (12 red pine (*Pinus resinosa*) and 6 Scots pine (*P. sylvestris*) were felled from six sites in central New York, cut into 0.5 m bolts and transported for dissection in the summer of 2008. Bolts were carefully split with a log splitter to recover all larvae of *S. noctilio*, native siricids, and parasitoids. A total of 1,972 siricid larvae were recovered, with densities of 24 per m^2 of bark surface area in red pine and 59 per m^2 in Scots pine. Parasitism of siricid larvae was 16 percent with *Ibalia leucospoides ensiger* causing the highest mortality (11 percent) followed by rhyssine parasitoids (*Rhyssa lineolata*, *R. persuasoria persuasoria*, and *Megarhyssa nortoni nortoni*). Based on these data, optimal sampling plans were developed, and as few as three bolts per tree yielded R^2 values >90 percent for siricids and their parasitoids.

In 2009, a total of 31 red pines and 31 Scots pines were felled from nine sites in central and northern New York to assess siricid densities and parasitism. Partial (based on a three-sample optimal sampling plan) and whole trees were cut into 0.5 m bolts and samples placed in rearing tubes under controlled conditions. In total, 2,901 *S. noctilio* were recovered as well as 2 native siricid species, 6 *S. edwardsii,* and 11 *S. nigricornis.* Four parasitoid species were recovered which included 959 *I. leucospoides,* 171 *R. lineolata*, 79 *R. persuasoria*, and 1 *M. nortoni*. Overall, parasitism was 31 percent, with *I. leucospoides* accounting for 24 percent. Results from 2008 and 2009 provide critical information on parasitism of *S. noctilio*, a potentially economically important invasive insect, by native parasitoids and elucidates interactions with native insects.

In 2010, nine 0.5 m bolts were removed from 15 red pine and 15 Scots pine from two sites, one in central and another in western New York. Insects were allowed to emerge in rearing tubes in an outdoor insectary at ambient environmental conditions to develop phenological models for *S. noctilio,* native siricids, and their parasitoids. In all, 391 *S. noctilio* emerged along with 246 native siricids and 216 parasitoids. Phenologies indicate *S. noctilio* and the native siricids minimally overlap in their emergence. Parasitoid phenologies indicate their emergence is timed to correspond with the introduced woodwasp, suggesting these parasitoids are exhibiting phenotypic plasticity triggered by a super abundant new host. This study provides novel insights into this complex and sets the stage for further research into how our native community is responding to increased numbers of *S. noctilio*.

RESPONSE OF EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE) TO GREEN AND PURPLE MULTIFUNNEL TRAPS

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

[1]USDA APHIS PPQ CPHST, Otis Lab, Buzzards Bay, MA 02542
[2]USDA APHIS PPQ CPHST, 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. A reusable, more user-friendly trap is desired by program managers, surveyors, and researchers. Field assays were conducted in southeastern Michigan to ascertain the feasibility of using nonsticky traps as survey and detection tools for emerald ash borer. Three nonsticky trap designs including multifunnel (Lindgren), modified intercept panel, and drainpipe (all painted purple) were compared with the standard purple prism trap; no statistical differences in capture of emerald ash borer adults were detected between the multifunnel design and the prism. In subsequent color comparison assays, green and purple painted multifunnel traps (and later, plastic versions of these colors) performed as well or better than the prism traps. Multifunnel traps coated with spray adhesive caught more beetles than untreated traps. The increased catch, however, occurred in the collection cups of the trap, 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.

WHAT HAVE WE LEARNED FROM OUR EXPERIENCE WITH *PHYTOPHTHORA RAMORUM?*

Susan J. Frankel

U.S. Forest Service, Pacific Southwest Research Station, Albany, CA 94710-0011

ABSTRACT

After more than a decade combating sudden oak death and its causal agent, *Phytophthora ramorum* (Werres, de Cock & Man in't Veld), both the disease and pathogen remain difficult to judge or categorize. The impact, spread rate, and response of the pathogen to management actions are all subject to interpretation. Has the pathogen spread significantly? A comparison of the 2010 vs. 2000 California statewide distribution map indicates the pathogen's current wildland boundaries have not expanded dramatically since 2000, yet within the infested areas, millions more tanoak (*Notholithcarpus densiflorus* (Hook & Arn.) Rehder Manos, Cannon, & Oh) and coast live oak (*Quercus agrifolia* Née) have been killed. How severe is the damage? On Mt. Tamalpais (Marin County), in one of the heavily impacted areas, tanoak has been virtually eliminated from stands that it previously dominated, but several miles away there are healthy stands. Likewise, from 2001 to 2010 the pathogen was detected in approximately 470 United States nurseries where it is subject to eradication. To those with zero tolerance, that is a large number, but as a percentage of all nurseries in the United States, the number of detections is far less than 1 percent.

Phytophthora ramorum was first recognized in 2000, so efforts to understand and manage it started from the basics. We have progressed quite far over the ensuing decade; in 2002 (October 5) a New York Times editorial stated that sudden oak death shows the limits of what we know about the world we live in. In contrast, in 2010, sudden oak death investigations were described as a model of scientific inquiry for a new pathogen, a demonstration of the effort that goes into science, and evidence that rational inquiry is important (Free 2010).

A quick search of the sudden oak death literature finds 650 references and well over 1,000 documents. How can we apply our knowledge to manage this invasive, exotic pathogen? What is the optimal timing and strategy for disease intervention? Which level of government (local, state or federal) should take action and at what time? How much taxpayer money should be spent to control or prevent forest diseases? The arguments and debates about whether host removal zones and wildland eradication treatments are effective for sudden oak death are eerily similar to those that raged in 1915 during anti-chestnut blight campaigns in the eastern United States (Freinkel 2007).

Advances in Diagnostics, Monitoring and Education and Outreach

While management remains somewhat elusive, our experiences with *Phytophthora ramorum* have contributed to improved diagnostics programs for regulated plant pathogens. From confusing lab results from samples taken at Tiffany Creek Preserve, (Nassau County, NY) in 2004, we learned that very sensitive diagnostic systems aimed at minimizing false negatives may result in false positives. Those difficulties as well as challenges interpreting 2004 samples from southern California nurseries contributed to numerous advances, including the activation of the National Plant Diagnostic Network, lab certification quality assurance programs, the addition of soil and water testing in response to nursery detections, and molecular assays based on more than one gene region.

Breakthroughs in monitoring and genetic analysis have documented pathways of pathogen movement. We have scientific evidence and official regulatory observations that *Phytophthora ramorum* can move from infested nurseries via runoff into adjacent streams to infect adjacent forest vegetation. The pathogen has moved from infested nurseries in the western United States, via shipments of infested stock to eastern states (Goss et

al. 2009b), and there have also been multiple pathogen migrations between Europe and North America and vice-versa (Goss and others 2009a). Improvements in monitoring also indicate that the pathogen is present, at low levels, in retail nurseries despite regulation of wholesale nurseries (USDA APHIS 2011).

Another highlight has been the development of strong education and outreach programs for sudden oak death, with the California Oak Mortality Task Force serving as a model of collaboration for effective communication of research results, policy and management updates, and disease status. The www.suddenoakdeath.org Web site provides one source for credible, science based information, and its more than 80 member organizations comprise a powerful coalition for disease prevention.

However, despite our research, management, and eradication efforts, the pathogen continues to spread in new, surprising and alarming ways, most notably on Japanese larch (*Larix kaempferi* (Lam.) Carrière), in the United Kingdom, where hundreds of hectares of plantations are being clear cut to prevent pathogen spread (Webber et al. 2010).

Lessons Learned

In 2004, the realization that more than a million potentially infected plants had been inadvertently shipped out from several West Coast nurseries prompted many lessons: the importance of a strong forest and plant health network; the need to build communication networks in advance; to communicate the importance of forests; to resolve broad underlying issues of disagreement regarding regulation of plant pathogens; to broaden the use of experts for regulatory design; foster collaboration; staunch the flow of infestations on nursery plants and firewood; reduce red tape for response programs; and to build capacity for forest health expertise (Frankel et al. 2005).

The U.S. Government Accountability Office (2006a, 2006b) looked at lessons learned from sudden oak death, emerald ash borer, and Asian longhorned beetle and recommended six actions: (1) expand efforts to monitor forest health conditions to include urban areas, particularly those deemed high risk for potential infestations; (2) regularly update and publish management plans for pests that include status information and funding needs; (3) implement written procedures that broadly define when and how to operate science panels for specific pests; (4) establish a process to identify and assess foreign pest risks and implement a staffing model to meet those risks, (5) improve the communication of pest alerts and other policies between agencies; and (6) improve the effectiveness of the canine inspection program.

Our review of the historical accumulation of nonindigenous forest pests in the continental United States (Aukema et al. 2010) showed that over the last century less than 20 highly damaging forest pathogens have been recorded, but among them, several have almost eliminated their host: chestnut blight, (*Cryphonectria parasitica* (Murrill) Barr); white pine blister rust (*Cronartium ribicola* J.C.Fisch), and Dutch elm disease (*Ophiostoma ulmi*, (Buisman) Nannf. and *Ophiostoma novo-ulmi* Brasier). Invasions by exotic pathogens like sudden oak death have a low likelihood of occurrence, but once they become established, there is no landscape scale control strategy to limit tree mortality and its cascade of impacts. Prevention of forest pathogen introductions is difficult, requiring a sustained preventive effort aimed at a microscopic target that may occur over a broad area and enter via any number of pathways. Many will consider the introduction of a highly damaging forest pathogen too unlikely, or too difficult to prevent, and focus on other more immediate concerns. But the story of *Phytophthora ramorum* and sudden oak death demonstrates that our forests are vulnerable and need protection from invasive pathogens.

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TRENDS IN LIVE PLANT TRADE

Lynn J. Garrett

USDA Agricultural Plant Health Inspection Service, Raleigh, NC 27606-5205

ABSTRACT

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) Plants for Planting Quarantine regulation is contained in Title 7 of the Code of Federal Regulations (CFR), Part 319, Section 37 (nursery stock, plants, roots, bulbs, seeds, and other plant products). The regulation also known as Quarantine 37, was promulgated in 1918 (becoming effective in 1919) "to reduce to the utmost the risk of introducing plant pests with plant importations" (Weber 1930). The regulatory design of the quarantine was based on particular conditions and assumptions including:

- Typical shipments would be small (less than 100 propagules) and infrequent
- Imports would mainly be for the establishment of domestic stock, not for direct resale
- Mandatory fumigation with methyl bromide would be applied for arthropod pests
- Imports would enter only through ports with specialized staff and inspection facilities
- Taxa known to carry pests that are difficult to detect, e.g., pathogens, would be prohibited or have special requirements

Although it was recognized that restrictions were necessary for certain plants[1] that could provide a pathway for the introduction of harmful exotic pests, the counterbalancing need to import new species and varieties to increase the germplasm available to U.S. growers was a powerful influence on the design of Quarantine 37. The horticulture industry favored a regulatory design that encouraged exploration of the plant world to discover new opportunities for agriculture and especially horticulture. There was little concern at the time for the invasive potential of certain plants and the magnitude of the risks posed by uncertainty regarding the plethora of pests that may be associated with propagative material in trade. Likewise, plant quarantine officials of that era could not have imagined that the phenomenal rate of globalization eight decades later would completely change the nature of trade in plant propagative material.

The current regulatory objective of Quarantine 37 is to manage the pest risk associated with the introduction of plant pests into the United States via the import of plants or parts of plants intended for cultivation, planting, or propagation. The regulation also extends to articles such as growing media and packing material associated with imported plants. Most plants for planting are allowed to be imported if accompanied by a phytosanitary certificate from the exporting country, and after visual inspection at a Federal plant inspection station or port of entry.

Currently, Quarantine 37 is limited to plants for planting as a pathway for the introduction of pests; it does not extend to plants as pests, *i.e.*, weeds. Pest plants are covered by 7 CFR 360 (Noxious Weed Regulations). This regulation is similar in that it is also based on a list of specific regulated articles (360.200 Designation of noxious weeds). However, the regulation is also significantly different because the regulated articles are themselves the pests rather than serving as the pathways for pest introduction. Thus, an imported plant may be regulated by APHIS under Quarantine 37 as a pathway for pests and/or also regulated under APHIS' Noxious Weed Regulations as a pest plant.

[1]"plants" is used in this document to refer to live plants, cuttings, bulbs described in the Harmonized Tariff Schedule of the United States—Chapter 6 Headings Subheading—04; 0601-Bulbs, tubers tuberous roots, corms, crowns and rhizomes, dormant, in growth or in flower chicory plants and other than roots of heading 1212 and 0602-Other live plants (including their roots), cuttings and slips, mushroom spawn.

Major trade trends identified in this study include:

- U.S. value of plant foreign trade between 1989 and 2005 in comparison to the crop receipt value of nursery and greenhouse crops is small, ranging from less than 2 percent for exports and nearly 3 percent in 1989 to 4 percent in 2004 for imports.
- U.S. continues the trend in being a net importer of live plants both on a quantity and value basis.
- U.S. plant trade measured in individual plant units for bulbs and live plant imports from 1967 to 2009 continues to increase with the share of live plants growing from <1 percent share in 1967 to over 50 percent in the last 3 years (2007 - 2009).
- U.S. average import quantity of bulb imports between two 5-year periods (1989-1993 and 2005-2009) increased by 36 percent with the most growth coming from origins in Europe, South America, Oceania, and Asia.
- U.S. average import quantity of live trees and plants (excluding orchid plants and mushroom spawn) between two 5-year periods (1989-1993 and 2005-2009) increased by 238 percent with significant increases coming from all world regions.
- U.S. states having ports with the largest share of aggregate shipping weights for plants for planting during the past 5 years (2005-09) were New Jersey with 31.9 percent California with 25.4 percent and Florida with 21.2 percent. California and Florida have multiple USDA APHIS Plant Inspection Stations (PIS).
- U.S. import of propagative material plants units (excluding flasks, seeds, and permits processed) inspected by the APHIS PIS during the five FY (fiscal year) period including 2005-2009 totaled 6.9 billion plant units with 74 percent being inspected at the PIS in Miami, Florida.
- U.S. regulated plant import shipments inspected by USDA APHIS Plant Inspection Stations during the FY 2006-2010 period totaled 504,257 shipments involving over 7 billion plants in 2,846 plant genera. During this period, 124 plant genera involved 1,000 or more shipments.
- U.S. regulated plant import shipments inspected by USDA APHIS Plant Inspection Stations during the FY 2006-2010 period annually had between 2.3 and 2.9 percent of shipments containing an actionable pest.

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RELEASE AND RECOVERY OF PARASITOIDS OF THE EMERALD ASH BORER (EAB), *AGRILUS PLANIPENNIS* IN MI, OH, AND MD

Juli Gould[1], Leah Bauer[2], Jian Duan[3], Ivich Fraser[4], Jason Hansen[5], Michael Ulyshen[5], and Jonathan Lelito[6]

[1]USDA APHIS PPQ, Center for Plant Health Science and Technology, Buzzards Bay, MA 02542
[2]U.S. Forest Service, Northern Research Station, East Lansing, MI 48823
[3]USDA ARS, Beneficial Insects Introduction Research Unit, Newark, DE 19713
[4]USDA APHIS PPQ, Brighton, MI 48116
[5]Michigan State University, Department of Entomology, East Lansing, MI 48824

ABSTRACT

After federal and state approval, release of three hymenopteran emerald ash borer parasitoid species native to China began in late July of 2007. These species are *Tetrastichus planipennisi* (Eulophidae), a gregarious larval endoparasitoid; *Oobius agrili* (Encyrtidae), a solitary parthenogenic egg parasitoid; and *Spathius agrili* (Braconidae), a gregarious larval ectoparasitoid.

Parasitoid Colonies

Researchers with the Forest Service Northern Research Station (FS-NRS) and Michigan State University (MSU) in East Lansing, MI initiated and continue to maintain laboratory colonies of *O. agrili* and *T. planipennisi;* scientists at the Animal and Plant Health Inspection Service (APHIS) facility in Buzzards Bay, MA established a colony of *S. agrili*. Comparatively few adult parasitoids were released at field sites in 2007 and 2008 because parasitoid rearing is labor intensive and laboratory space and staff are limited.

Parasitoid Releases

In 2007, a combined total of 2,857 adult female parasitoids were released at seven field sites in Lower Michigan. In 2008, the number of release sites increased to 10 in lower Michigan plus two each in Ohio and Indiana, but with similar parasitoid numbers being released. By 2009, parasitoid rearing methods were greatly improved; the Agricultural Research Service (ARS) in Delaware started a colony of *T. planipennisi*, and the APHIS EAB parasitoid-rearing facility in Brighton, MI became operational. These actions enabled the release of 33,833 female parasitoids in 2009 at research sites in lower Michigan, Ohio, and Indiana, with new sites established in Maryland and Illinois.

Parasitoid production at the Brighton facility continued to increase, and in 2010, a combined total of 103,734 female parasitoids were released at 24 sites in areas with previous releases and at new sites in West Virginia, Kentucky, and Minnesota.

Overwintering

The first step in assessing the success of parasitoid releases is determining the ability of each species to overwinter. Given the cryptic habitat of EAB eggs and larvae, detecting the presence of parasitoids is difficult and generally requires destructive sampling methods. In early spring the year following parasitoid releases, we sampled ash trees near the release epicenter of selected study sites to assess overwintering. Each tree was cut into sections and debarked, EAB larvae were collected, and parasitoids were reared to the adult stage for identification. Instead of debarking, some ash logs were placed in rearing tubes, and emerging insects were collected and identified; *O. agrili,* the egg parasitoid, was also reared from ash bark samples. Additional methods used at select sites included sentinel logs containing EAB eggs or larvae, yellow pan traps, and direct collection of EAB eggs (see Bauer et al. abstract). By the spring of 2010, using a combination of methods, we determined the three parasitoid species could successfully overwinter at study sites in Michigan, Ohio, and Maryland. It is important, however, to distinguish overwintering (recovery one year after release) from actual establishment (recovery two or more years after the last release).

Larval Parasitoids

S. agrili has successfully overwintered at eight release locations in Michigan and Ohio, and individuals were

recovered using bark peeling, yellow pan traps, and sentinel logs containing EAB larvae. Parasitism ranged from 1-45 percent. *S. agrili* has not yet been recovered in Maryland. *T. planipennisi* overwintered at five sites in Michigan and Ohio and was recovered by bark peeling, yellow pan traps, and larval sentinel logs. Parasitism ranged from 0.1-50 percent. *T. planipennisi* also overwintered in Maryland. This species was found ~800 m from the nearest release trees at two of the Michigan sites.

Egg Parasitoid

O. agrili was detected at seven research sites in 2010 (five in Michigan, one in Ohio, and one in Maryland). A total of 400 *O. agrili* females had been released over a 2-year period (2008 and 2009) at each of two sites in Michitan and one in Ohio. Overwintering was confirmed by rearing parasitoids from ash logs or bark sampled in early spring the year following release. At each of three more intensively sampled sites in Michigan, we had released a total of 2,400 *O. agrili* females over the same time period. During the summers of 2009 and 2010, we detected a range of 1 to 6 percent EAB egg parasitism by *O. agrili* at these sites. Yellow pan traps were attractive to EAB larval parasitoids but not *O. agrili*.

In general, the process of recovery yields highly variable outcomes, but some factors we observed that might have affected the likelihood of parasitoid recovery include the number of insects released, the sampling method, EAB density, and woodpecker predation. Other factors that could affect establishment and/or recovery include ash tree diameter, bark thickness, availability of nectaring plants or honeydew, competition with other parasitoids, and the tendency to disperse. We will continue to monitor the effect of biotic factors and site characteristics on the probability of establishment.

Impact of Biocontrol on Ash Health

Documenting parasitoid overwintering is the first step in evaluating the success of the EAB biocontrol project. The next steps will be to verify establishment and impact on ash health. We will continue monitoring release sites for parasitoid establishment and spread. Methods are being developed to evaluate the impact of parasitoids on EAB population density using life table analyses (see Duan et al. abstract) and indirect evidence of EAB activity. Because EAB density is difficult to determine without destructive sampling, we use indirect evidence of EAB population size at release and nearby nonrelease control sites. At each of these plots, we collect data annually on the health of 50 ash trees including: crown class, number of epicormic shoots, presence of EAB exit holes, woodpecker feeding, and bark splits. We found that from 2008 to 2010, ash health declined at all EAB monitoring sites, and no significant differences were found between ash health at the release and control plots. This is not unexpected because of the small number of parasitoids released prior to 2009 when EAB populations were already high and ash health rapidly declining. Moreover, the parasitoid species are still in the establishment phase of introduction, thus their numbers are currently insufficient to alter the trajectory of EAB population dynamics and ash mortality.

Our findings on ash health demonstrated that our control and release plots are similar in terms of ash condition and EAB densities, and these methods will allow us to monitor the impact of EAB biocontrol over time, although additional data on ash seedling and sapling growth and health will also be needed.

Conclusions

Three parasitoid species were confirmed to overwinter in Michigan, Ohio, and Maryland. These parasitoids are difficult to recover from field sites, although release of greater parasitoid numbers throughout the field season increases their probability of establishment and our ability to detect them. Recovery methods such as yellow pan traps, sentinel logs, or pheromones are easier to deploy, and, if more effective in recovering parasitoids, will become increasingly important as the number of ash trees available for sampling declines. Future releases will be made at locations where EAB population density is low, and monitoring of ash health will continue.

REDBAY AMBROSIA BEETLE AND LAUREL WILT: BIOLOGY AND HOST INTERACTIONS

Jim Hanula[1], Albert Mayfield III[2], and Stephen Fraedrich[1]

[1]U.S. Forest Service, Southern Research Station, Athens, GA 30602-2044
[2]U.S. Forest Service, Southern Research Station, Asheville, NC 28806

ABSTRACT

The exotic redbay ambrosia beetle (RAB), *Xyleborus glabratus* Eichhoff, and its fungal symbiont, *Raffaelea lauricola* Harrington, Fraedrich and Aghayeva, are responsible for widespread mortality of redbay (*Persea borbonia* (L.) Spreng.) in the southern United States. Originally introduced near Savannah, GA, RAB has spread to northeast South Carolina, throughout much of the Florida peninsula, and is now found in an isolated infestation in southern Mississippi. Tree mortality is rapid and 90 percent or more of the mature redbay trees over 2.5 cm diameter are killed within 5 years of the beetle's arrival. Once mature redbay trees have been eliminated from an area, RAB populations drop to very low levels, but they have persisted in those areas for up to 5 years now.

Peak flight of RAB occurs in late August and early September, but in 2010 the peak flight occurred in May at three monitoring sites in Georgia and South Carolina. The cause of this change is unknown. In the lab, RAB took approximately 60 days from initial attack to first brood emergence from galleries, with the last brood emerging approximately 150 days after initial attack.

We conducted a series of experiments to find the best trap design, color, lure, and trap position for detection of RAB. The best lure and trap combination was then used to trap throughout the year at three locations with varying population levels. These traps were also deployed at seven locations along the coast of Georgia and South Carolina during the peak flight period, and catches from them were compared to sticky traps, and beetle population densities were determined by counting the numbers of attacks on freshly cut redbay bolts. Manuka oil proved to be the most effective lure tested, particularly when considering cost and availability. Traps baited with manuka oil lures releasing 5 mg/d caught as many beetles as those baited with lures releasing 200 mg/d. Distributing manuka oil lures from the top to the bottom of 8-unit funnel traps resulted in similar numbers of RAB as a single lure in the middle. Trap color had little effect on captures in sticky traps or crossvane traps. Funnel traps caught twice as many beetles as crossvane traps and three times as many as sticky traps, but mean catch per trap was not significantly different. When comparing height, traps 1.5 m above the ground captured 85 percent of the beetles collected, but a few were caught at each height up to 15 m. Funnel trap captures exhibited a strong linear relationship (R^2=0.79) with RAB population density, and they performed well throughout the year in areas with high, medium, and low beetle densities.

Catching beetles at low densities is important to port of entry monitoring programs where early detection of infestations is essential. Our trials show that multiple funnel traps baited with a single manuka oil lure were effective for capturing *X. glabratus* even when no infested trees were visible in the area.

ASH DEMOGRAPHY IN THE WAKE OF THE EMERALD ASH BORER: WILL REGENERATION RESTORE ASH OR SUSTAIN THE INVASION?

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

[1]The Ohio State University, Department of Entomology, Ohio Agricultural Research and Development Center, Wooster, OH 44691
[2]The Ohio State University, Department of Horticulture and Crop Science, Ohio Agricultural Research and Development Center, Wooster, OH 44691
[3]U.S. Forest Service, Northern Research Station, Delaware, OH 43015
[4]Current address: University of Georgia, Warnell School of Forestry and Natural Resources, Athens, GA 30602
[5]Current address: Ohio Department of Natural Resources, Division of Forestry, Columbus, OH 43229
[6]Michigan State University, Department of Entomology, East Lansing, MI 48824

ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, has killed millions of ash (*Fraxinus* spp.) trees in urban and forested environments since its accidental importation from Asia. We have monitored the impact of EAB on forest communities of the Upper Huron River watershed near the epicenter of the invasion in southeast Michigan since 2004, including patterns of ash decline, mortality, and regeneration. We established transects through 38 stands across a moisture gradient of hydric, mesic, and xeric forests containing black (*F. nigra*), green (*F. pennsylvanica*), and white (*F. americana*) ash. Each transect consisted of three replicate circular plots (0.1 ha).

By 2009, overall mortality of ash trees greater than 2.5 cm diameter at breast height (dbh) exceeded 99 percent. Ash are the most common woody species in the seedling and sapling layers of these forests, which has led us to pose these questions: (1) will this regeneration restore ash if EAB is locally extirpated due to depletion of its food resource; or (2) will ash regeneration maintain an EAB population indefinitely as the supply of susceptible saplings is continually replenished? To address these questions, we quantified: (1) ash regeneration by sampling the ash seed bank from 2005-2008; (2) ash seedling and sapling dynamics in 2008-2010; and (3) EAB populations in relation to ash density through annual sampling with purple panel traps from 2008-2010. We measured densities of four demographic classes of ash: newly germinated seedlings (cotyledons present), established seedlings (at least 1-year-old but less than 25 cm tall), saplings (25 cm tall to dbh of 2.5 cm), and trees large enough to support EAB (dbh > 2.5 cm).

Four years of intensive soil sampling (432 samples/yr) suggests that the ash seed bank in these stands is rapidly depleted as overstory trees die. Small numbers of seeds were found in 2005 and 2006; however, no ash seeds were found in soil cores or on the soil surface in 2007 or 2008, and no ash seedlings emerged from germination of soil cores in either year. Our observations also indicate, contrary to some earlier speculation, that ash trees do not increase seed production as they become stressed by EAB. Patterns of ash demography were consistent with conclusions reached from seed bank sampling. Density of new ash seedlings (with cotyledons) was less than one plant/ha in Michigan plots in 2008 and 2009, respectively, and only one newly germinated seedling was found in 2010 inside or outside the monitoring plots. In contrast, density of new ash seedlings routinely exceeded 800 plants/ha in Ohio plots where EAB mortality was still low or nonexistent, and exceeded 20,000 plants/ha in some plots in 2009 following mast seed production on a regional scale in 2008. Established ash seedlings (no cotyledons but less than 25 cm tall) were far more abundant than new seedlings in the Michigan plots. However, this pattern was reversed in Ohio plots where new seedlings greatly exceeded established seedlings, except in stands where mortality of mature ash approached 100 percent, in which case the density of established seedlings greatly exceeded that of new seedlings.

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

In summary, EAB-induced mortality of trees with dbh > 2.5 cm now exceeds 99 percent in the Huron River Watershed in southeast Michigan. Our data suggests that the ash seed bank does not persist after overstory trees succumb to EAB. Ash regeneration has ceased in these plots, as newly germinated seedlings have been virtually nonexistent since 2008. An orphaned cohort of established seedlings and saplings remains the only demographic class of ash remaining in these plots. However, EAB continues 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-induced mortality. Alternatively, a dynamic equilibrium may establish in which some trees reach reproductive maturity before succumbing to EAB, especially if biological control agents prove capable of regulating EAB at very low densities.

CHEMICAL COMMUNICATION IN THE ASIAN LONGHORNED BEETLE

Kelli Hoover[1], Melody Keena[2], Maya Nehme[1], Aijun Zhang[3], Talbot Trotter[2], and Alan Sawyer[4]

[1]Penn State University, Department of Entomology and the Center for Chemical Ecology, University Park, PA 16802
[2]U.S. Forest Service, Northern Research Station, Hamden, CT 06514
[3]USDA ARS, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD 20705
[4]USDA APHIS PPQ, Otis Pest Survey, Detection, and Exclusion Laboratory, Buzzards Bay, MA 02542

ABSTRACT

Tree surveys to identify signs of infestation by the Asian longhorned beetle (ALB), *Anoplophora glabripennis*, an introduced pest from China, rely either on ground surveys or tree climbing. The first method is not very accurate (about 20 percent), and the second is time consuming and expensive. As a consequence, there have been efforts for several years to develop a more efficient method to detect ALB in the field using a trap baited with an effective lure.

After two seasons of testing a potential trapping system in China, we evaluated the best of the lure/trap combinations in the Worcester, MA, ALB infestation during the summers (adult flight periods) of 2009 and 2010. From the work in China, we learned that the most effective lure consisted of a combination of the male-produced pheromone (a blend of 4-(*n*-heptyloxy) butan-1-ol and 4-(*n*-heptyloxy)butanal), and a mixture of 5 plant volatiles: (-)-linalool, *cis*-3-hexen-1-ol, linalool oxide, *trans*-caryophyllene and *trans*-pinocarveol. Intercept panel traps baited with this lure caught significantly more females than control traps, pheromone alone, or plant volatiles alone when tested in China. In addition, 85 percent of females trapped were virgin.

In 2009, we deployed 85 Intercept® panel traps in Worcester, MA, using six different lure treatments and unbaited control traps at nine different sites from mid-June to mid-September. Traps were hung on trees that had been evaluated by tree climbers and labeled uninfested. Traps were hung using a simplified pulley system and checked every 2 weeks. Lures were changed monthly. In 2009, nine ALB were trapped, all female, between August 21 and September 27. The most effective lure (6 beetles caught in 16 traps) was the same combination of male pheromone and plant volatiles that proved most effective in China, consisting of the male pheromone at a release rate of 10 μg/day and the same five plant volatiles described above at a release rate of 1 mg/day. Among the other lure combinations, we trapped 1 beetle/6 traps using the male pheromone alone at the highest release rate of 1 mg/day; 1 beetle/14 traps using the male pheromone alone plus five plant volatiles replacing *trans*-pinocarveol with δ-3-carene; and 1 beetle/16 traps using the five plant volatiles alone (with *trans*-pinocarveol) at 1 mg/day. No beetles were trapped in the 13 empty control traps.

In 2010, we deployed 40 Intercept® panel traps in Worcester using a simplified pulley system (line thrown over a limb). This simplified approach reduced the amount of time it took to install the traps. Traps were placed near recent infested tree finds where no pesticide had been applied. Traps were coated with Fluon® to improve trap slipperiness, and India ink to darken the light Fluon. The first of two lure treatments, the male-produced pheromone (0.1 mg/day) and the five plant volatiles (1 mg/day) used in 2009 caught 3 beetles/20 traps. The second lure, which was the same as the first except that trans-pinocarveol was left out, caught 1 beetle/10 traps. No beetles were trapped in the 10 empty controls. All beetles were caught in late July through early September in 2010 and all were female.

For both 2009 and 2010, we recorded the latitude/longitude, diameter at breast height, and species for each tree that contained a trap. Using data provided by APHIS, which contains street addresses (based on property records for Worcester, MA) for all infested trees for both study years, including data on when the positive tree was found and when it was removed, we are working to determine the distance over which the trap can attract beetles. This analysis has not been completed,

but to date we have found that traps that failed to catch beetles were located at sites where known infested tree finds had been removed before the traps were deployed. In some locations where beetles were trapped, surveys had indicated all infested trees had been removed. After ALB were trapped and reported to APHIS, host trees in the areas around the trap were resurveyed, leading to the discovery of one or more additional infested trees. In one example, near the intersection at Parkton and Lansing Avenue, an area that was surveyed in the spring of 2010, traps near this location caught beetles on July 27 and August 11, 2010, prompting a second survey by tree climbers in early January of 2011. During this survey, an oviposition pit was found on a tree 84m from the trap, and two exit holes in a tree on Drummond Avenue 177 m from the trap. It is possible that these two beetles came from the exit holes on the tree 177 m from the trap. However, the oviposition pit shows active females within ~80 m of the trap, suggesting the traps may have a draw radius of ~80 m. These results indicate that the most effective lure and trap combination tested can be used as a quality control check following removal of infested trees, for early detection around high risks sites, or as a presence/absence delimiting survey.

Over the next few months we plan to optimize the plant volatile blend used in combination with the pheromone, evaluate lure release rates, and to complete our data analysis to determine the effective range of the lure based on the 2009 and 2010 Worcester trapping results. These results will be used as we plan our trapping study for the 2011 season in Worcester to determine if this method can be used in conjunction with surveys to increase the efficiency of locating infested trees.

DO WESTERN CAMPERS BRING OUT-OF-STATE FIREWOOD TO STATE AND NATIONAL PARK CAMPGROUNDS

W.R. Jacobi, B.A. Goodrich, and C. M. Cleaver

Colorado State University, Department of Bioagricultural Sciences and Pest Management, Fort Collins, CO, 80523-1177

ABSTRACT

Untreated firewood has the potential to harbor insects or pathogens lethal to trees in urban and natural forest ecosystems. Campers at 15 campgrounds in 7 Colorado State Parks and 30 campgrounds in 13 National Parks in Arizona, Colorado, Nevada, Utah, and Wyoming were surveyed in 2007-2009 to determine camper home states, firewood presence, firewood state origins, and risks of firewood harboring pests. Sixty-six percent of Colorado State Park campers had firewood, but only 4 percent had firewood brought from out-of-state origins. Sixty percent of National Park campers had firewood, and 39 percent had firewood from out of state, equating to 329,919 campers bringing out-of-state firewood in one year to surveyed parks. Forty-one percent of out-of-state firewood was brought by campers from nonneighboring states, indicating long distance transport of firewood occurs. Of all firewood present in National Parks, 32 percent was purchased inside the park, 25 percent was purchased outside the park, and 17 percent was from camper residences. Fifty-three percent of firewood had evidence of previous insect presence and 39 percent had fungal infestation. Camper movement of untreated firewood has the potential to be a high risk pathway for distribution of live tree pests throughout North America, and educational and mitigation actions should be implemented.

USE OF ARBORETA SURVEYS AND SENTINEL TREE PLANTINGS IN ASIA TO IDENTIFY POTENTIAL FOREST PESTS IN EUROPE

Marc Kenis[1], Alain Roques[2], Jiang-hua Sun[3], Jian-ting Fan[4], Natalia Kirichenko[5], Yuri Baranchikov[5], Maria Tomoshevich[6], Svetlana Gorokhova[7], Pavel Ostrogradsky[7], Annie Yart[2], Keith Holmes[1], and Christelle Péré[1]

[1]CABI Europe-Switzerland, DelémontSwitzerland
[2]Institut National de Recherche Agronomique (INRA), Station de Zoologie Forestière, France
[3]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[4]School of Forestry, Zhejiang Agricultural and Forestry University, Lin'an, 311300, China
[5]V.N. Sukachev Institute of Forest, Siberian Branch of the Russian Academy of Sciences, Krasnoyarsk 660036, Russia
[6]Central Siberian botanical garden, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, 630090, Russia
[7]V.L. Komarov Mountain-Taiga Station, Far Eastern Branch of the Russian Academy of Sciences, Primorskiy kray, 692533, Russia

ABSTRACT

An important weakness in Pest Risk Analysis (PRA) is the fact that PRAs usually focus on well known pests or on pathways that are known to carry serious pests. However, most serious invasive plant pests and pathogens are not pests in their region of origin, partly because they are often introduced without their natural enemies, but also because their original host plants are more resistant to the pest than the newly encountered hosts.

A novel method has been developed to detect new potential pests of European woody plants in their region of origin before they are introduced into Europe. This method consists of two approaches: (1) Sentinel European trees were planted in two areas in China and monitored at regular intervals for damage caused by indigenous arthropods and pathogens; and (2) In Siberia and the Russian Far East, surveys were carried out in several arboreta and botanical gardens to detect arthropods and pathogens attacking European trees and shrubs. These two methods are complementary since the sentinel tree method provides statistically robust data on pests colonizing young trees but is logistically difficult to implement. In contrast, arboretum surveys are easier to carry out and can provide data on pests attacking mature trees. However, observations in arboreta are not easy to analyze statistically because they often refer to observations on single trees that may not be representative of the tree species variability. Both methods require strong local links with entomologists and pathologists to be successfully implemented.

Investigations in China and Russia have resulted in the discovery of severe arthropod pests and diseases of European woody plants. Some are already identified and can be the target of new PRAs while others need further research on their identification and impact. To further develop this method, we strongly support the suggestion of Britton et al. (2010) to develop an early warning system through a global network of arboreta and botanical gardens, which would allow them to inform the international community of any serious pest occurring on alien woody plants, but also to request help for pest identification.

This research was initiated during the EU-funded project ALARM (GOCECT-2003-506675) and has been developed in the framework of two other EU project, PRATIQUE (212459) and ISEFOR (245268). Work in Russia was also partly funded by the grant of President of the Russian Federation (MK-7049.2010.4) and the fund of Siberian branch of the Russian Academy of Sciences (Lavrentiev's grant, grant No. 19).

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STRATEGIES FOR SELECTING AND BREEDING EAB-RESISTANT ASH

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

[1]U.S. Forest Service, Northern Research Station, Delaware, OH 43015
[2]U.S. Forest Service, Northern Research Station, East Lansing, MI 48823
[3]The Ohio State University, Department of Entomology, Ohio Agricultural Research and Development Center, Wooster, OH 44691

ABSTRACT

Breeding for pest resistance in forest trees is a proven approach for managing both native and nonnative insects and diseases. A recent study by the Food and Agriculture Organization of the United Nations reports 255 forest tree breeding programs for insect or disease resistance in 33 different countries (http://www.fao.org/forestry/26445/en/). Advantages to incorporating breeding as a management approach to nonnative insects such as the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire include: (1) wide public acceptance; (2) a proven successful approach (many examples of success); (3) not dependent on prior knowledge of mechanisms of resistance; and (4) not dependent on the number of genes involved (qualitative or quantitative traits are equally successful), or the mode of inheritance of resistance (can select for dominant or recessive traits).

We are employing two different strategies to breeding for resistance: hybrid and traditional breeding. In hybrid breeding, the desired resistance trait is found in a different species, often an exotic species. The exotic species is crossed with the native species to create hybrids. The hybrids go through subsequent rounds of testing, selection, and backcrossing to the native species to carry only the resistance genes from the exotic parent into the native population while retaining all of the traits of the native species, as is being done in the American Chestnut Foundation's breeding program (Hebard 2006). In traditional breeding, the parents are both from the same species, generally the native species, and either one or both of them have the resistance trait of interest. This approach has been used successfully in other species (see FAO forestry reports link above for detailed examples).

Hybrid Breeding

Initial reports after discovery of the EAB infestation indicated that all native ash species were highly susceptible. The theory that ash species that coevolved with EAB developed mechanisms of resistance that allowed them to coexist indicates that Asian species of ash would be the most likely source of genetically heritable resistance (Rebek et al. 2008). The first step in initiating a hybrid breeding program is to identify and confirm genetic control of such EAB-resistant species for selection as breeding parents. A common garden study demonstrated that a cultivar of *F. mandshurica*, 'Mancana', was resistant to EAB relative to North American species, and anecdotal evidence indicates the same may be true of *F. chinensis* (Liu et al.,2007, Rebek et al. 2008). To identify additional EAB-resistant species, we have been accessioning Asian ash species across a wide geographical and ecological range. Each accession is confirmed for the proper species identity through the use of DNA-based technologies including ITS sequencing and AFLPs. This step is important because there is no comprehensive global taxonomic key, and few traits are diagnostic between closely related but geographically isolated species. Our current exotic ash collection includes 85 independent accessions of exotic ash, including 11 Asian species and 2 European species. In the process of confirming species identities, we uncovered seven different Asian ash accessions from reputable botanical gardens and arboreta that were incorrect, illustrating the difficulty of distinguishing *Fraxinus* species, even by experts. Additionally, six different seed lots (representing both Asian and North American species) from two different commercial seed companies were found to be incorrectly identified. Once the species identity is confirmed, the individual

accessions are replicated through grafting and eventually assessed for their EAB resistance through bioassays and field plantings.

Inter-specific hybridization of ash has not been widely reported. Hybridization of *F. nigra* and *F. mandshurica* resulted in the release of two horticultural cultivars (Davidson 1999). To determine what other species combinations may readily hybridize, we pollinated as many combinations as possible (dependent on availability of viable male/female flowers) under controlled conditions. In 2010, 42 different species combinations were crossed and 15 combinations successfully produced seed which was collected and put into stratification for germination. Resulting seeding families will be screened for EAB resistance. A total of 971 hybrid seed were collected representing full-sib family sizes up to 450 seeds. The hybrid parentage of one small family has been confirmed with DNA markers, and as tissue becomes available, the parentage of all full-sib families will be confirmed. Such large family sizes will be very useful for segregation analysis to confirm the genetic basis of resistance, linkage mapping, marker development, and the identification of proteins and metabolites that segregate with the resistance phenotype. Association of a protein or metabolite of interest that segregates with only resistant progeny in a full-sib family allows the identification of truly functional differences from among the many differences due only to the species differences between parents and the novel combination of these species.

Breeding work in 2010 also included the backcrossing of an interspecies hybrid cultivar (Davidson 1999) to both parental species. The seed from these crosses, should they successfully germinate, will be the most advanced *Fraxinus* interspecies pedigree, and a demonstration that at least some F1 hybrids are reproductively competent providing support for the potential of the hybrid/backcross breeding approach.

Traditional Breeding

Initial EAB infestations were in urban areas where a few ash cultivars were planted in high numbers. Although a large number of trees were killed, these represented a small number of genotypes. As EAB moved into natural stands and monitoring plots were established, it became apparent that there are trees that are still alive and rare trees that support a healthy crown even when over 99 percent of ash trees in the area are dead. These "lingering ash" trees may simply be the last to die. Alternatively, they may be trees with rare phenotypes that are less preferred, tolerant or resistant to EAB infestation. We have been identifying, grafting to preserve and replicate, and testing these trees. We believe it is important to investigate these trees and to verify if the lingering ash phenotype has an underlying physiological or biochemical basis that can be utilized in a breeding program.

We have identified, collected, and grafted 33 lingering ash trees (primarily green and white ash) from 2008-2010. Clonal ramets of each of these select trees will be generated and outplanted for field testing and bioassays to confirm their EAB phenotype. Initial bioassays have been conducted for a few lingering ash selections that had been replicated enough to provide an adequate quantity of tissue for experimentation. Adult choice feeding bioassays were conducted in two separate experiments with seven different green ash individuals selected as lingering ash. Unselected green ash seedlings (pe-H880), the EAB-susceptible green ash cultivar 'Summit'(pe-sum), and a selection of *F. mandshurica* (man-19) a known host species for EAB in Asia and a known EAB-resistant control were included in both experiments. Adult feeding, expressed as the proportion of the leaf area fed over 2 days, was analyzed by combining the data from both experiments and fitting a Generalized Linear Model. Genotype was found to be a significant effect, so least square means were estimated for each genotype and compared to the seedling control, pe-H880 (Fig. 1). Four trees had significantly different least square means from the control seedlings. Both *F. mandshurica* (man-19) and 'Summit' (pe-sum) had higher proportions fed, and two of the lingering ash selections (pe-24 and pe-22) had significantly lower proportions fed relative to the seedling control.

The feeding preference data from the choice bioassay was further supported by a natural EAB infestation

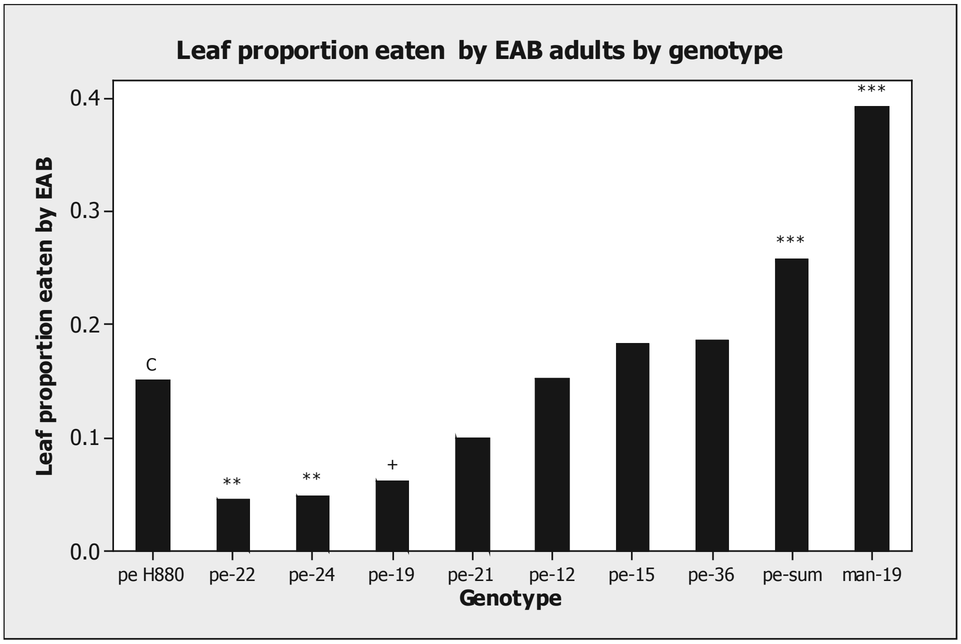

Figure 1.—Leaf proportion eaten by EAB adults by genotype. Least squares means for each genotype were calculated and compared to the seedling control, pe-H880. Four trees were significantly different from the control seedlings and a fifth very nearly different (***highly significant, $p<0.003$; **significant, $p<0.02$; + nearly significant, $p=0.0052$).

in the nursery growing area at the U.S. Forest Service laboratory in Delaware, OH where some lingering ash selections (including the significantly less-preferred lingering ash pe-22 from the feeding bioassay) remained uninfested while known susceptible selections in the same nursery bed were infested. Taken together with the feeding bioassay, this suggests that the lingering ash phenotype is real, and one component of this phenotype may be the reduced feeding preference to EAB adults of these trees.

Future work will focus on attempting to further enrich the lingering ash phenotype through breeding with other lingering ash or with exotic EAB-resistant trees in a combination of traditional and hybrid breeding. Two lingering ash grafts flowered and were cross-pollinated in 2010, successfully producing seed. The resulting F1 families of 'lingering x lingering' seedlings will be grown and assessed for their EAB phenotype so that patterns of inheritance can be discerned. Segregation of phenotype and potential underlying physiological or biochemical features will be analyzed for indications of the possible mechanisms involved.

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THE INFLUENCE OF EMERALD ASH BORER SATELLITES ON DAMAGE IN U.S. COMMUNITIES, 2010-2020

Kent F. Kovacs[1], Robert G. Haight[2], Deborah G. McCullough[3], Rodrigo J. Mercader[3], Nathan W. Siegert[4], and Andrew M. Liebhold[5]

[1]University of Minnesota, Department of Applied Economics, St. Paul, MN 55108
[2]U.S. Forest Service, Northern Research Station, St. Paul, MN 55108
[3]Michigan State University, Department of Entomology, East Lansing, MI 48824
[4]U.S. Forest Service, Northeastern Area State & Private Forestry, Durham, NH 03824
[5]U.S. Forest Service, Northern Research Station, Morgantown, WV 26505

ABSTRACT

The invasion spread of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is characterized by the formation of satellite populations that expand and coalesce with the continuously invading population front. As of January 2010, satellite infestations had been detected in 13 states and 2 Canadian provinces. Understanding how newly established satellite populations may affect economic damage can help program managers design prevention and control strategies. We estimate the economic damage caused by EAB for the 10-year period from 2010-2020 for scenarios of fewer EAB satellite populations in 2005-2010 and slower expansion of satellite populations found in 2009. Damage is measured by the projected discounted cost of treatment, removal, and replacement of ash trees (*Fraxinus* spp.) growing in managed landscapes in U.S. communities. Estimated damages for the base scenario with the full complement of satellites in 2005-2010 and no management is $12.5 billion. Fewer EAB satellites in 2005-2010 reduce economic damage by $1.0 to 7.4 billion. Slower expansion of 2009 satellite populations reduces economic damage by $0.1 to 0.7 billion. Satellite populations that are both distant from the core EAB infestation and close to large urban areas cause more economic damage in our simulations than do other satellites. Our estimates of reduced economic damage suggest that spending tens of millions of dollars per year on activities that prevent establishment of new satellite EAB populations or slow expansion of existing populations can be cost-effective, and that continued research on the cost and effectiveness of prevention and control activities is warranted.

BIOLOGICAL CONTROL OF MILE-A-MINUTE WEED, *PERSICARIA PERFOLIATA*: SIX YEARS OF POST-RELEASE RESULTS FROM SOUTHEASTERN PENNSYLVANIA

Ellen Lake[1], Judith Hough-Goldstein[1], Kimberley Shropshire[1], and Vincent D'Amico[2]

[1]University of Delaware Department of Entomology and Wildlife Ecology, Newark, DE 19716
[2]U.S. Forest Service, Northern Research Station, Newark, DE 19716

ABSTRACT

Mile-a-minute weed [*Persicaria perfoliata* (L.) H. Gross (Polygonaceae)], is an annual Asian vine that established in the United States in the late 1930s in Pennsylvania via a contaminated shipment of holly seed from Japan. Mile-a-minute's current range includes 12 states from Massachusetts to North Carolina. Backward projecting thorns enable mile-a-minute to climb up and over other vegetation, form a dense canopy, and outcompete other plants in a variety of habitats. Mile-a-minute is a prolific seed producer, and its seed can be viable for 6 years in the seedbank. The host specific weevil *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae) was first released in the United States in 2004. Adult weevils feed on mile-a-minute leaves and the larvae are stemborers; at least 3-4 generations are produced per year in the mid-Atlantic United States.

Three replicated releases of 450 weevils each were conducted in southeastern PA in June 2005. Weevil dispersal from the central release point, and impact on mile-a-minute weed percent cover and production of seeds and seedlings were monitored in 1 m^2 quadrats located on concentric circles surrounding the point of release. Mile-a-minute cover has declined significantly at two of the three release sites. Spring seedling counts, an indicator of mile-a-minute reproduction and release from the seedbank, decreased from 2005 to 2008. A very cold and wet spring in 2009 provided good growing conditions for mile-a-minute and slowed the development of weevil populations. This weather contributed to an increase in mile-a-minute seedlings and cover in 2009. The larger mile-a-minute populations in 2009 led to elevated seedling counts at two of the three sites in 2010.

Rhinoncomimus latipes is negatively impacting mile-a-minute weed, but when mile-a-minute populations decline, they are often replaced by a different invasive weed rather than native vegetation. The Hough-Goldstein lab is researching integrated weed management strategies to break this invasive species treadmill and restore native plant communities.

A DISPARATE TALE OF TWO MOTHS: LIGHT BROWN APPLE MOTH AND EUROPEAN GRAPEVINE MOTH INVADE CALIFORNIA

David Lance[1] and Roxanne Broadway[2]

(presented by Vic Mastro[1])

[1]USDA APHIS PPQ CPHST, Otis Laboratory Buzzards Bay, MA 02542
[2]USDA APHIS PPQ WR Sacramento, CA 95814

ABSTRACT

Two exotic tortricid moths of regulatory concern to the United States have been discovered in California in the past few years. In early 2007, two light brown apple moths (LBAM), *Epiphyas postvittana* (Walker), were identified from materials that had been captured the previous year in a backyard light trap in Berkeley, CA. In 2009, larvae of the European grapevine moth (EGVM), *Lobesia botrana*, were identified from a vineyard in central Napa Valley. Both of these species are known pests of fruit within their native ranges and in other areas they have invaded. As a result, both had been relatively well studied prior to their discovery in the United States., and pest management tools were available, including pheromone-baited traps, mating disruption methods, and effective insecticides.

Light brown apple moth is highly polyphagous and has been recorded as a pest of pome fruit, stone fruit, grapes, citrus, cane berries, strawberries, kiwi, and other crops. It is native to Australia but has for some time been established in New Zealand, Hawaii, and Great Britain. LBAM is a leaf-roller by habit but also attacks fruit where foliage comes in contact with fruit, or in some crops where fruit grow in tight bunches, such as grapes and kiwis. The larvae also feed on numerous ornamental plants as well as forest and shade trees, including broadleaves and conifers. LBAM is not generally considered a direct agricultural pest of plants other than fruit crops, but potential movement of the insect on such items as cut flowers and nursery stock make it a phytosanitary problem on those commodities as well.

Following its discovery, the LBAM population in California was delimited using pheromone-baited traps. California Department of Food and Agriculture (CDFA) maintains, in cooperation with county agencies, extensive detection trapping systems for a number of pests such as exotic fruit flies, and LBAM traps were rapidly deployed across broad areas by piggybacking onto those existing efforts. In 2007, most LBAM in California were found either in the area around the Monterey Bay or in the central San Francisco Bay area (San Francisco, Albany, Berkeley), with numerous pockets of infestation between and around those areas. CDFA also placed restrictions on moving LBAM host materials out of the known infested area, which affected primarily nursery stock and fruit crops.

A Technical Working Group (TWG) was convened to provide USDA and CDFA with science-based advice on mitigation efforts. Despite the relatively widespread nature of the LBAM population, the TWG recommended attempting eradication as long as effective tools were available to carry out the program and cost-benefit analyses indicated that eradication was warranted. The program they proposed used an integrated approach, relying on mating disruption as the primary suppression tool. This decision drew fire from some parts of the scientific community and the public, but appeared to be the only feasible option at the time: (1) mating disruption was known to be effective at population levels present in CA; (2) mating disruption becomes increasingly effective as populations decline, making it a theoretically sound choice for suppressing and eliminating the last vestiges of a population; and (3) a nontoxic and species specific suppression method was needed. This last item was critical as LBAM existed throughout a very broad area that encompassed urban habitats as well as remote wild areas. Aerial application was considered necessary for cost-effective program delivery.

After securing federal funding, CDFA applied mating disruption formulations by air to nearly 90,000 acres (38,000 of those twice) in the fall of 2007. The only available formulation at the time was a microencapsulated product, which was applied without sticker. The applications appeared to suppress trap catch, but the TWG believed that the effect was neither strong enough nor persistent enough for programmatic use. Applications were suspended, and the USDA funded a pilot-scale, replicated field trial of four aerially applied formulations in a New Zealand pine forest, using a commercially available hand applied formulation as a positive control. Two aerially applied formulations were identified that appeared suitable for program use.

In California, however, there had been strong public backlash against the initial applications. Over 600 health related complaints had been registered thru the internet or other unofficial media, with over 400 claiming symptoms that they believed had been caused by the applications. In no case was there any direct evidence linking the applications to the symptoms, most of which were mild respiratory symptoms, such as those associated with allergies or colds, which are present in substantial portions of the population on any given day. There was no spike in hospital or doctor visits around the time of the applications. Still, local reporters repeatedly stated that over 600 people had been sickened by the applications. The treatments were also blamed, in internet blogs and related media, for death of >600 seabirds, a red tide outbreak, death of songbirds, bees, and pond fish, and for a large buildup of foamy material in Monterey Bay. Moreover, a widely quoted analysis claimed that the applications could have elevated the levels of small (<10-µm) airborne particles (which can cause respiratory problems) to levels that the EPA claims are unhealthy. The analysis, though, was based on an erroneous understanding of the formulation, resulting in a 4-fold overestimate of quantity of particles (this alone would reduce levels to what EPA considerers normal background), and also made the questionable assumption that all particles would remain suspended within 2 m of the ground. The resistance to the spraying, which was tracked back to a small core of activists, produced not only public outcry against treatments, but also a number of lawsuits that blocked further applications for extended periods. Program managers from USDA and CDFA eventually decided to give up on aerial applications of mating disruptants, and instead focused on developing Sterile Insect Technique, which was then in its infancy as a technology for LBAM.

In the absence of control efforts, the LBAM infestation in California has continued to grow. There were ~16,800 moths captured in 2007, 47,000 in 2008, and 208,000 in 2009, at which point most trapping within the infested area was suspended due to budgetary reasons. The geographic area infested has increased several-fold, and satellite infestations have arisen in distant locations such as San Diego and Los Angeles. The program has continued detection trapping and regulatory restrictions on movement of host materials, but eradication is no longer its stated goal.

European grapevine moth is native to Europe and was found in Chile several years ago. In both areas, it is a key pest of grapes. Unlike LBAM, EGVM has a fairly restricted host range: olive flowers and some stone fruits and ornamentals are occasional alternate hosts and are considered to pose potential regulatory risks, but the insect is really only a pest of grapes. The larvae feed on grape flowers early in the season, and later generations attack the fruit directly. The attacks also leave the fruit susceptible to *Botrytis* infestation.

To date, EGVM and LBAM programs in California have gone quite differently. As was the case with LBAM, USDA and CDFA responded to the EGVM find with an extensive delimitation program based on pheromone-baited traps and with phytosanitary restrictions on movement of host materials. The trapping program found that EGVM had spread to a number of sites beyond Napa. Some of these sites were in the neighboring counties of Sonoma and Solano, but others were in more distant counties such as Fresno and Mendocino. Captures beyond the Napa Valley area represented a very small proportion of the moths caught—about 125 of over 100,000 total moths. In all cases, these remote finds were associated with grape production.

Although funding for this program was limited in 2010, the USDA, CDFA, county agricultural agencies, and affected growers worked closely together to design treatment plans to suppress EGVM populations. Napa County hired a treatment coordinator to work with growers to help ensure appropriate coverage and timing of treatments, and additional counties followed suit. The growers, who rarely apply insecticides at all under normal conditions, agreed to treat their vineyards at their own expense to knock down EGVM populations. Most used insect growth regulators, but some growers chose more conventional toxins, while organic growers used either Bt or spinosad. Growers in the central Napa Valley were also encouraged to apply twist-tie pheromone dispensers for mating disruption. The treatments appeared to knock the populations back substantially. There were three "flights" (generations of adult moths) during the season. The first, insects that had overwintered as pupae, continued until June and produced nearly 100,000 males in traps. During the second flight, which lasted until mid-August, just over 1,300 males were captured, and for the third flight, which ran into early October, there were only 291 males caught. The treatments will continue in 2011, and plans are being drafted to get sites of outlying infestations out from under regulation as quickly as possible.

The difference in the courses taken by the LBAM and EGVM programs is striking. Part of this has resulted from differences in industry support. Grape growers (especially wine grapes) were very cooperative and very motivated to try to eliminate this pest. With LBAM, the program heard little from fruit producers; in contrast, some nursery owners were vocal in complaining about a program that placed restrictions on them for an insect that they didn't even consider a pest. The difference was exacerbated, we believe, by observed levels of damage at detection. LBAM had not produced commercial damage in the United States when the program started, whereas detection of EGVM was associated with complete loss of a grape crop in a vineyard in central Napa Valley, as well as losses in at least one other vineyard in the area. This alarmed the local wine grape producers and their county agricultural agencies. LBAM has since caused economic damage to cane berry producers in the Monterey Bay area (who have been grateful for mating disruption technology) but still hasn't moved into California's major production areas for many of its host crops.

Interactions of biological and social factors also affected program direction. The host range and habitat of LBAM resulted in the initial stages of the battle being fought in residential neighborhoods, whereas EGVM program activities, to date, have been primarily in agricultural areas. In addition, levels of public concern about LBAM program activities were unexpected to many of us who believe that mating disruption is a nontoxic and species-specific method of pest control. The broad public interface and public worries with the LBAM program hit full force in an information war the program was not prepared to fight. To be fair, the program did a very good job, by historic standards, of public outreach and education regarding program activities. However, information (accurate or otherwise) by opponents of the program moved, morphed, and was assimilated on the internet almost instantaneously. Government agencies involved in these types of programs need to check and cross-check public statements for accuracy and consistency. By then, the bloggers were another 10 steps down the road and it was too little, too late. Information management in the internet age will continue to challenge future programs.

As a final note, there was one disturbing similarity between the two programs: both pests were relatively widespread by the time the populations were detected. Eradication or even containment of widely distributed pests is expensive, often has a broad interface with the public, can put restraints on tools available for mitigation, and may have limited chances of ultimate success. LBAM and EGVM are pests of regulatory concern to USDA and have been targeted by the Cooperative Agricultural Pest Survey since the mid 1980s. Pheromone based trapping systems have been available throughout that period. Despite this, relatively little survey had been done for either insect. California, for example, had never trapped for EGVM until embarking on the current program. Detection trapping, done properly (i.e., to catch a population early enough to simplify mitigation) can be expensive, making it a tough sell for pests that don't have a history of being introduced into an area. Still, proper integration of risk assessment and survey technology should allow us to balance survey effort and cost against predicted costs of mitigating the effects of introductions. If the LBAM population in California had been discovered when it was confined to one or a few square miles, what would resulting programs have looked like, what would it have cost, how would public reaction have differed, and what would the likely outcome have been?

HOW IMPORTANT IS PROPAGULE PRESSURE IN INVASION ECOLOGY?

Julie Lockwood

Rutgers, The State University of New Jersey, Ecology, Evolution and Natural Resources, New Brunswick, NJ 08902

ABSTRACT

Propagule pressure is defined as the number of individuals of a nonnative species released into any single region, and is the sum over the total number of discrete releases to the region (propagule number) and the number of individuals in each release event (propagule size). This definition stems from the work of Mark Williamson published in 1996, although the roots of the term are clearly held within botany. Although the influence of propagule pressure on establishment success is widely known today, the subject was absent from early seminal works in invasion ecology such as that produced by Charles Elton. Nevertheless, there is theoretical and empirical reason to suggest that higher propagule pressure serves to directly increase the likelihood that an incipient nonnative population will establish a self-sustaining population.

The mechanisms behind the influence of propagule pressure on establishment reflect well-known principles in conservation biology. Higher numbers of individuals released ensures that the incipient population does not become extinct via the influence of environmental and demographic stochasticity, or Allee effects. Higher numbers of independent release events increases the chance that the nonnative species encounters local conditions that are favorable for establishment. Finally, higher numbers of individuals released may ensure that the incipient population has a relatively high level of genetic diversity which should, in turn, prevent inbreeding depression and allow the population to evolve to match local conditions. Although there is a clear logical connection between these mechanisms in invasion ecology and conservation biology, they do not always reflect the opposite sides of the same coin. There continues to be a need for nuanced exploration of these mechanisms as they relate to the realities of species invasions.

As an example of the influence of propagule pressure on invasion success, I reviewed work by Phillip Cassey and colleagues on the successful introduction of birds worldwide. The dataset these authors explore includes information on the numbers of individuals released in 1,920 independent introduction events, which encompasses 416 bird species. Propagule pressure was statistically evaluated as an explanation for the fate of these introductions (successful or failed) against a suite of life history and location level information. In this dataset, the predominate explanation for success was propagule size; the larger the numbers of individuals released in any one event, the more likely that incipient population would successfully establish. Also of note was a correlation between propagule pressure and taxonomic family, and between pressure and island/mainland status. Some bird families have had relatively many introduction events that involved large numbers of individuals. Similarly, when birds were released onto islands, these events tended to involve far more individuals than when (even the same species) they were released onto mainlands. These and similar correlations suggest that, in the absence of information on propagule pressure, statistical tests associated with identifying suites of factors that influence establishment will be highly biased. Indeed, in this dataset, when information on propagule pressure is removed from statistical tests, a suite of life history and location level traits appear to influence success. However, when propagule pressure is kept in the models, only it and greater dietary generalism provide any explanatory power. Based on this evidence, I suggest that research in invasion ecology that fails to account for the influence of propagule pressure may produce quite flawed, and overly complicated, models of what influences establishment success.

The limiting factor for incorporating propagule pressure in most models of invasion success comes not from

a failure of scientists to recognize its importance, but in the lack of available data. Most invasions of nonnative species are accidental, and thus information on the numbers of individuals released is nonexistent. In response to this dilemma, several ecologists have developed surrogate measures of propagule pressure using information that is available. Good examples include the use of port inspection information, price and availability information from seed catalogs, and the number of oceangoing ships that arrive in any given port. There is a deep need to continue developing such surrogates, and exploring their strengths and weaknesses relative to the goal of predicting invasion success.

Finally, I posed the question of whether propagule pressure had any bearing on how one might manage already successful species invasions. This topic has not seen much attention in the literature; however, there are several possible avenues worth exploring. For example, species released in high numbers may be (1) more likely to be detected using standard surveillance protocols, and/or (2) more likely to increase quickly away from small numbers after being detected. Similarly, species released in higher numbers may be more likely to occur in large enough numbers, and spread over enough geographic space, that they more quickly surpass the threshold beyond which eradication is a realistic option. Neither of theses suppositions have been explored as of yet, however, but such an effort should pay good rewards for those responsible for managing invasive species.

PREDICTING MOVEMENT, RISK, AND ECONOMIC IMPACT OF EMERALD ASH BORER IN MARYLAND

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

[1]University of Maryland, Department of Entomology, College Park, MD 20742
[2]Maryland Department of Agriculture, Annapolis, MD 21401
[3]USDA APHIS PPQ CPHST, Otis Laboratory, Buzzards Bay, MA 02542

ABSTRACT

Since its detection in Michigan in 2002, the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), has spread to several surrounding states, and satellite populations have been found across the Midwest and mid-Atlantic. EAB was introduced to Maryland from one shipment of infested nursery stock in 2003. Survey and eradication efforts by the Maryland Department of Agriculture (MDA) have provided spatially explicit data on its movement shortly after its introduction to the state (Sargent et al. 2010). In this study, we investigated whether there has been a recent change in the rate of EAB spread, and we estimated the predicted arrival dates of EAB in local municipalities. We assessed potential risk to these cities and towns by estimating the size-class distribution and total numbers of municipally-managed ash trees. Finally, we considered the environmental and aesthetic benefits provided by those trees and the management options available at this time to prepare for EAB arrival.

To quantify the spatial spread of EAB in Maryland and predict when the beetle might arrive in local municipalities, we used linear and polynomial regression. Using the MDA data on the four farthest detections each year from the original point of EAB introduction, we quantified rate of spread by regressing distance from the introduction site against time. For this study, we evaluated the linear regression models of Sargent et al. (2010) with 2010 data from the MDA surveys of landscape and naturally occurring ash trees in Prince Georges and Charles Counties. We found that the linear model predicting an average rate of spread of 1.00 km per year (Sargent et al. 2010) underpredicted the most recent detections, and that a polynomial, increasing rate of spread model provided a better fit to the data.

Clearly, the distance a municipality is from the site of EAB introduction will influence its risk for damage and the timing of the beetle's arrival. For example, EAB will likely arrive in Washington, DC and other nearby municipalities such as Upper Marlboro (both 15 km away) between 2013 and 2018, depending on the true rate of spread of the beetle. However, cities such as Annapolis and Baltimore have substantially more time to prepare for the arrival of EAB. These predictions are based on spread from the known point of introduction in Maryland, and would certainly change with secondary introductions to the state or with an increase in human-mediated long distance dispersal within the state.

To demonstrate how cities and towns might prepare for the arrival of EAB and appropriately manage their ash populations, we obtained U.S. Forest Service street tree data for several cities and conducted sample street tree surveys in several municipalities in the region. We then estimated management costs and environmental and aesthetic benefits provided by ash trees. Following the i-Tree Streets survey protocols (i-Tree Streets v. 3.1), we randomly selected 6 percent of all possible street segments in a municipality for surveys using ArcMap 9.3 (ESRI, Redlands, CA). Survey crews recorded the species, DBH, and condition for all municipally-managed street trees on those segments. Municipal trees were defined as those located in street medians, between the street and sidewalk or utility poles, or within 10 feet of the street. Data were entered into a PDA in the field, and later downloaded into i-Tree Streets, which was used to estimate total population sizes of street trees, with a 10 percent standard error, as well as the environmental and aesthetic contributions of the trees.

Municipalities differed greatly in the size of their city managed ash populations, and consequently, the benefits provided by these trees, as well as the management costs that will be incurred in the future. The small municipality of Upper Marlboro was estimated to have no city managed ash in the random street segment survey, whereas Baltimore City was estimated to have 302,051 city managed ash (data from USDA FS 2001).

Municipalities have several options for managing their ash populations. We estimated the 5-year and 20-year cumulative costs of four different management options for these ash populations with the EAB Cost Calculator (Sadof 2009). Cities can: (1) remove all ash trees without replacement; (2) remove and replace all ash with a nonsusceptible species; or (3) treat all ash trees yearly with insecticides. Alternatively, cities could consider hybrid approaches in which they treat certain trees and remove or replace others. Here, we considered such a hybrid strategy to treat all large ash but remove ash smaller than 24 inches DBH. This last approach was found to be optimal in recent dynamic economic modeling by Kovacs et al. (2010), under the assumption that pesticides will be effective in treating large diameter trees.

Overall, removing ash without replacement was the cheapest option but would leave the municipality with a reduced urban canopy cover and the loss of associated environmental and aesthetic benefits. Treating trees yearly was the most expensive option over 20 years but would leave the entire urban canopy intact and have consistent annual costs. Assuming large trees can be effectively treated with insecticides, the hybrid strategy of treating large (>24 inch DBH) and replacing small and medium sized trees added some expense on top of replacing all trees, but would leave more canopy intact. The optimal management strategy will have to be selected by each municipality in light of their budget, their ash population size, tree benefits they hope to preserve, and the estimated time until EAB arrival.

Conclusions

Emerald ash borer is a threat to all native ash trees, and the urban ash populations in local municipalities must be managed. The overall risk to a municipality will be a function of the distance from the point of introduction, the size and size class distribution of the ash population, and their environmental and aesthetic benefits. To assess both risk and economic benefits, cities can conduct street tree inventories and estimate population sizes with the i-Tree Streets program. For management options, city managers can utilize the free online Purdue EAB Cost Calculator for management options that provide annual and cumulative estimated costs. Cities that will not have EAB for many years should use this preinfestation period to conduct street tree inventories and prepare management plans for the future.

Acknowledgments

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SYSTEMIC INSECTICIDES FOR EAB CONTROL ACROSS VARYING SCALES: TREES TO LANDSCAPES

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

[1]Michigan State University, Department of Entomology, East Lansing, MI 48823
[2]Michigan State University, Department of Forestry, East Lansing, MI 48823
[3]U.S. Forest Service, Northern Research Station, East Lansing, MI 48823

ABSTRACT

Our ability to use systemic insecticides to protect ash (*Fraxinus* sp.) trees from emerald ash borer (EAB), A*grilus planipennis* Fairmaire, has advanced considerably, representing one of the few bright spots in the EAB saga in North America. Systemic insecticides, applied to the soil or directly into the tree, are preferred to cover sprays for protecting trees in landscapes because there are no problems with drift. In addition, systemic insecticides are associated with minimal applicator exposure and nontarget concerns, and provide a greater chance of controlling insects in the upper canopy of large trees.

The three insecticides most commonly used for EAB control contain imidacloprid, dinotefuran or emamectin benzoate. Several products with imidacloprid, a neonicotinoid insecticide, are available and are applied either as a soil drench around the trunk of the tree or via injection into the base of the tree trunk. Imidacloprid is not highly soluble in water and moves into and through trees relatively slowly. Effectiveness of imidacloprid products varies with tree size; results are less consistent for large trees (e.g. > 30 cm DBH) than for small trees. This likely reflects the exponential relationship between the area of trees that must be protected by the insecticide and tree diameter (McCullough and Siegert 2007), which determines the application rate of systemic insecticides. A relatively new product used for soil application (Xytect®), is labeled for higher application rates than other imidacloprid products registered for soil drenches. Studies by other scientists indicate the high rate is likely to be more effective than the lower rates for medium and large trees. Cost of treatment, however, also increases with application rates, and treatment costs for large trees may become prohibitive. Imidacloprid can also be applied as a trunk injection. Many products and application devices are marketed by various companies, but efficacy of these products varies considerably.

Dinotefuran, a new generation neonicotinoid, is sold as Safari®. It is registered for soil applications and can also be applied to ash trees as a basal trunk spray. This insecticide is 80 times more soluble in water than imidacloprid and moves through trees more rapidly than imidacloprid. The basal trunk spray is becoming popular among arborists because it is efficient, requires only a garden sprayer, and does not require wounding the tree or introducing insecticide into the soil. Bark on the trunk of the tree is sprayed from the base up to approximately 1.5 m high, using low pressure to avoid any backsplash, until the appropriate rate has been delivered. Data from our studies has shown the insecticide moves through the bark and is readily translocated to branches and foliage in the canopy.

Emamectin benzoate is an avermectin insecticide that attained full EPA registration for EAB control on ash trees in 2010. It is sold as TREE-äge® and is applied as a trunk injection. We completed a study in 2009 that was designed to compare EAB control provided by emamectin benzoate, dinotefuran, and imidacloprid products. Results showed that density of EAB larvae on trees treated with the emamectin benzoate was >99 percent lower than larval density on control trees, even 2 years after treatment. Untreated control trees in this study were heavily infested, and canopy decline was apparent in the second year. The neonicotinoid products reduced larval density by 30-70 percent, but only if trees were treated annually.

A more recent study was designed to evaluate EAB control on trees that were treated with a low rate of emamectin benzoate, a higher rate of emamectin benzoate, the dinotefuron basal trunk spray, or imidacloprid injected with Mauget capsules. Preliminary results indicate that both rates of emamectin benzoate provided nearly 100 percent control of EAB, even 3 years post treatment. Other studies in Michigan and Ohio have similarly documented 3 years of highly effective EAB control with emamectin benzoate. The multiyear control provided by emamectin benzoate reduces logistical issues and costs associated with treating large numbers of trees.

Emamectin benzoate is currently being used to slow EAB population growth and delay ash mortality in the SLAM Pilot Project currently underway in selected EAB outlier sites in Upper Michigan (EABSLAM.info). Goals of the SLAM project, which stands for SLow Ash Mortality, are to reduce the onset and progression of ash mortality in localized, recently established EAB outliers. Results from simulation models have shown that using emamectin benzoate is substantially more effective at slowing EAB population growth and spread than using girdled ash trees as population sinks ,or felling ash trees to reduce phloem availability (Mercader et al., in press a, in press b). Ideally, however, emamectin benzoate, girdled trees, and ash removal are employed in an integrated strategy such that the management options act in a synergistic manner. Girdling trees in the center of a newly established outlier site, for example, should act to attract and retain ovipositing female EAB beetles. Ash trees within and surrounding the girdled trees can be treated with emamectin benzoate to control beetles that would otherwise disperse or colonize nontreated trees.

Lethal trap trees provide another option for reducing EAB population growth. In major studies conducted in 2009 and 2010, we compared EAB larval densities on ash trees that were untreated, girdled, injected with emamectin benzoate, or injected with emamectin benzoate then girdled 3 weeks later. We hypothesized that the girdling would attract adult EAB, but the trees treated with emamectin benzoate then girdled would kill adult beetles that fed on foliage and control larvae if beetles laid eggs on the trees. Results in both years confirmed our hypothesis. Girdled trees were heavily infested and control trees were also colonized. In contrast, there were almost no live larvae on trees treated with emamectin benzoate, even the lethal trap trees that were girdled 3 weeks after injection. Lethal trap trees, therefore, represent another option for slowing EAB population growth and spread, particularly in outlier sites.

Currently, we are applying simulation models of EAB population growth, dispersal and ash mortality to evaluate optimal allocation of resources in urban settings. The high efficacy and multiyear control provided by emamectin benzoate makes it economical to treat ash trees in municipalities and residential areas, rather than removing and replacing those trees. We created an artificial environment to represent a subdivision where up to 50 percent of the trees growing along boulevards could be ash trees. We assumed EAB was introduced by a specific homeowner, then we allowed beetles to disperse and reproduce over a 10- year period. We compared ash tree survival under different management scenarios to assess effects of: (1) treating 10 percent versus 20 percent of the ash trees annually; (2) beginning treatment 1 year versus 3 years after the EAB introduction; and (3) targeting trees near the introduction area for treatment versus treating the same number of randomly selected trees. Preliminary results suggest randomly selecting trees for treatment protected more trees than targeting a specific area for control. Treating 20 percent of trees protected substantially more trees over the 10-year period than treating only 10 percent of trees. Results also demonstrated the need to begin treating trees as soon as possible after EAB is detected. We are planning to expand on this work and consider larger and more realistic scenarios for our simulations. Results should be useful to city foresters and municipal arborists in the eastern United States where EAB populations threaten high value, abundant landscape ash trees.

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CHEMISTRY OF CERAMBYCID BEETLE PHEROMONES FOR PRACTICAL APPLICATIONS

Jocelyn G. Millar[1], James D. Barbour[2], Ann M. Ray[3], and Lawrence M. Hanks[4]

[1]University of California, Department of Entomology, Riverside CA 92521
[2]Southwest Idaho Research and Extension Center, Parma, ID 83660-9637
[3]Xavier University, Department of Biology, Cincinnati, OH 4520
[4]University of Illinois at Urbana-Champaign, Department of Entomology, Urbana IL, 61801

ABSTRACT

Cerambycid beetles comprise a large group of insects that are relatively well known to naturalists and collectors because they are frequently large and colorful. However, much of their biology is still relatively unknown, despite the fact that some species can be serious pests of forest, orchard, and ornamental trees and other woody plants, as well as lumber and wooden structures. Equally or more important, a number of species have the potential to be highly invasive because immature stages can be readily moved to new countries and continents while hidden in wooden packing materials and products. Several recent examples of invasive species that have been introduced into the United States include the notorious Asian longhorned borer (*Anoplophora glabripennis*), several species of Eucalyptus borers (*Phoracantha sp.*), the small Japanese cedar borer (*Callidiellum rufipenne* (Motschulsky)), and the brown spruce longhorned beetle (*Tetropium fuscum*).

Over the past several decades, pheromone baited traps have been developed as highly sensitive tools for detection and population monitoring of pests in many insect families. However, despite their economic importance and potential invasiveness, little is known about pheromones and pheromone use by cerambycid beetles. For example, a 2004 review of cerambycid semiochemistry listed less than 10 known pheromones, of which none had been properly developed for practical applications. Consequently, around 2004, we initiated a project to take a closer look at pheromone use within the cerambycids, with a particular focus on exploiting pheromones for practical purposes. We identified three long-term goals:

1. To determine the roles and importance of pheromones and related compounds in cerambycid beetle biology. This included such questions as: which species produce pheromones, what are the functions of these pheromones, and in what environmental and temporal contexts are pheromones used? Are there behaviors and morphologies that are associated with and possibly diagnostic for pheromone use in these beetles?
2. To develop an overview of the pheromone chemistry, such as: what types of compounds do cerambycid beetles use as pheromones, and how similar or different are the pheromones of closely related species?
3. To develop predictive tools for practical applications by forest managers and regulatory personnel charged with excluding and/or eradicating invasive species. For example, given a species taxonomic placement, can we predict whether it will use pheromones, and if so, which sex will produce them and in what context, and what are the pheromone structures likely to be?

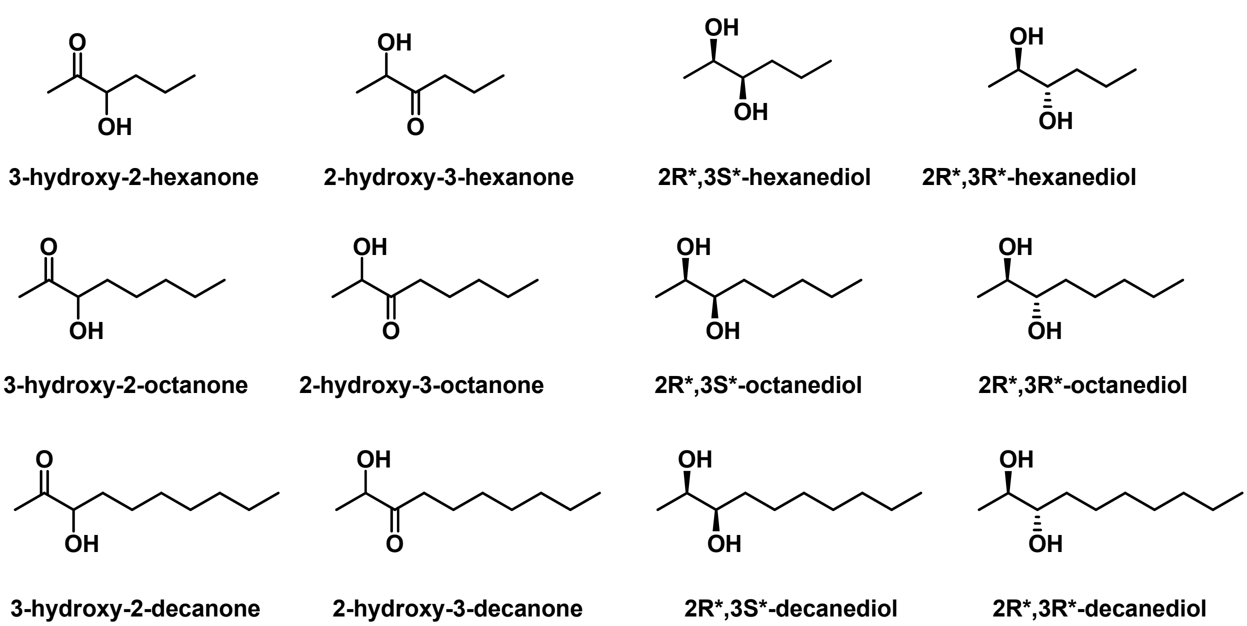
Thus, we hoped to develop a much better understanding of the behaviors and chemistry associated with pheromone use in this important beetle family, not only to increase our basic knowledge of their biology, but also to develop practical tools for detection and management of both native and exotic pest species.

We attacked these goals from two complimentary and synergistic angles. First, we synthesized a library of all the known or suspected cerambycid pheromones and a number of analogs and homologs, and started testing them in field screening trials. Second, we specifically targeted species in tribes and subfamilies in which no pheromones had been identified, to fill the gaps in knowledge of pheromone use within the various taxonomic subgroups. Then, as we identified new pheromone structures, they could be incorporated into the screening library as well, thereby continually expanding the array of compounds to test. Both approaches have turned out to be extremely successful, and in the space of 6 years, we have identified pheromonal attractants for several hundred cerambycid species, including a number of invasive species.

The first library of compounds that we synthesized and screened is shown below.

The results from the first screening studies were immediately successful, with more than 50 species being attracted, primarily to 3-hydroxy-2-hexanone and the 2,3-hexanediols, and to a lesser extent, the 8-carbon homologs. Several other points also became clear:

1. Many closely related species were attracted to the same or very similar compounds, with multiple species being attracted simultaneously to a single lure. This extreme biosynthetic parsimony, whereby one chemical is used as a pheromone by multiple species, is extraordinarily useful for practical purposes.
2. The species that were attracted were restricted to certain subfamilies, indicating that there are different pheromone chemistry motifs for different subfamilies and tribes.



3. For some species, pheromone release rates were high (>0.1 mg per beetle per hour). Consequently, to be effective, lures had to release synthetic pheromone at similar rates (i.e., several mg per day).
4. For species in the subfamilies Cerambycinae, Lamiinae, and Aseminae, pheromones were produced by males, whereas in the Prioninae, pheromones were produced by females.
5. Proper trap design was critical, and to be most effective at both catching and retaining beetles, traps should be coated with an aqueous dispersion of Teflon® (Fluon®), to increase the slipperiness of trap surfaces and prevent escapes. Fluon® treatments can improve trap captures by more than an order of magnitude (Graham et al. 2010).

Since our initial successes with the screening trials in 2004-2005, we have expanded our screening library to include more compounds. We have also worked with collaborators in different parts of the United States, and in 2010, overseas, to develop an overview of how broadly attractive the pheromones might be. The results have again exceeded our expectations, with less than a dozen chemicals attracting several hundred species in total. Some highlights of this work include:

- In 2009, collaborator Sven-Eric Spichiger (Pennsylvania Department of Agriculture) caught 77 species of cerambycids using only five of our library compounds. Furthermore, several noncerambycid species (e.g., bark beetles, predatory beetles) were attracted in substantial numbers to some lures. Continued testing in 2010 with a total of six compounds resulted in capture of more than 7,500 cerambycid beetles of 112 species.
- In 2010, collaborators Elizabeth Graham, Therese Poland, and Debra McCullough deployed three chemicals in Michigan, and caught 3,700 beetles in 78 species.
- In 2010, at sites around the United States, project collaborators caught several *Monochamus* species.
- In the first field trials of our pheromone library in China, project collaborators caught three species (*Anoplophora chinensis*, *Monochamus alternatus*, and *M. urussovi*) that are among the 13 listed species that USDA APHIS considers the most damaging, potentially invasive species of Asian woodboring insects. For another four species on the list, our pheromones caught congeners, providing strong leads as to the pheromone structures of those target species.

In parallel with the screening work, we have been able to identify pheromones for species within several subfamilies and tribes for which no pheromones at all had been known. Some highlights from this part of the project include the identification of the first female produced pheromone within the entire cerambycid family, for *Prionus californicus*, a primary pest of the multimillion dollar hops industry. Unlike the male produced pheromones of species in other subfamilies, female *P. californicus* produce the pheromone in minute amounts, and so it took several years of work to finally identify it as 3,5-dimethyldodecanoic acid. Since then, with the help of collaborators, we have determined that this compound is a pheromone for at least seven other *Prionus* species, including a European species. This again highlights how highly the pheromone structures are conserved within these beetles. However, we have also identified a series of unrelated compounds as the female produced pheromones of prionine species in the genus *Tragosoma*, so there are clearly different pheromone motifs within this subfamily. As another example, in 2010 we identified the first pheromone for a species in the subfamily Lepturinae, for *Ortholeptura valida*, as *cis*-vaccenyl acetate. Identification of additional entirely new pheromone structures are in progress for other species. As each of these compounds is identified, it will be incorporated into the screening library so that we can continually expand the spectrum of beetles that we are sampling, and determine whether each compound is broadly attractive to a number of related species, as has generally been the case to date, or whether it is quite specific and only attracts one or a few species.

So what remains to be done to translate this work from the research to the operational level? Right now, there are several important issues that need to be resolved. First, working with pheromone companies, we need to develop pheromone release devices that have the correct release rates, good shelf life, and a field lifetime of at least several weeks. This is one of our major priorities for 2011 so that commercially available operational lures will be available for 2012.

Second, we need to determine whether a number of pheromones can be mixed in one lure, to develop a generic lure that will attract a large number of species simultaneously. Preliminary trials with these generic lures appear promising, and further trials are ongoing to define which compounds can be safely blended, and which might interfere with others.

Third, we need to develop a better understanding of the role of host plant volatiles in combination with the pheromones. So far, almost all of our trials have only used pheromones, and as successful as they have been, data from our group and other groups has indicated that for at least some species, blends of host volatiles with the pheromones will be critically important for optimizing attraction.

Our team will be actively working on these three questions this coming field season, with the goal of having robust and practical protocols for trapping multiple cerambycid species ready for operational use in 2012.

Acknowledgments

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EVALUATION OF TREE INJECTIONS AND OTHER METHODS TO ATTRACT WOOD BORERS TO TRAPS

Jason Oliver[1], Alicia Bray[1], Chris Ranger[2], Sam Ochieng[1], Nadeer Youssef[1], Vic Mastro[3], Michael Reding[2], Peter Schultz[4], William Klingeman[5], James Moyseenko[2], and Joshua Basham[1]

[1]Tennessee State University, School of Agriculture and Consumer Sciences, McMinnville, TN 37110
[2]USDA ARS, Horticultural Insects Research Laboratory, Wooster, OH 44691
[3]USDA APHIS PPQ, Otis ANGB, Buzzards Bay, MA 02542
[4]Virginia Tech, Hampton Roads Agricultural Research and Extension Center, Virginia Beach, VA 23455
[5]The University of Tennessee, Department of Plant Sciences, Knoxville, TN 37996

ABSTRACT

Woodboring insects are important pests of forests, nurseries, and landscapes. Numerous nonindigenous species continue to be introduced into North America, posing a threat to tree resources. Early detection of newly introduced borers is a critical part of the regulatory process for preventing invasive species from establishing and causing economic damage. Our research group has been investigating ethanol treatments of wood as a potential method to enhance attraction of woodboring insects to traps. Ethanol is a well-known attractant of many woodboring insects, especially some Scolytinae. The injection of ethanol into trees attracts numerous species of Scolytinae and rapidly induces attacks by ambrosia beetles including *Xylosandrus* and *Xyleborinus* species.

Based on these findings, multiple tests were performed during spring and summer of 2010 in Tennessee to evaluate the attraction of ethanol treated wood to Scolytinae (Curculionidae), Buprestidae, and Cerambycidae borers. In two tests, containerized red maple (*Acer rubrum* L.) trees injected using an Arborjet Tree I.V. with a 75 ml volume of 50 percent or 90 percent ethanol had significantly greater Scolytinae attacks than trees injected with water, or noninjected controls. Ethanol injected trees were significantly more attractive to Scolytinae than noninjected trees when trees were cut into 25 cm long bolts and placed in traps (i.e., 2-liter soda bottle traps [Reding et al. 2010], 4-funnel Lindgren traps, or semi-transparent corrugated plastic triangle traps [15 by 29.5 cm] covered with insect glue), and placed about 1 m above the ground surface. Ethanol injected trees or bolts were also significantly more attractive to Scolytinae than a commercial ethanol lure pouch with a release rate of 65 mg/d. The predominant Scolytinae species attacking trees in one of the tests were *Xylosandrus crassiusculus* (Motschulsky) (70.6 percent), *Xylosdandrus mutilatus* (Blandford) (6.1 percent), *Xylosandrus germanus* (Blandford) (1.1 percent), and other unidentified Scolytinae beetles (22.2 percent). In one of the maple injection tests, low numbers of buprestid (n=36) and cerambycid (n=55) borers were also collected, but ethanol injections did not appear to increase the capture of any particular species in traps.

In another test, the effect of host plant species injected with ethanol on response of borers to traps was evaluated. Containerized red maple (M), white oak (*Quercus alba* L.) (O), and white pine (*Pinus strobus* L.) (P) trees were injected with a 75 ml volume of 50 percent ethanol using an Arborjet. Trees were then cut into 20 cm long bolts and placed in the center of semitransparent corrugated plastic triangle traps covered with insect glue at about 1 m above the ground. Treatments included six bolts of all maple, all oak, all pine, or the combinations of OMOMOM, MPMPMP, OPOPOP, or MOPMOP arranged in a completely randomized block design with four replicates. Traps with all maple bolts caught significantly more total Scolytinae than traps with all pine or a commercial ethanol lure (65 mg/d). No statistical differences were detected among the other bolt treatments or the commercial ethanol lure. All treatments had significantly greater Scolytinae captures than a blank trap control. Low numbers of buprestid (n=21) and cerambycid (n=34) borers were captured and no distinct treatment effects were apparent. However, the

MPMPMP treatment was the only treatment that did not capture any buprestid or cerambycid borers, and therefore, may have reduced beetle attraction to traps.

During August 2010, the walnut twig beetle (WTB),*Pityophthorus juglandis* Blackman, was first detected in the Tennessee counties of Knox and Blount, where it has caused substantial mortality of black walnuts (*Juglans nigra* L.). A final ethanol injection test was performed late in the summer (September to October) in an effort to determine if WTB could be attracted to traps. Black walnut branches from trees in White County, TN were injected with a 75 ml volume of 50 percent ethanol. Other branches were hollowed from one end with a 1.6 cm diameter drill bit to a depth of 14 cm and then filled with 22 ml volume of 50 percent ethanol. All branches were cut into 20 cm long bolts and then placed inside 2-liter soda bottle traps. Traps were deployed in the lower canopy of walnut trees in Knox and Blount counties in a completely randomized design with five replicates. One WTB was captured in a trap with a hollowed and ethanol filled bolt during September 3–16. Other Scolytinae captured between September 3–October 1 included *X. crassiusculus* (n=13) and *Xyleborinus saxeseni* (Ratzeburg) (n=19). Because traps were tested in autumn, ethanol treated walnut baits may be more effective at capturing WTB in followup trapping this spring and summer of 2011.

Overall, ethanol injected trees were more attractive to Scolytinae than noninjected trees or commercial ethanol lures, but we still need to quantify the release rate of ethanol (and other volatiles) from injected trees for better comparisons with commercial ethanol lures. Buprestid and cerambycid trap captures were low and did not appear to be enhanced by ethanol injections in these tests. Lastly, some host related effects on Scolytinae captures were indicated based on the results from one ethanol injection test that compared multiple tree hosts.

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FRAXINUS CONSERVATION AND GENETIC MODIFICATION FOR RESISTANCE TO THE EMERALD ASH BORER

Paula M. Pijut

U.S. Forest Service, Northern Research Station, West Lafayette, IN 47907

ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, poses substantial risk to the ash resource in North America. Native ash species include green ash (*Fraxinus pennsylvanica* Marsh.), white ash (*F. americana* L.), and black ash (*F. nigra* Marsh.), trees which are major components of the landscape, and blue ash (*F. quadrangulata* Michx.) and pumpkin ash (*F. profunda* (Bush) Bush), which are less common species. The development of transgenic *Fraxinus* spp. exhibiting resistance to the EAB will have great economic and ecological benefits. The objectives of this research were to: develop adventitious shoot regeneration and rooting protocols for conservation of *Fraxinus* spp.; develop an *Agrobacterium*-mediated transformation protocol; and develop a genetic construct containing the Cry8Da protoxin of *Bacillus thuringiensis* (*Bt*) for genetic transformation and recovery of ash with resistance to the EAB. Adventitious shoot regeneration was initiated by culturing hypocotyls on Murashige and Skoog (MS) medium containing 0 to 22.2 μM 6-benzylaminopurine (BA) plus 0 to 4.5 μM thidiazuron (TDZ). Proliferating shoot cultures were established on MS medium with Gamborg B5 vitamins supplemented with various plant growth regulators or organic supplements. Rooting of shoots was achieved on woody plant medium with 4.9 μM indole-3-butyric acid plus 0 to 8.6 μM indole-3-acetic acid. Plants of green, white, and black ash were successfully acclimatized to the greenhouse, and adventitious shoots initiated on pumpkin ash hypocotyls. A genetic transformation protocol was developed for green ash and is currently being optimized for white and black ash. Hypocotyls were transformed using *Agrobacterium tumefaciens* strain EHA105 harboring binary vector pq35GR containing the neomycin phosphotransferase (*nptII*) and β-glucuronidase (GUS) genes. Hypocotyl explants were transformed in the presence of 100 μM acetosyringone using 90 s sonication plus 10 min vacuum-infiltration. Transgenic green ash shoots regenerated on MS medium with 13.3 μM BA, 4.5 μM TDZ, 50 mg/l adenine sulfate, and 10 percent coconut water. Kanamycin at 20 mg/l was used for selecting transformed shoots. The presence of the GUS and *nptII* genes in GUS-positive shoots was confirmed by polymerase chain reaction, and copy number determined by Southern blotting. Three transgenic green ash plantlets were acclimatized to the greenhouse, and one putative transgenic white and black ash were regenerated. Genetic transformation studies are underway using the construct containing the Cry8Da protoxin of *Bt*, and one putative *Bt* green ash has been regenerated.

EVALUATION OF DIFFERENT TRAP TYPES AND LURES FOR CAPTURING EMERALD ASH BORER ADULTS IN LOW DENSITY POPULATIONS

Therese M. Poland[1,2], Deborah G. McCullough[2,3], Andrew J. Storer[4], Jordan M. Marshall[4,5], and Ivich Fraser[6]

[1]U.S. Forest Service, Northern Research Station, East Lansing, MI 48823
[2]Michigan State University, Department of Entomology, East Lansing, MI 48824
[3]Michigan State University, Department of Forestry, East Lansing, MI 48824
[4]School of Forest Resources and Environmental Science, Houghton, MI 49931
[5]Current Address: Indiana University-Purdue University Fort Wayne, Department of Biology, Fort Wayne, IN 45805
[6] USDAAPHIS, Otis PSDEL, Buzzards Bay, MA 02542

ABSTRACT

Effective methods for early detection of newly established, low density emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, infestations remain a critical need (Poland and McCullough 2006). Following the discovery of EAB in 2002, most field studies to evaluate traps and lures were conducted in areas where *A. planipennis* populations were at moderate to high densities (Francese et al. 2005, 2008; Crook et al. 2008, 2009; Lelito et al. 2008). In such sites, however, 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. Once promising traps and lures have been identified, ideally, they should be assessed in sites where EAB density is at low levels and few, if any, trees are symptomatic or stressed by larval feeding (Marshall et al. 2009, 2010).

A number of studies have been conducted to compare the most promising trap types and lures for EAB at sites with low levels of EAB infestation. In one study, light green and purple double-decker traps were compared to purple prism traps hung about 3 m above ground in the canopy, or girdled trees in a 16.2 ha forested site in central Michigan with no symptoms of EAB infestation. Each double-decker trap consisted of 3 m tall 10 cm diameter PVC pipe with a prism panel mounted at the top and a second prism panel 60 cm below it. Double-decker traps were set on T-posts in the open, approximately 3 m from the edge of ash stands. The site was divided into 16 blocks each with four 50 by 50 m cells. One trap type was randomly assigned to each cell within a block. A total of 87 EAB were captured during the experiment. Purple double-decker traps baited with a blend of ash leaf volatiles (*cis*-3-hexenol, *trans*-2-hexenol, hexanal, and *trans*-2-hexenal), Manuka oil, and ethanol captured more EAB (65 percent of all EAB captured) than similarly baited, green double-decker traps (18 percent) and sticky bands on girdled trees (11 percent). Purple traps baited with Manuka oil and placed in the canopies of live ash trees captured the fewest beetles (5 percent). At least one EAB was captured on 81 percent of the purple double-decker traps, 56 percent of the green double-decker traps 42 percent of sticky bands, and 25 percent of the purple canopy traps .

In another study, light green or purple double-decker traps were compared to light green or purple prism traps hung about 4.5 m above ground in the canopy of ash trees. All traps were baited with *cis*-3-hexenol and an 80:20 blend of Manuka and Phoebe oils. There were 21 replicates at six different field sites that ranged in EAB infestation level from extremely low with no symptoms to moderate or high populations. Traps were spaced at least 15 m apart with 25 m between replicates. Light green canopy traps caught significantly fewer EAB than the other trap types. Overall, double-decker traps captured more EAB than canopy traps, and purple traps were more attractive than light green traps. Differences were pronounced at the low density sites while at sites with higher EAB populations, differences among trap designs were not significant. All of the double-decker traps (both colors) and purple canopy traps captured at least one EAB each, while only 64 percent of the light green canopy traps did.

Different lures were also compared for light green or purple prism traps hung at least 10 m above ground in the canopy of ash trees. Traps were baited with *cis*-3-hexenol, an 80:20 blend of Manuka and Phoebe oils, or both. There were 17 replicates at three sites with EAB populations that ranged from low/moderate to high. For all sites and both trap colors combined, traps baited with *cis*-3-hexenol or both lures captured more EAB than unbaited traps, while traps baited with the 80:20 blend were intermediate. Males were significantly more attracted to traps with lures that contained *cis*-3-hexenol than to the 80:20 blend alone or unbaited traps. The number of females captured did not differ significantly between treatments. For both sexes combined, more EAB were captured on purple than on light green traps. Females were significantly more attracted to purple traps but there was no difference in number of males captured between light green and purple traps. The differences in male and female responses to the different treatments and colors were most pronounced at the site with low EAB populations.

In a similar study, light green prism traps baited with *cis*-3-hexenol or the 80:20 blend of Manuka and Phoebe oils were compared to purple prism traps baited with the 80:20 blend. All traps were hung at least 6 m above ground in the canopies of ash trees. There were 160 replicates at sites with low EAB densities in Indiana, Kentucky, Maryland, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, West Virginia, and Wisconsin. More males were captured in light green traps baited with *cis*-3-hexenol than in purple traps baited with the 80:20 blend, while light green traps baited with the 80:20 blend were intermediate. There were no differences in female trap catches between treatments, but they tended to prefer purple traps baited with the 80:20 blend. Detection rates were similar for all three treatments; 52 percent of light green traps baited with *cis*-3-hexenol, 59 percent of light green traps baited with the 80:20 blend, and 57 percent of purple traps baited with the 80:20 blend captured at least one EAB.

Overall, at sites with low EAB densities, double-decker traps appear to be more attractive than prism traps hung 3-5 m above ground in the canopy. Studies using only prism traps hung at least 6 m above ground in the canopy showed males tended to prefer light green traps baited with *cis*-3-hexenol, while females tended to prefer purple traps baited with an 80:20 blend of Manuka and Phoebe oils. A combination of trap designs and lures may improve the likelihood of attracting both sexes, which could increase the potential for capturing at least one beetle.

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UPDATE ON USDA FOREST SERVICE EMERALD ASH BORER ACTIVITIES

Robert J. Rabaglia

U.S. Forest Service, Forest Health Protection, Arlington, VA 22209

ABSTRACT

As the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, footprint across the United States changes, the response by the USDA agencies, Animal and Plant Health Inspection Service (APHIS) and Forest Service (FS), is changing. APHIS and FS, along with state representatives from the National Plant Board and the National Association of State Foresters are developing a National EAB Framework. This document will align key roles and responsibilities for the agencies with regards to EAB. Within the four strategic goals of prevention, preparedness, response and recovery, the Framework will identify lead agencies responsible for interagency coordination and management in EAB infested areas and areas not known to be infested.

APHIS is the lead agency on regulatory activities and survey, and is also involved in biocontrol and methods development. The Forest Service assists APHIS and states on regulatory actions, provides technical assistance on EAB management to state, tribal and Federal land managers, and conducts research and methods development of tools to detect and control EAB. Both agencies provide outreach and public information on EAB.

Forest Service Technical Assistance

Preparing for the arrival of EAB is one of the most important activities that states, local governments and land managers can do. With FS assistance, projects were implemented in Minnesota and Iowa to help communities prepare for the arrival of EAB, and assistance was provided to North Dakota, South Dakota, Nebraska, and Kansas to develop EAB readiness plans and assess rural and urban ash resources. The detection of EAB infestations is still one of the greatest challenges in EAB management. In cooperation with APHIS and state partners, FS has implemented zip code-based surveys in high risk areas in 10 states. In areas already affected by EAB, such as Illinois, Michigan, New York, Ohio, and Wisconsin, FS has continued to provide technical and financial assistance to help state and local governments replant EAB affected urban areas.

Forest Service Outreach and Public Information

An informed public can be an important asset in detecting EAB infestations. Since EAB was first identified in the United States, FS has provided information to states, communities and landowners on identifying EAB and its impacts. FS has continued the production of EAB kits to help identify the signs of EAB infestations. In recent years, FS has worked with Master Gardeners on EAB outreach and information sessions for communities. Related to this effort, FS has piloted citizen detection and outreach efforts in Kentucky and Virginia. Also in Kentucy, FS has provided assistance to develop EAB preparedness meetings for urban foresters, arborists and public land managers. Some of the most well received outreach efforts have been activities on the internet. FS has supported the emeraldashborer.info website, and just completed the first year of the EAB university online training in cooperation with Purdue University. Finally, EAB monitoring and management online training modules are available on the National Plant Diagnostic Network website.

Forest Service Research and Methods Development

Forest Service has continued its commitment to research and methods development to better understand EAB biology, behavior, and host relationships, and support regulatory actions, surveys, insecticidal management and biological control. FS Northern Research Station labs in East Lansing, MI and Hamden , CT have been looking at EAB life cycles, dispersal, mate-finding behavior as well as rearing methods, genetics and cold-

hardiness. In support of regulatory efforts, studies have been conducted on firewood treatments, such as heat, vacuum, and microwave.

FS Research and Development is also working on EAB-host relationships such as host range, host stress, nutrition, defense chemistry, as well as host resistance and breeding and genetic modification. Research and methods development of control methodologies is an important aspect of FS EAB activities. Work is continuing on insecticidal control, such as bark spray and trunk injections, and microbial control utilizing *Bt* and *Beauvaria*. In cooperation with APHIS, FS researchers are making progress on biological control utilizing native and introduced natural enemies. Research and methods development into enhancing survey and detection capabilities is important to the successful management of EAB. FS and partners have been making progress on understanding the chemical ecology and attraction of EAB and developing artificial traps and trap trees.

Methods development work on the management of EAB infestations is continuing in the Upper Peninsula of Michigan where the second year of a Slowing-Ash-Mortality (SLAM) project was completed. The SLAM project has been a cooperative effort with universities, states, APHIS and the Canadian Forest Service.

INTERACTIONS BETWEEN SOIL CALCIUM, PLANT INVASIONS, AND BREEDING FOREST BIRDS: A FRAME STUDY (FOREST FRAGMENTS IN MANAGED ECOSYSTEMS)

Christine Rega[1], W. Gregory Shriver[1], and Vincent D'Amico[2]

[1]University of Delaware, Department of Entomology and Wildlife Ecology, Newark, DE 19716
[2]U.S. Forest Service, Northern Research Station, Newark, DE 19716

ABSTRACT

Recent studies suggest that Neotropical migratory songbird population declines may be related to the negative effects of soil acidification caused by acid rain. In addition, the harmful effects of forest fragmentation could be compounded by soil acidification, which directly results in the loss of calcium to the ecosystem.

Little is known about the relationship between the availability of calcium and the invasibility of nonnative plant species, and how this may impact the bird community. In this study, we strive to understand the interactions between soil calcium availability in urban forest fragments with the invasion of nonnative plant species and the territory density of breeding forest birds. The availability of calcium was determined by the molar ratio of calcium:aluminum, due to aluminum's antagonistic effects on calcium uptake by vegetation. The threshold of ecological damage occurs when the Ca:Al ratio falls below a value of one.

Even after one year of data collected from locations in Newark, DE (n=120), the relationships between the availability of soil calcium and the presence of nonnative plants and forest breeding birds are quite clear. More nonnative shrubs were present in calcium-rich soils (Ca:Al >1) than the calcium-poor (Ca:Al <1) locations. In addition, bird species which forage on the forest floor increase in territory density with an increase in calcium availability.

Currently we are in the process of collecting more data on the soil, vegetation, and breeding bird composition of the same forest fragments to get a more comprehensive understanding of the flora and fauna that occur in each site. The exploration of the interactions between soil chemistry and nonnative invasive plants, snails, and native songbirds is important to better understand potential causes of songbird declines and plant invasions.

EXPANSION OF THE AMERICAN ELM RESTORATION EFFORT TO VERMONT

James M. Slavicek[1] and Christian O. Marks[2]

[1]U.S. Forest Service, Northern Research Station, Delaware, OH 43015
[2]The Nature Conservancy, Connecticut River Program, Northampton, MA 01060

ABSTRACT

The American elm (*Ulmus Americana)* was once widely distributed throughout the eastern United States and was a preferred tree for use in the urban landscape. The Dutch elm disease (DED) fungal pathogen *Ophiostoma ulmi* (Buisman) Nannf., introduced into the United States in 1930, and *Ophiostoma novo-ulmi,* Brasier has destroyed tens of millions of American elm trees in the United States and Canada. One line of research on the American elm from the 1970s to the present focused on the identification of American elm isolates that could withstand the DED pathogen. Over 100,000 American elm trees were tested for resistance to Dutch elm disease. No trees were found that were resistant to DED; however, at least eight selections were identified that exhibited good levels of tolerance to the disease. Two of these selections, the St. Croix and Prairie Expedition are recently patented selections from the University of Minnesota and North Dakota State University, respectively.

To develop methods to restore the American elm in forested landscapes, five DED tolerant selections, the Valley Forge, Princeton, New Harmony, R18-2, and Delaware 2, were used in experimental plantings in areas where the trees could exist undisturbed and be allowed to regenerate. Plantings sites were on State Forests, natural parks, and conservation lands managed by the Army Corps of Engineers and private foundations. Additional selections will be added to the sites as they become available with the goal of having 25 different genotypes at each test site.

The following aspects will be monitored at the restoration sites: tree growth, determination of basis for loss of planted trees, dates of first seed formation, number of regenerating trees and their distance from the planting site, incidence of DED at the restoration site in planted and seedling trees, and genetics of survivor trees. In addition, information on the suitability of selected cultivars for different regions will be discerned as well as identification of currently unknown pathogens and pests that could impact a future American elm restoration effort.

In 2010, three test sites were established in Vermont in partnership with The Nature Conservancy. The test sites are on Nature Conservancy land on the LaPlatte River, Shelburne, the Connecticut River, Maidstone, and Otter Creek, Cornwall. All three sites are floodplains where the American elm was a dominant overstory tree before being lost to DED.

GENERIC PHEROMONES AND HOST VOLATILE LURES FOR ENHANCED DETECTION OF EXOTIC BARK AND WOOD BORING BEETLES

Jon Sweeney[1], Peter J. Silk[1], Leland Humble[2], Reggie Webster[1], Krista Ryall[3], Peter de Groot[3], Jerzy M. Gutowski[4], Vasily Grebennikov[5], Bruce Gill[5], Qingfan Meng[6], Peter Mayo[1], and Troy Kimoto[7]

[1]Natural Resources Canada, Canadian Forest Service, Fredericton, NB
[2]Natural Resources Canada, Canadian Forest Service, Victoria, BC
[3]Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, ON
[4]European Centre for Natural Forests, Białowieża, Poland
[5]Canadian Food Inspection Agency, Ottawa, ON
[6]Beihua University, Jilin China
[7]Canadian Food Inspection Agency, Vancouver, BC

ABSTRACT

International agreements on the phytosanitary treatment and certification of solid wood packaging material will mitigate but not eliminate new introductions of exotic woodborers. The host volatile attractants currently used in trapping surveys for invasive forest insects in Canada and the United States have provided some first records of new incursions, but more often than not, new infestations of invasive species have been detected by chance by a member of the general public, many years after the estimated year of establishment. Improved tools for early detection and survey of invasive forest insects are, therefore, critically needed.

In the last 10 years, much progress has been made in the identification of long-range sex and aggregation pheromones for several species of longhorn beetles, mainly in the subfamily Cerambycinae. In the Cerambycinae the pheromones are usually 6-, 8- or 10-carbon hydroxy ketones (ketols) or hexanediols (diols) with specific chirality, emitted by males. Hanks et al. (2007) hypothesized the ketol/diol structural motif was highly conserved in the Cerambycinae and predicted that racemic blends of these compounds ("generic pheromones") would attract several species in the subfamily, and therefore, be useful for their survey and detection.

We tested this prediction in 2008, 2009, and 2010, in field trapping experiments in a variety of forest habitats in Canada, Poland, China and Russia, with the ultimate goal of improving the suite of semiochemical lures used for surveillance and early detection of exotic bark and wood boring beetles in North America. We report here results from Canada and Poland, testing the response of cerambycids to racemic 3-hydroxyhexan-2-one (C6-ketols), racemic 3-hydroxyoctan-2-one (C8-ketols), and a blend of four diastereomers of 2,3 hexanediol (C6-diol), alone or combined with ultra-high release rate lures of ethanol, alpha-pinene or both. Ethanol and alpha-pinene + ethanol (aP+E) are two of the standard semiochemical lures currently used in operational surveillance in Canada and the United States for early detection of exotic bark and wood boring beetles. If traps baited with generic pheromones plus ethanol or alpha-pinene + ethanol attracted similar or greater numbers of target species as traps baited with either lure alone, survey costs could be reduced and surveillance efficacy enhanced. Our objectives were to: (1) test racemic ketols and diols for significant attraction of Cerambycid species in trapping bioassays; (2) determine the effects of adding host volatile lures to traps baited with ketols/diols; and (3) determine which lure combinations detected the greatest number of species of Cerambycids and Scolytines.

Release devices (ConTech, Delta, BC) emitted the ketols at rates of 20-25 mg/d and the diols at a rate of 1 mg/d, at 20 °C. Two lures were placed on each trap for release rates of 40-50 mg/d and 2 mg/d for ketols and the diols, respectively. Traps were either black panel intercept traps (AlphaScent) or Lindgren 12-funnel traps (ConTech) with saturated NaCl solution, 50 percent propylene glycol, or 50 percent ethylene glycol in the collecting cups, depending on local availability.

All trapping experiments were replicated in randomized complete block designs with about 30 m spacing between traps.

As predicted, several species of cerambycine beetles were significantly attracted to the generic pheromone lures: eight species to the C6-ketols and four species to the C8-ketols. The C8-ketols were also significantly attractive to two species of longhorn beetles in the Lepturinae subfamily. In addition, traps baited with C6- or C8-ketols detected nine species of cerambycids at sites where these species were considered rare. No species were attracted to traps baited with the C6-diols; this may have been because our release rate was too low or because presence of all four diastereomers is known to reduce attraction of some cerambycine species.

The effects of combining these generic pheromone lures on traps baited with standard surveillance lures such as ethanol, was generally positive. Presence of an ethanol lure on ketol-baited traps significantly increased catch of about one quarter of the cerambycine species that were attracted to the ketols, significantly decreased attraction of another quarter of species, and had no effect on the remaining species. Adding generic pheromone lures (C6-ketols, C8-ketols, C6-diols or all three) to traps baited with the standard ethanol lure usually increased the total number of species of Cerambycids and Scolytines detected. Results were varied when generic pheromones were added to traps baited with aP + E. In New Brunswick, the addition of all three generic pheromone lures to traps baited with aP + E more than doubled the number of Cerambycid species detected, but in British Columbia, the same lure combination detected fewer species of both Cerambycids and Scolytines than traps baited with aP + E.

Our results indicate that use of generic pheromone lures in trapping surveys should increase the probability of detecting species of Cerambycinae, including those that may be exotic and potentially invasive. Further work is required to determine the most effective way to incorporate these with the standard lures currently used for early detection of invasive bark- and wood boring species.

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EVALUATION OF THE TOXICITY AND TOXIN BINDING PROPERTIES OF THE CRY1A CLASS OF *BACILLUS THURINGIENSIS* TOXINS IN DOUGLAS-FIR TUSSOCK MOTH

Algimantas P. Valaitis[1] and John D. Podgwaite[2]

[1]U.S. Forest Service, Northern Research Station, Delaware, OH 43015
[2]U.S. Forest Service, Northern Research Station, Hamden, CT 06514

ABSTRACT

Bacillus thuringiensis (Bt), an entomopathogenic bacterium, is one of the most successful microbial agents produced commercially for control of agricultural and forest insect pests. The Bt group bacteria produce a variety of insecticidal proteins, referred to as Cry toxins. *Bacillus thuringiensis* subsp. *kurstaki* (Btk) produces several Cry1A classes of proteins, which show different toxicities against target insects. Although Btk is known to be active against Douglas-fir tussock moth larvae (DFTM), *Orgyia pseudotsugata* (McDunnough), the potency of the individual Btk toxins against DFTM larvae has not previously been evaluated.

In this study, biotin-labeled Cry1Aa, Cry1Ab, and Cry1Ac toxins were used to examine irreversible binding to brush border membrane vesicles (BBMV) prepared from DFTM larvae. Binding assays revealed that the extent of irreversible binding among the toxins was different: more Cry1Ac was incorporated into the toxin-BBMV complex in comparison with Cry1Aa and Cry1Ab. Western blot analysis revealed the presence of two large molecules on the larval gut membrane that bind Cry1Aa and Cry1Ab toxins, which may function as specific receptors for these Bt toxins. Although the Cry1Ac toxin bound to one of these molecules, its major target appeared to be an aminopeptidase N not observed with the other two toxins. A bioassay was performed with the individual recombinant Cry1Aa, Cry1Ab and Cry1Ac toxins purified from *Escherichia coli* using a diet-incorporated protocol. The toxicity of these toxins to DFTM larvae were Cry1Aa > Cry1Ab >> Cry1Ac. Comparison of the bioassay and the binding study data revealed an inverse relationship between toxicity with the extent of toxin binding of Cry1Ac and Cry1Aa or Cry1Ab to DFTM BBMV. These results suggest that binding to aminopeptidase N contributes to reduced insecticidal activity of Cry1Ac toxin to DFTM larvae.

GONE WITH THE TRAIN: FAR EASTERN BARK BEETLE AND ASSOCIATED BLUE STAINED FUNGI OUTBREAK IN SOUTHERN SIBERIA

Yuri Baranchikov, Natalia Pashenova, and Vladimir Petko

Sukachev Institute of Forest, Siberian Branch Russian Academy of Science, Krasnoyarsk, Russia

ABSTRACT

Polygraphus proximus Brandford (Coleoptera: Scolytidae), a typical bark beetle on Far Eastern firs, was distributed in northeastern China, Korea, Japan, Kurile and Sakhalin Islands, and the southern part of the Russian Far East. During the last 15 years, the beetle expanded its range to the west and has reached eastern Europe (Moscow and Saint Petersburg). The beetle was moved with the row logs transported by trains from the Russian Far East, and settled local populations expanded to the south and north from the Trans-Siberian railroad.

Currently, the beetle is distributed in the southern regions of Krasnoyarsk Kray, eastern part of Kemerovo and Novosibirsk Oblasts, and the southeastern part of Tomsk Oblast. There is a high probability that the pest has also occupied the fir stands along the railroad on the southeastern banks of Lake Baikal and at the Middle Urals.

Now, *P. proximus* is considered to be the most aggressive bark beetle ever found on firs in Siberia. Previously, only *Monochamus urussovi* Fish., a cerambycid species, was known to be able to attack and kill healthy firs. The invader is recently responsible for a huge, 30,000-hectare fir dieback in Kemerovo Oblast and a few local outbreaks (up to 3,000 hectares) in Krasnoaysrsk Kray.

In Japan, 11 species of phytopathogenic ophiostomal fungi are associated with *P. proximus* on firs. Our surveys of fungi from beetle galleries in Siberia have shown that it carries a suite of fungi, including some that are associated with it in its area of origin. *Ophiostoma aoshimae* Ohtaka, Masuya & Yamaoka, a recently described fungus from firs in Japan, was found in 48-91 percent of beetle nests in Krasnoyarsk Kray. This fungus was never recorded in Siberia before and appears to be extremely aggressive on the local fir *Abies sibirica* Ledeb. More intriguingly, when *P. proximus* was introduced into this new environment, it acquired fungi with which it was not associated in the Far East. For example, a local fungus, *Leptographium sibirica* Jacobs & Wingfield, was found in 52-56 percent of *P. proximus* nests and is evidently transported by the beetle from tree to tree. Previously, *L. sibirica* was associated exclusively with the most aggressive pest of firs in Siberia, the sawyer beetle *M. urussovi,* where it was believed to be a main cause of firs weakening. The introduced insect has thus received a novel suite of fungal associates, and this might explain why a relatively nonaggressive insect pest in the Far East has become a serious and damaging pest in Siberia.

This work was supported in part by the Russian Fund for Fundamental Research (grant # 10-04-00196a) and FP7 ISEFOR project.

A SIMPLIFIED GAS CHROMATOGRAPHY-MASS SPECTROMETRY METHOD FOR QUANTITATION OF METHYL JASMONATE IN ASH SPECIES

Sourav Chakraborty[1], Alifia Z. Merchant[1], Justin G. A. Whitehill[1], Shane Whitacre[2], and Pierluigi Bonello[1]

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

ABSTRACT

The invasive insect pest, emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), threatens to decimate North American ash trees (*Fraxinus* spp.). The larval stage of this pest kills trees by feeding in the secondary phloem of the main stem, thereby cutting off nutrient translocation. Our group is actively engaged in elucidating molecular and biochemical mechanisms of ash resistance to EAB larvae. As part of this work, we are trying to determine the role methyl jasmonate (MeJA), a phytohormone, plays in governing defense mechanisms in ash. MeJA is thought to be a key signaling molecule in the induction of plant defense responses against chewing herbivores such as EAB. However, MeJA has never been measured in ash phloem, in part due to the cost and complexity of existing analytical methods. Here we describe a relatively inexpensive, simple and reproducible gas chromatography-mass spectrometry (GC-MS) protocol for detecting MeJA in ash phloem. By using an internal standard (bifenthrin) and employing selective ion screening (SIS), we demonstrate that this simple yet robust protocol is more sensitive than other reported methods, with a detection limit of 1 picomole per injection. The usefulness of this method was demonstrated in an experiment in which MeJA was detected in the phloem of ash trees treated with MeJA. This method promises to simplify quantification of MeJA in ash as well as other plants, including model systems.

SYSTEMATICS AND BIOLOGY OF *AGRILUS PLANIPENNIS* FAIRMAIRE (EMERALD ASH BORER) AND ITS RELATIVES: A NEW USDA ARS-FS INTERNATIONAL INITIATIVE

M. Lourdes Chamorro[1], Steven W. Lingafelter[1], Robert A. Haack[2], Therese M. Poland[2], Mark G. Volkovitsh[3], and Runzhi Zhang[4]

[1]USDA Agriculture Research Service, c/o National Museum of Natural History, Washington, DC 20560
[2]U.S. Forest Service, Northern Research Station, East Lansing, MI 48823
[3]Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia
[4]Chinese Academy of Sciences, Institute of Zoology, Beijing 100101, China

ABSTRACT

We presented an overview of a 3-year project between international programs of the U.S. Forest Service and Agriculture Research Service to define the species group to which emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) belongs, provide an identification manual of all related species, and transfer information through identification workshops in China and the United States. Detailed morphological study of imaginal and preimaginal stages will form the basis of the identification manual and phylogenetic study. Molecular data will also be included. The following hypotheses will be tested: (1) Species in *Uragrilus* are the closest relatives of EAB; (2) Species in *Agrilus cyaneoniger* group are the closest relatives of EAB. Fieldwork in China and southeast Asia is planned to collect specimens and biological data.

This study will generate a wealth of information on taxonomy, morphology, and evolution in both the larval, pupal and adult stages of EAB, a highly destructive beetle in North America, and its relatives. By basing our study on evolutionary principles, we may be able to predict potential new invasive pests with similar habits to EAB.

MALE AGGREGATION PHEROMONE IN THE EUROPEAN WOODWASP *SIREX NOCTILIO* (HYMENOPTERA: SIRICIDAE)

Miriam Cooperband[1], Katalin Böröczky[2], Victor C. Mastro[1], Joceyln Millar[3], Tappey H. Jones[4], Kelley Zylstra[5], and Jim Tumlinson[2]

[1]USDA APHIS PPQ CPHST, Buzzards Bay, MA 02542
[2]Pennsylvania State University, Center for Chemical Ecology, University Park, PA 16802
[3]University of California, Department of Entomology, Riverside, CA 92521
[4]Virginia Military Institute, Chemistry Department, Lexington, VA 24450
[5]USDA APHIS PPQ CPHST, Syracuse, NY 13212

ABSTRACT

The major component of a male aggregation pheromone was identified for the European woodwasp, *Sirex noctilio* (Hymenoptera: Siricidae). Males in a Y-tube olfactometer were attracted to natural emanations from groups of other males. Males displayed excitatory behaviors such as everting their genitalia, fanning their wings, vibrating their abdomens, walking, and flying. Headspace volatiles from groups of males were collected on SuperQ filters, Porapak®, charcoal, and SPME fibers. An unsaturated short-chain alcohol, (Z)-3-Decen-1-ol, was discovered and identified. This alcohol was produced in abundance by males that were 2 or more days old, but was not detected in volatiles from groups of females. The natural emanations from males that were 2 or more days old were attractive to other males in the Y-tube, whereas emanations of males less than 2 days old were not attractive to other males. Gas chromatography-electroantennographic detection (GC-EAD) verified antennal activity of the alcohol by both male and female antennae. There is evidence of at least one minor component that is antennally active, and the identification of that compound is currently being investigated.

WORLDWIDE DIVERSITY OF PARASITOID GUILDS OF *AGRILUS* WOODBORERS (COLEOPTERA: BUPRESTIDAE)

Jian J. Duan[1], Phil Taylor[1], Roger Fuester[1], Mark Hoddle[2], and Roy Van Driesche[3]

[1]USDA ARS, Beneficial Insects Introduction Research Unit, Newark, DE 20705
[2]University of California Riverside, Department of Entomology, Riverside, CA 92521
[3]University of Massachusetts, Department of Plant, Soil and Insect Sciences, Amherst, MA 01003

ABSTRACT

Agrilus is the largest genus within the family Buprestidae (Coleoptera), with nearly 3,000 described species worldwide. *Agrilus* adults normally lay eggs under the loose bark or in crevices of host plant tissues, and larvae bore into the living tissue of their host plants. While a few species feed on herbaceous plants, generally attacking root or stem tissue, most *Agrilus* species attack the cambial tissue of woody trees or shrubs. In their native habitats, *Agrilus* populations are generally suppressed by a diverse group of natural enemies and/or host tree resistance, and rarely become serious pests. However, when introduced into ecosystems where host plants lack coevolutionary resistance, or where appropriate specialized natural predators and parasites are absent, they can become severe pests. The recent invasion of North America by the emerald ash borer, *Agrilus planipennis* Fairmaire, from northeast Asia is an excellent example of this.

In the present study, we reviewed literature studies in North America (United States and Canada), Europe, and Asia (particular Russia, China, Japan, and the Korean peninsula) to identify parasitoid guilds associated with *Agrilus* woodborers. There are at least 10 species of hymenopteran parasitoids attacking eggs of *Agrilus* beetles and 38 species attacking *Agrilus* larvae infesting various host plants (trees) in North America, Asia, and Europe. While most of the egg parasitoids (8 species) belong to the family Encyrtidae, a majority of the larval parasitoids are members of three families: Braconidae (18 species/5 genera), Ichneumonidae (9 species/8 genera), and Eulophidae (4 species/1 genus). The highest rate of *Agrilus* egg parasitism (>50 percent) was recorded for four species of encyrtid wasps reported in North America, Asia, and Eurpoe. In contrast, the highest rate of *Agrilus* larval parasitism (>50 percent) was caused by species in two genera of braconid wasps: *Atanycolus* (in North America) and *Spathius* (in Asia), and one of Eulophidae, *Tetrastichus* (in Asia and Europe). Although ichneumonid wasps were frequently reported attacking *Agrilus* woodborers, primarily in North America, the reported rate of *Agrilus* larval parasitism by this group of parasitoids has been generally low (<1 percent). Potential for success in biological control of emerald ash borer (*A. planipennis*) in the United States with North American native parasitoids and old-associations Asian parasitoids is discussed.

INTERACTION BETWEEN A BEETLE AND ITS PATHOGEN: DO ASIAN LONGHORNED BEETLES BEHAVIORALLY FEVER?

Joanna J. Fisher and Ann E. Hajek

Cornell University, Department of Entomology, Ithaca NY 14853-2601

ABSTRACT

Asian longhorned beetles (ALB), *Anoplophora glabripennis* (Motschulsky), are invasive woodborers from China that have the potential to become serious tree pests if they become established in North America. ALB have caused extensive tree mortality in China due to the widespread planting of nonnative susceptible trees. Entomopathogenic fungi have shown promise in controlling these insects. It has been shown that flies, locusts and grasshoppers are able to elevate their body temperatures through basking (behavioral fever) in response to fungal infections. By elevating their temperatures, these insects are able to fight off or delay fungal infection, and therefore live longer compared to infected controls. The entomopathogenic fungus *Metarhizium brunneum* (formerly *M. anisopliae*) is pathogenic to ALB. Since our laboratory is developing this fungus for control of ALB, and since these beetles have been reported exhibiting basking behavior at times in China, it is important to know if ALB adults have a behavioral fever response to fungal infection.

Adult ALB infected with *M. brunneum* were placed in temperature gradients to determine whether they exhibit behavioral fever and what impact this has on their longevity. Overall, infected males died one day earlier than infected females. Our preliminary results indicate that ALB do not behaviorally fever in response to infection with *M. brunneum*. However, infected male ALB that were allowed to bask lived an average of 2.5 days longer than infected males that were not allowed to bask. These results suggest that although male ALB that were allowed to bask did not choose significantly higher temperatures than the controls, brief exposures to high temperatures may have retarded fungal growth. Additional experiments are in progress to further explore these findings. Our preliminary results suggest that *M. brunneum* could provide successful control for ALB, as infected females do not appear to exhibit behavior fever, and even though infected males lived longer when they had access to higher temperatures, it does not appear that they preferentially chose these temperatures.

GUIDE TO IMPLEMENTATION OF PHYTOSANITARY STANDARDS IN FORESTRY

Food and Agricultural Organization (FAO) Forestry Guide Core Group[1]

[1]Adnan Uzunovic, FP Innovations, Canada; Andrei Orlinski, European and Mediterranean Plant Protection Organization (EPPO), France; Brian Zak, Canada Wood Group, Canada; Edson Tadeu Iede, Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Brazil; Eric Allen, Canadian Forest Service, Canada; Hesham A. Abuelnaga, USDA Foreign Agricultural Service, USA; Hugh Evans, Forest Research Wales, UK; Keng-Yeang Lum, CABI Southeast and East Asia Regional Centre, Malaysia; Kerry Britton U.S. Forest Service, USA; Roddie Burgess, Forestry Commission, UK; Sarah A.H. Olembo, African Union Commission, Ethiopia; Shiroma Sathyapala, Ministry of Agriculture and Forestry (MAF), New Zealand; Su See Lee, Forest Research Institute Malaysia (FRIM), Malaysia; Ana Peralta, Brent Larson and Shane Sela, IPPC Secretariat; Beverly Moore and Gillian Allard, FAO.

ABSTRACT

Expanded global trade through the exploitation of new market opportunities is increasing the movement of forest pests globally, and local climatic change may increase the potential for these pests to become established in new areas. Extreme weather events also threaten forests, forest health and peoples' livelihoods. Management of pests and preventing their spread plays a key role in helping ensure forests remain healthy, meeting sustainable forestry objectives.

The governing body of the International Plant Protection Convention (IPPC), the Commission on Phytosanitary Measures (CPM), adopts International Standards for Phytosanitary Measures (ISPMs) to help prevent pest introduction and spread while facilitating trade. Most IPPC member countries have established their own national plant protection organizations (NPPOs) which have historically dealt mostly with agricultural crops. In recent years, however, forest pests have become a more prominent concern, and while communication between the forest sector and NPPOs has increased, more is needed. Forest sector personnel have a vital role in developing and implementing phytosanitary standards, and therefore need to develop a better understanding of what the IPPC is and how NPPOs work.

Since the regulatory language of ISPMs targets phytosanitary experts, foresters may benefit from plain language descriptions of the ISPMs. FAO coordinated a multistakeholder process to prepare a "Guide to implementation of phytosanitary standards in forestry" which offers clear and concise guidance on forest health practices and suggestions for improved national implementation of phytosanitary standards. The guide was prepared through a consultative approach involving an international group of scientists, phytosanitary authorities and forest sector representatives, and is supported by the IPPC Secretariat at FAO. It went through two cycles of review and comments were received from more than 100 specialists from 46 countries. Copies will be available in six languages by April 2011 at www.fao.org/forestry/56879.

Implementation of the guide is underway. The main goals are to widely disseminate the key messages of the guide and to integrate people from forestry and regulatory worlds to improve communication and collaboration. The guide will be used in training to provide basic information on management practices that reduce pest spread.

COLONIZATION PREFERENCES OF THE EUROPEAN WOODWASP, *SIREX NOCTILIO*, ON SOUTHEASTERN PINE SPECIES

Kamal J.K. Gandhi[1], Jamie E. Dinkins[1], John J. Riggins[2], Laurie Schimleck[1], Brian Sullivan[3], Jeff F. Dean[1], Kelley E. Zylstra[4], and Vic Mastro[5]

[1]University of Georgia, Warnell School of Forestry and Natural Resources, Athens, GA 30602
[2]Mississippi State University, Department of Entomology and Plant Pathology, Mississippi State, MS 39762-9775
[3]U.S. Forest Service, Alexandria Forestry Center, Pineville, LA 71360
[4]USDA APHIS PPQ, Otis PSDEL, Buzzards Bay, MA 02542
[5]USDA APHIS PPQ, Pest Survey, Detection and Exclusion Lab, North Syracuse, NY 13212

ABSTRACT

We assessed the colonization and oviposition preferences of an exotic woodwasp species, *Sirex noctilio* Fabricius, on conifer species that are present in varying degrees in southeastern forests. Two species of commercially important southeastern pines, loblolly pine (*Pinus taeda* L.) and Virginia pine (*P. virginiana* Mill.), were used for the bioassay study in 2009. Pine trees were felled in Georgia in late May and cut into 1 m long logs in two diameter classes (10-15 and 20-25 cm), to also assess which log size is optimal for such bioassays. Logs were transported to Syracuse, NY within 5 days of cutting, where Scots pines (*P. sylvestris* L.), the control species, were also cut into logs of two diameter classes. We conducted host choice and no-choice experiments on *S. noctilio*. For the host choice experiment, logs of each of the three pine species (loblolly, Virginia, and Scots) in two diameter classes (small and large) were placed in random locations within an arena where males and females of *S. noctilio* were released. Observations of the activity of adult *S. noctilio* on logs (e.g., ovipositing, resting, or walking) were taken every hour (three times) for 3 days until most of the woodwasps died. Logs were then individually enclosed in mesh sleeves to allow progeny emergence. For the host no-choice study, only large diameter class logs of each of the three pine species were individually enclosed in mesh sleeves, where males and females of *S. noctilio* were introduced. Similar observations to that of host choice experiments on the activity of adult *S. noctilio* were also taken. All the logs were stored in Syracuse until the adults of S. *noctilio* started emerging in September 2009, indicating rapid emergence of *S. noctilio* from our logs. In early December 2009, we dissected the logs completely to collect all immature stages, and count the number of exit holes to determine reproductive success of *S. noctilio* on southeastern pines. Adults of the parental and progeny generations were also measured, including attributes such as diameter of the pronotum, and lengths of whole insect, ovipositor, and abdomen.

Preliminary results from 2009 indicate that in the host choice experiment, significantly more males and females of *S. noctilio* were observed resting/walking on Virginia pine than on Scots or loblolly pines. Female *S. noctilio* were observed drilling with their ovipositor in the southeastern pine logs, especially on Virginia pine, indicating that they may be laying eggs. More adults of *S. noctilio* were found resting on larger than smaller diameter pine logs. Our experiments indicate that *S. noctilio* can reproduce successfully on two southeastern pine species: in fact, this may be the first record of *S. noctilio* emerging from Virginia pine. In general, more adults of *S. noctilio* emerged from Virginia pines, especially from larger than smaller diameter logs, indicating both a host and size class preference.

In 2010, we continued our studies on the colonization and oviposition preferences of *S. noctilio* on southeastern pines including six major species: loblolly, shortleaf (*P. echinata* Mill.), slash (*P. elliottii* Engelm.), longleaf (*P. palustris* Mill.), white (*P. strobus* L.), and Virginia pine. Similar to 2009, we conducted host choice and no-choice experiments on *S. noctilio*, but with greater replication and number of species. Further, attributes of the southeastern tree species were also determined to provide mechanisms for colonization preferences

of *S. noctilio*. From each of the tested trees, we cut a 3 cm thick cookie towards each end of the logs, and are currently measuring wood attributes such as wood density, moisture content, and number and size of resin canals. We also collected resin from each tree species at DBH height in late summer to determine differences in monoterpenes among the southeastern pines.

Preliminary results from 2010 mostly support the host preference data for *S. noctilio* from 2009. In the colonization experiment, more adult females of *S. noctilio* were drilling with their ovipositor on Scots, white and Virginia pines, followed by longleaf and shortleaf pines, with loblolly and slash pines at the lowest levels. More females of *S. noctilio* were found resting on white and Virginia pines followed by Scots, slash, and longleaf in decreasing numbers. The adults of *S. noctilio* started emerging in less than five months, indicating that diapause may not be facultative for this species. In spring 2011, we will dissect these logs to collect data on the reproductive success of *S. noctilio* on southeastern pines, and also measure adult characteristics of parental and progeny generation from 2010. Our overall goal is to incorporate these biological data for *S. noctilio* into the existing risk maps for better predictability about which areas are most susceptible to invasion by *S. noctilio*, and where to focus our survey and management efforts for the southeastern U.S. region.

TEMPERATURE REQUIREMENTS TO BREAK THE EGG DIAPAUSE OF *SCYMNUS CAMPTODROMUS* (COLEOPTERA: COCCINELLIDAE)

Melody A. Keena[1], R. Talbot Trotter[1], Carole Cheah[2], and Michael Montgomery[1]

[1]U.S. Forest Service, Northern Research Station, Hamden, CT 06514
[2]The Connecticut Agricultural Experiment Station, Windsor, CT 06095

ABSTRACT

Scymnus (*Neopullus*) *camptodromus* Yu and Liu is a predator from the native range of the hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand), which is being evaluated to determine its potential use as a classical biological control agent for release against HWA in eastern North America. This species has a broad geographic range in China (Yunnan and Sichuan Provinces), where it is one of the most abundant predators of HWA, and is consistently found over a wide range of habitats and HWA densities. The biology of this species is similar to that of the two *Scymnus* (*Neopullus*) species previously released (i.e. *S. sinuanodulus* and *S. ningshanensis*) with the exception that the eggs of *S. camptodromus* enter a summer diapause and do not hatch until the following spring. This summer egg diapause may be an adaptation to allow it to survive the summer when the stages of HWA it feeds on are not available. Experiments were conducted to evaluate the effects of temperature (10, 15, and 20±1 ºC) and photoperiod (16:8 or 12:12 (L:H) h) on the egg diapause of this beetle (30-50 eggs per treatment), and to determine the length of chill at 5±1 ºC (7, 28, 56, and 84 d) required for eggs to hatch.

Almost all *S. camptodromus* eggs go through a diapause (with no obvious embryo development until diapause is complete) that is broken by exposure to temperatures at or below 10 °C, regardless of the photoperiod to which they have been exposed. Only two eggs (4 percent) hatched when held at a constant 20 °C, and no eggs hatched at a constant 15 °C. Eggs held at a constant 10 °C required an average of ~200 d to hatch. As the length of time eggs were exposed to 5 °C increased, the time to hatch once they were moved to either 10 or 15 °C decreased. Hatch rate also increased (maximum of ~80 percent) with increased time at 5 °C which suggests diapause is broken during exposure to this temperature. Of the temperatures evaluated, the best temperature regimeto break diapause and achieve >80 percent hatch was holding eggs at 5 °C for 56-84 d and then allowing them to hatch at 10 °C. Based on these data, the estimated lower threshold for accumulation of heat units following exposure to 5-10 °C is -5.3 °C. Thus there is a considerable range of temperatures (-5 to 10 °C) over which eggs can both accumulate chill to break diapause, and subsequently accumulate heat to proceed to hatch (probably not simultaneously). The ability of the *S. camptodromus* eggs to develop at these low temperatures may facilitate their hatching in the spring in synchrony with the initiation of oviposition by the sistens generation of HWA. Further studies are needed to determine exactly how long eggs must be exposed to ≤10 °C in order to break diapause.

DO INDIGENOUS LEAF MINING INSECTS COLONIZE MORE NATIVE OR ALIEN WOODY PLANTS IN EUROPEAN AND ASIAN ARBORETA?

Natalia Kirichenko[1], Christelle Péré[2], and Marc Kenis[2]

[1]V.N. Sukachev Institute of Forest, Siberian Branch of Russian Academy of Sciences, RUSSIA
[2]CABI Europe-Switzerland, SWITZERLAND

ABSTRACT

Alien trees planted in arboreta provide great tools to test various ecological hypotheses linked to biological invasions (Kenis et al. 2009). We surveyed alien and closely related native woody plants in Asian and European arboreta to check whether: (1) alien plants are less colonized by native leaf mining insects compared to native plants, following the enemy release hypothesiswhich suggests that invasive insects are more successful in the area of introduction because they are released from the natural enemies that control them in the area of origin (Joshi and Vrieling 2005); and (2) some native leaf miners may do better on alien plants because they have not coevolved with them, according to the new association theory (Hokkanen and Pimentel 1989).

The study was carried out in 2008-2010 in six arboreta in Siberia and Russian Far East, and in two arboreta in Switzerland on about 200 woody plant species from 16 families and 25 genera originating from various continents. Plant pairs (alien vs. native congeneric plant species) were surveyed for leaf miners attacks and their taxonomic diversity.

1. Alien plants were significantly less attacked by native leaf miners than indigenous congeneric plants in all regions, supporting the enemy-release hypothesis. The level of attacks and species richness were 2-3 times lower on alien trees. In Europe, North American, and East Asia, plants were about 2 times less colonized by native leaf miners than European plants ($Z = 3.1$, $N = 21$, $p = 0.001$ and $Z = 3.9$, $N = 50$, $p = 0.0001$,respectively). In Siberia, alien plants from distant regions (Europe, North America) were also less damaged by leaf miners than native plants ($Z = 3.1$, $N = 19$, $p = 0.001$). However, no significant differences in leaf miner attacks were found for species of East Asian and North Asian (native plant) origin ($Z = 1.5$, $N = 30$, $p = 0.12$).
2. No native leaf miner was significantly more damaging to alien trees than native trees in Asian and European arboreta. The only remarkable case was *Phyllonorycter populifoliella*, which was found severely attacking the North American *Populus balsamifera* in Siberia, but it is not clear if this pest is native or introduced from Europe. Thus, leaf miners showed no evidence for the new association theory.

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A MANAGEMENT STRATEGY FOR BEECH BARK DISEASE: EXPLOITING NATIVE RESISTANCE

Jennifer L. Koch[1], Mary E. Mason[2], and David W. Carey[1]

[1]U.S. Forest Service, Northern Research Station, Delaware, OH 43015
[2]Ohio State University, Department of Entomology, Ohio Agricultural Research and Development Center, Wooster, OH 44691

ABSTRACT

Beech bark disease (BBD) is an insect disease complex that has been killing American beech (*Fagus grandifolia* Ehrh.*)* trees since the accidental introduction of the beech scale insect (*Cryptococcus fagisuga* Lind.) to Canada around 1890. Insect infestations are followed by infection with *Neonectria ditissima* Samuels & Rossman or *Neonectria faginata* Castlebury. Mortality levels in the first wave of the disease can be as high as 50 percent, with consequent loss to stand health, merchantable timber, and many wildlife and ecosystem services. It is currently estimated that between 1 and 5 percent of the native American beech are resistant to beech bark disease, and resistance has been shown to be to the insect part of the complex.

Beech bark disease-resistant American beech are currently being identified, grafted, and propagated as part of a cooperative effort among several of the National Forests, including the Allegheny (PA), the Hiawatha (MI), the Chequamegon-Nicolet (WI), and the Monongahela (WV), as well as the Michigan Department of Natural Resources, the Michigan Tree Improvement Cooperative, the Pennsylvania Department of Conservation and Natural Resources, and the Holden Arboretum (Kirtland, OH). Selected beech trees are tested using the artificial infestation technique to screen for scale insect resistance. Confirmed resistant genotypes are replicated to appropriate numbers and will be used to establish seed orchards that will supply regionally adapted disease-resistant beechnuts for use in restoration plantings and BBD management. Previous studies have shown only crosses between two resistant parents produce significant improvement over unselected seedlots. Between 20 and 25 unique genotypes with confirmed resistant phenotypes will be included in the seed orchards, allowing collection of a genetically diverse nut crop with sufficient percentage of R nuts for genetic improvement. We are currently working with cooperators to establish the first orchards in Michigan and Pennsylvania.

Grafted mature beech may flower the year of grafting, or resume cyclic flowering quickly on potted grafts. As beech trees flower, controlled crosses are performed between the selected parents to produce new progeny families for testing. Results from these progeny tests will be used to estimate the level of improvement expected from the seed orchards.

ASIAN LONGHORNED BEETLE (ALB), *ANOPLOPHORA GLABRIPENNIS*, ADVANCEMENTS IN ERADICATION PROGRAM

Christine Markham[1] and Brendon Reardon[2]

[1]USDA Animal Plant Health Inspection Service, Raleigh, NC 27606
[2]USDA Animal Plant Health Inspection Service, Riverdale, MD 20737

ABSTRACT

Asian longhorned beetle detections in the United States include Brooklyn, NY (August 1996), Chicago, IL (July 1998), Jersey City, NJ (October 2002), and Worcester, MA (August 2008). The eradication program declared eradication of infestations in Chicago, IL., and Jersey City, NJ, in 2008. There are currently 271 square miles regulated for ALB in the United States. New York has 142 square miles under regulation. Survey inspections continue, but only small pockets of infestation have been detected in the last few years. Islip, NY is scheduled for eradication in 2011 pending final survey results. A total of 6,275 infested trees have been detected, and 18,467 infested and high-risk host trees have been removed. Approximately 44,100 trees were treated in 2010. New Jersey has 25 square miles under regulation. Survey inspections continue with the last detection being made in 2006. To date, 729 infested trees have been detected, and 21,981 infested and high-risk host trees have been removed. Preventative chemical applications were finished in 2009. Massachusetts has 104 square miles under regulation. Survey inspections continue, and over 720,000 host trees have been surveyed. Over 19,000 infested trees have been detected, and over 28,000 infested and high-risk host trees have been removed. In 2010, more than 62,320 trees were treated. The goal of the ALB program is to eradicate the pest in the United States to protect the hardwood forests of North America. To achieve this goal, the ALB program has developed and implemented eradication protocols using an area-wide, science-based strategy including the following: (1) exclusion; (2) visual survey of host trees; (3) tree removal; (4) chemical treatment; (5) regulatory activities to prevent the pest's spread; (6) replanting to mitigate effects of trees lost to ALB; (7) outreach efforts; (8) quality assurance to ensure survey, removals, and treatments are done correctly so that these actions are effective; and (9) methods development to improve program effectiveness and delivery.

MULTISTATE COMPARISON OF EMERALD ASH BORER DETECTION TOOLS: A FIVE YEAR SYNTHESIS

Jordan M. Marshall[1,2], Jessica A. Beachy[1], Ivich Fraser[3], Andrew J. Storer[1], and Victor C. Mastro[4]

[1] Michigan Technological University, School of Forest Resources and Environemtnal Science, Houghton, MI 49931
[2] Current Address: Indiana University-Purdue University Fort Wayne, Department of Biology, Fort Wayne, IN 46805
[3] USDA APHIS PPQ, Brighton, MI 48116
[4] USDA APHIS PPQ, Otis ANGB, MA 02542

ABSTRACT

The need for effective detection of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, early in the infestation timeline has been evident since this pest was first discovered in 2002. Since 2006, we have compared potential trap and lure combinations at low emerald ash borer population densities in order to identify which traps may have the highest detection efficiency. The objectives of this study over the years 2006-2010 were to: (1) compare the effectiveness of different detection tools across various states; (2) identify the most effective of the available tools for EAB detection at low density; and (3) develop monitoring and trapping recommendations for managers in states with and without EAB infestations.

States where traps were established and the trap types tested varied across years. Indiana, Michigan, and Ohio, had sites throughout the experiment (2006-2010), while Kentucky (2010), Minnesota (2010), Missouri (2009-2010), New York (2009), Pennsylvania (2008-2010), Virginia (2009), Wisconsin (2009-2010), and West Virginia (2010) had trapping sites during 1-3 years. The only trap consistently used across all years was a current year girdled tree with a plastic, sticky trap wrapped around the bole of the tree.

While the mean number of EAB captured per trap type in 2006 was not significantly different, girdled trees wrapped with purple glue had the greatest capture rates, as well as the highest rates of detection. However, due to the high variability of capture rates, identification of a single successful trap was not possible in 2006. Five of the eight trap types resulted in a third or less of the traps detecting EAB, suggesting that they may not be effective detection tools. Girdled, mature ash trees (>30 cm DBH), wrapped with a plastic, sticky trap captured the greatest number of EAB adults in 2007. In addition, the mature trees also had the highest detection rates. While these trees appear effective, it can be difficult to find ash trees of suitable size, and to acquire permission from property owners or managers to girdle such large trees. The large mature trees also become hazards when girdled and are difficult to remove.

As with the traps in 2006, there was no significant difference in the number of EAB captured per trap in 2008. However, the purple prism traps hung at 6 m with manuka oil lure and at 1.5 m with phoebe oil lure had detection rates above 0.50, suggesting that after a single flight season, those traps may be effective. Natural fluctuations in population size and EAB behavior may play important roles in the effectiveness of detection traps. This is evident in the variability of detection rates in current and previous year girdles in 2006, 2007, and 2008. For 2009, only the current year girdle had significantly fewer EAB captured than the green prism trap hung at 13 m. The green traps at 13 m had the greatest number of EAB captured, but had lower detection rates than the purple prism trap at 6 m, orthe double-decker tower trap. Both the purple prism trap at 6 m and the double-decker tower trap detected EAB in more than 50 percent of cases. The variability in effectiveness of the current year girdle continued from previous years, with less than a quarter of the traps detecting EAB in 2009, with previous years ranging from 1/3 to 1/2 of the traps.

Similar to 2006 and 2008, there was no significant difference between trap types in 2010. The different green and purple prism traps had detections with over half of the traps. However, for each prism trap type individually, only approximately one-third of the sites

had detections on all three replicates of the same trap type. This is an indication that landing behavior of EAB influences the success of a trap. It may be necessary for numerous traps to be placed within a stand to effectively and operationally identify a stand as being negative for EAB.

The current year girdled tree was the only trap used in all 5 years of the study, but it was not consistent in capture or detection rates. Since the mean diameter was not significantly different between years for the current year girdle, EAB population density most likely varied enough between years and sites to cause variation in the effectiveness of the current year girdle. The degree at which EAB population density and behavior influences the effectiveness of an individual trap type is unknown. Because of this uncertainty, the longterm, multiyear use of a single trap type will likely result in unclear detection efforts. Increasing trap diversity, numbers of traps, and total trapping surface area may be the most effective strategy for EAB detection. Analysis of detections and the number of traps used each year showed that the odds of detecting EAB increased by 27 percent with each addition of a trap. Continued refinements of traps, lures, and combinations will also improve EAB detection efforts.

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PREDICTING EMERALD ASH BORER LANDING BEHAVIOR ON UNWOUNDED ASH TREES

Jordan M. Marshall[1,2], Melissa J. Porter[1], and Andrew J. Storer[1]

[1] Michigan Technological University, School of Forest Resources and Environemtnal Science, Houghton, MI 49931
[2] Current Address: Indiana University-Purdue University Fort Wayne, Department of Biology, Fort Wayne, IN 46805

ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, detection has relied on visual surveys, artificially stressed trap trees, and plastic panel traps. Previous studies have illustrated that unwounded ash trees can be similar in effectiveness for adult EAB trapping densities to girdled trap trees. The objectives of this study were to: (1) model the capture rates of EAB adults on ash trees without an artificial wound; (2) test the predictive power of the resulting models; and (3) use the resulting model to predict the likelihood of EAB landing on an ash tree.

In spring 2008, a total of 386 ash trees were identified at Burt Lake and Harrisville State Parks, MI, and in spring 2009, another 42 ash trees were identified at Farnsworth and Providence Metroparks, MI. Each tree was coated with a band of Tanglefoot® Trap Coating. Traps were checked every 2 weeks, and adult EAB were collected. For each tree, crown light exposure (CLE) and vigor rating were assessed, and density of EAB adults captured was calculated (EAB/m^2 of trap surface). Vigor was rated on a scale of 1-6, with 1 being a healthy tree and 6 being a dead tree. Two multiple regressions were created for the two trapping years (2008 and 2009 models) for EAB/m^2 (dependent) using the categorical variables CLE and vigor (independents). These regression models were tested by predicting EAB/m^2 for trees at Deford and Shiawassee State Game Areas, MI, during spring 2009. Forty ash trees were identified at each Deford and Shiawassee, and each tree was wrapped, traps were checked, and tree health was assessed as described above. Differences in the observed and predicted values of EAB/m^2 were tested. In spring 2010, another 30 trees at both Deford and Young State Parks were identified as either low or high likelihood of capturing EAB to test a simplification of the models.

For both 2008 and 2009, trees that did not capture EAB adults had significantly lower CLE and vigor values (less direct sunlight and healthier) than trees that captured EAB. Two significant multiple regression models were produced (2008 model, $R^2 = 0.082$, $F_{9,363} = 3.61$, $P < 0.001$, EAB/m^2 = 6.77 + 0.62*CLE0 - 1.06*CLE1 - 0.33*CLE2 - 0.51*CLE3 + 0.83*CLE4 - 5.97*vigor1 - 6.18*vigor2 - 2.21*vigor3 - 0.18*vigor4; 2009 model, $R^2 = 0.717$, $F_{10,33} = 10.46$, $P < 0.001$, EAB/m^2 = 334.09 + 26.48*CLE0 - 2.11*CLE1 + 7.00*CLE2 + 26.13*CLE3 + 34.30*CLE4 - 333.52*vigor1 - 305.33*vigor2 - 65.25*vigor3).

For Deford, the predicted EAB/m^2 values from the 2008 model did not differ from the observed values, while the 2009 model predicted values were significantly greater than observed. For Shiawassee, the 2008 model predicted EAB/m^2 values were significantly lower than the observed values, while the 2009 model predicted values did not differ significantly from the observed. The 2008 and 2009 model predicted values for Deford and Shiawassee were not significantly correlated with the observed values at these sites. Based on the two models, a decision matrix was created to predict the likelihood of EAB capture by 2010 trees. At Deford, 16 trees were subsequently categorized as high likelihood and 14 as low. At Young, 13 were categorized as high and 17 as low likelihood. Trees capturing ≥ 5 adult EAB accounted for 44.8 and 22.6 percent of high and low category trees, respectively. In addition, trees capturing ≥10 adults accounted for 34.5 and 12.9 percent of high and low category trees, respectively. Trees categorized as high likelihood of capturing EAB adults captured significantly more male and female beetles than low likelihood trees.

Population size variability adds considerable difficulty to effectively model and predict actual EAB/m^2. This is evidenced by the fact that only one model was effective at each of Deford and Shiawassee. The utility of the models may come in the form of selecting trees that will have the greatest likelihood of capturing EAB adults. While the actual predicted values for the two models were very different, the greatest number of EAB/m^2 for both models was for a tree with a CLE of 4 and a vigor rating of 4 (approximately half of crown is dead). Some natural resource managers and private land owners decline the request to girdle ash trees for EAB trapping. Girdling ash trees as a trap can be expensive to establish and evaluate, as well as a hazard to workers and the public when the tree is felled, peeled, and inspected for EAB larvae. By leaving the tree ungirdled and wrapping a plastic trapping surface around the bole, the expense and hazard can be greatly reduced or essentially eliminated. An unwounded tree is a less expensive alternative to an artificial trap and a simpler alternative to a girdled trap tree. While not a replacement for federal and state agency detection program artificial traps, using unwounded trees may provide an additional trapping tool for use in survey and detection programs where resources, both financial and natural, limit the use of other trapping techniques.

NEUROPHYSIOLOGICAL CHARACTERIZATION OF GUSTATORY NEURONS OF GYPSY MOTH LARVAE

Timothy L. Martin and Vonnie D.C. Shields

Towson University, Biological Sciences Department, Towson, MD 21252

ABSTRACT

The gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), is considered to be a serious pest since it defoliates many tree species including forest, shade, fruit, and ornamentals. It is highly polyphagous, but prefers leaves from red oak and sweet gum trees. Lepidopteran larvae, such as the gypsy moth, depend largely on their gustatory and olfactory sensory organs (sensilla) to find food sources. Feeding behavior is controlled by input from the mouthpart gustatory sensilla. These larvae possess taste sensory organs, the medial and lateral galeal styloconic sensilla, which are thought to be the primary sensory organs involved in feeding. These sensilla play important roles in host plant selection through the detection of different phytochemicals and are in continuous contact with plant sap during feeding. Each sensillum houses four taste receptor cells including a sugar, salt, deterrent, and inositol cell. Using a single cell electrophysiological tip-recording method, our aim was to characterize the temporal firing patterns and sensitivities of the taste receptor cells within each sensillum when exposed to selected phytochemicals. Gustatory sensory input is encoded as patterns of nerve impulses by neurons housed in these sensilla and sent to higher processing centers in the brain of the insect. These neural messages signal acceptance or rejection of food. Our results revealed that these cells responded to alkaloids, (i.e., strychnine, aristolochic acid, nicotine, and caffeine), sugars and sugar alcohols, (i.e., sucrose and inositol), and salt (i.e., potassium chloride). The deterrent cell exhibited a robust temporal firing pattern and displayed varying sensitivity to alkaloid stimulation. We also examined the effects of mixture interactions on food palatability to address the fact that foods contain both positive and negative factors which stimulate or deter feeding, respectively. This study offers insights into the role of phytochemicals, especially alkaloids, in the taste physiology of this larval insect.

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THE DESERT LOCUST CONTINGENCY PLANNING ASSISTANT

Bruce J. Miller[1] **and Max W. McFadden**[2]

[1]The Heron Group, LLC, North East, PA 16428
[2]The Heron Group, LLC, Santa Fe, NM 87508

ABSTRACT

In 2008, The Heron Group, LLC was contracted by the Desert Locust Group (DLG), a part of the United Nations Food and Agriculture Organization (FAO) located in Rome, Italy. In early discussions with the DLG, members of The Heron Group learned that perhaps the greatest problem confronting the DLG was the immediate need for funding if an outbreak was imminent or already in progress. Donors have been reluctant to release funds because it has been difficult for the DLG staff to provide needed information in a timely manner. This delay in providing funds has led to greater damage to crops over a much greater area. The challenge to The Heron Group was to develop a contingency planning tool that could be used to display the current state of preparedness for approximately 103 countries that could be impacted by an outbreak of desert locust (*Schistocerca gregaria* Forskal). A report on preparedness for these countries would be developed annually and combined with a request for funding based on country needs.

The completed Desert Locust Contingency Planning Assistant was developed initially from documents provided by DLG members. Periodic contact with the DLG through emails and the internet allowed for interaction such as review of our approach to developing the tool and actually testing the planning assistant over a 2-year period. At that time Miller attended a weeklong meeting in Cairo, Egypt, and demonstrated the planning assistant and provided copies of the user's manual to national group representatives. These individuals were requested to review the planning assistant and users manual and recommend changes if needed. After the country reviews were returned, changes were made and the final product delivered to the DLG. Members of the DLG then planned to work with individual countries to complete the planning assistant questionnaire and have a state of preparedness for each country ready for the next outbreak as well as a well-prepared request for immediate funding.

ATTACHING LURES FOR SAPROXYLIC BEETLES TO FUNNEL TRAPS: INSIDE OR OUTSIDE FUNNELS

Daniel R. Miller[1], Christopher M. Crowe[1], Brittany F. Barnes[2], Kamal J.K. Gandhi[2], and Donald A. Duerr[3]

[1]U.S. Forest Service, Southern Research Station, Athens, GA 30602
[2] University of Georgia, Warnell School of Forestry, Athens, GA 30602
[3]U.S. Forest Service, Forest Health Protection, Atlanta, GA 30309

ABSTRACT

Our objective was to determine the effect of hanging lures within multiple-funnel traps compared to hanging them outside the funnels. Two trapping experiments were conducted in stands of mature pine in Georgia, each with the following treatments: (1) lures inside the funnels; and (2) lures outside the funnels. In each experiment, we used 20 multiple-funnel traps grouped into 10 replicate blocks of 2 traps per block. Traps were hung between trees by rope and spaced 8-12 m apart. Each collection cup contained about 250 ml of RV & Marine antifreeze (i.e., propylene glycol). All traps in Experiment 1 were baited with ipsenol and ipsdienol bubblecap lures, and ethanol and (-)-α-pinene ultra-high release rate (UHR) pouches, whereas all traps in Experiment 2 were baited with ipsenol and ipsdienol bubblecap lures, and a low-release rate (-)-α-pinene bottle lure (15 mL) . We used 8-unit multiple-funnel traps in Experiment 2, whereas we used 11-unit multiple-funnel traps in Experiment 1. In Experiment 1, traps with treatment (1) were modified in order to place the UHR lures within the funnels. The diameter of the center hole of each funnel with treatment (1) was increased from to 5.5 to 12.0 cm. The trapping periods for Experiments 1 & 2 were 24 July-29 August 2008 and 29 August-7 October 2008, respectively. Bark and wood boring beetles were either unaffected by the location of lures on traps, or preferred traps with lures placed within funnels. Hanging lures on the outside of traps did not result in increased catches of any species that we monitored.

TRADING PLACES: FUNGUS AND NEMATODE SWITCH OFF AS PREDATOR AND PREY

E. Erin Morris and Ann E. Hajek

Cornell University, Department of Entomology, Ithaca, NY 14853-2601

ABSTRACT

Sirex noctilio, an invasive pest of pine (*Pinus* spp.) trees, was first found in New York State in 2004, and currently threatens 200 million hectares of U.S. and Canadian forests. *S. noctilio* has been well known as an invasive in the Southern Hemisphere for many decades. In many cases in the Southern Hemisphere, it has been successfully controlled by biological control programs using the parasitic nematode *Deladenus siricidicola*. The North American invasion is the first introduction of *S. noctilio* to a location where *Sirex* and conifer hosts are native, which increases the complexity of developing a control program.

S. noctilio in North America carries with it two IGS-based strains of its symbiotic fungus *Amylostereum areolatum* that indicate *S. noctilio* was likely introduced from Europe to North America. Additionally, this white rot fungus is presumed to be native to North America as a symbiotic fungus of *Sirex nitidus*. This fungus is injected into trees by *S. noctilio* during oviposition, after which it grows throughout the tree, ultimately causing lethal pine wilt.

D. siricidicola, the nematode control agent of *S. noctilio*, has a complex life cycle involving two forms, a fungal-feeding form and a parasitic form. The fungal-feeding form persists in pine trees, where it spreads throughout the tree and eats the symbiotic fungus of *S. noctilio*. In the presence of *S. noctilio* larvae, preinfective nematodes develop into a parasitic form which invades and eventually sterilizes the woodwasp. Because the nematode spends part of its life cycle feeding on the *A. areolatum* injected into trees by *Sirex* woodwasps, there is potential that nematodes released for biological control might encounter diverse strains of *A. areolatum*.

We studied interactions between the nematode (from Ecogrow, Australia) and five different isolates of *A. areolatum* that it may encounter upon North American field release, in order to assess whether a proper balance between fungal growth and nematode inoculum would lead to differential production of nematodes. Fungal isolates grew for 1, 5, or 10 days on media in petri dishes at 23 ˚C prior to inoculation of 500 nematode eggs per plate. Inoculated plates were then kept at 23 ˚C in darkness for 25 days, at which time nematodes were washed off each plate to enumerate total nematodes produced.

Days of fungal growth prior to nematode egg inoculation did not consistently have a significant effect on nematode yield, but fungal isolate had a significant effect. A fungal isolate from *S. nitidus* produced the most nematodes despite being the slowest growing isolate. Notably, the fastest growing fungal isolate produced few nematodes. However, a fungal isolate with an intermediate growth rate produced very few nematodes. These results indicate that different fungal isolates of *A. areolatum* have an impact on growth of *D. siricidicola*, which may be an important factor regarding potential release of the nematode in a control program in North America. Additionally, these findings may be useful in mass rearing of the nematode for biological control.

EFFECTS OF TWO INTRODUCED PESTS ON FOLIAR TERPENES OF EASTERN HEMLOCK

Joshua Pezet[1], Joe Elkinton[1], Sara Gómez[2,3], and Evan Preisser[2]

[1]University of Massachusetts, Organismic and Evolutionary Biology, Amherst, MA 01003
[2]University of Rhode Island, Department of Biological Sciences, Kingston, RI 02881
[3]Tufts University, Department of Biology, Medford, MA 02155

ABSTRACT

Hemlock woolly adelgid (*Adelges tsugae*) and elongate hemlock scale (*Fiorinia externa*) are two introduced insect pests of eastern hemlock (*Tsuga canadensis)* that often co-occur on the same trees, but have very different effects on their hemlock hosts. Adelgid usually cause swift and dramatic declines in tree health, often resulting in tree death within a few years, while quite heavy infestations of scale result in only mild declines in tree health. Previous evidence indicates that infestation of eastern hemlocks by the scale has a paradoxically beneficial effect on the tree by protecting it in some unknown manner from the more damaging effects of the adelgid. The role of oleoresin metabolites has been frequently implicated in conifer resistance against bark beetles, pathogens, and other pests. Here we report preliminary foliar terpene profiles from a manipulative study investigating why the adelgid is so much more destructive and how elongate hemlock scale protects hemlocks from hemlock woolly adelgid. We predicted that the terpene profiles of hemlocks infested with scale or adelgid would differ from uninfested control trees in divergent ways, in agreement with the very different effects of the two insects on their host hemlocks. Forest saplings were transplanted from Pelham, MA to a plantation in Kingston, RI, and were artificially inoculated each spring since April 2007 with adelgid crawlers, scale crawlers, or neither insect. Samples of previous year's growth were collected, flash frozen in the field, and solvent extracted. The 10 major monoterpene and sesquiterpene resin components were tentatively identified and quantified by GC/FID. Mean individual and total terpene amounts for the three treatment groups were calculated. Although we found no significant differences in concentrations of individual terpenes between the three treatment groups, principal component analysis revealed potentially significant differences between overall terpene profiles of control and infested trees. The current study examined oleoresin in needles of eastern hemlock repeatedly infested with elongate hemlock scale, hemlock woolly adelgid, or neither insect. Studies of other conifers suggest there may be greater changes in oleoresin concentrations induced by herbivory in stem secondary xylem compared to foliage. A parallel study of terpene accumulation in stems and woody tissue is underway. This is especially relevant given that hemlock woolly adelgid, the greatest threat to eastern North American hemlocks, feeds on hemlock twigs.

CAN SUBMERGING BLACK ASH LOGS KILL EMERALD ASH BORER AND PRESERVE WOOD FOR NATIVE AMERICAN BASKETRY?

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

[1]U.S. Forest Service, Northern Research Station, East Lansing, MI 48823
[2] Michigan State University, Department of Entomology, East Lansing, MI 48823
[3] USDA APHIS, Otis PSDEL, Buzzards Bay, MA 02542

ABSTRACT

Black ash, *Fraxinus nigra,* has ring-porous wood that allows the annual layers of xylem to be easily separated and used for basketry by many Native American cultures. The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is threatening North America's ash resource including black ash, and a centuries-old traditional native art form. There is grave concern 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. We submerged EAB infested black ash logs in running water for different lengths of time and evaluated EAB mortality as well as the color and quality of wood splints from the submerged ash logs. 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 five treatments with eight 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. 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 ^{o}C during the first month of the study. The temperature increased to 21.1 ^{o}C 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. Splint pliability and quality were not impaired after 3 months of submergence. Based on these results, submerging logs in spring and summer for 2 to 3 months is effective at killing overwintering mature EAB larvae and prepupae and for preserving wood quality for basket making. Criteria may differ for logs cut and submerged at other times of year.

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]U.S. Forest Service, Northeastern Area State & Private Forestry, Durham, NH 03824
[2]Michigan State University, Department of Entomology, East Lansing, MI 48824
[3]U.S. Forest Service, Northern Research Station, East Lansing, MI 48823
[4]Michigan Department of Natural Resources, Marquette, MI 49855

ABSTRACT

Early detection of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), increases the likelihood that management tactics will effectively reduce population growth 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. Here, we present preliminary results from three large-scale field studies that examine various aspects of using girdled trees as detection tools in the field: (1) the detection ability of 4 girdled trees per 4 ha (10 ac) compared to 16 girdled trees per 4 ha; (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 and colonized at higher rates than nongirdled 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 4 trees per 4 ha was as effective at detecting low densities of EAB as establishing girdled ash trees at 16 trees per 4 ha. Also, establishing 3-tree clusters of girdled ash trees was as effective at detecting low densities of EAB 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 emergence from girdled trees a year after being used as trap trees was very low on trees that were bucked into meter lengths (0.7±0.2 per m^2) and was positively correlated with both prepupal emerald ash borer density (R^2=0.81) and overall emerald ash borer density (R^2=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.

ARKANSAS *SIREX*: BIOLOGY, PHENOLOGY AND NATURAL ENEMIES

Fred M. Stephen, Larry D. Galligan, Jessica A. Hartshorn, Danielle M. Keeler, Ace Lynn-Miller, and Donald C. Steinkraus

University of Arkansas, Department of Entomology, Fayetteville, AR 72701

ABSTRACT

The overall goals of our research are to investigate the biology of native Arkansas *Sire*x spp. (Siricidae), their insect natural enemies, fungal symbionts, parasitic nematodes, and their interactions among other insect and fungal species known to colonize phloem and xylem of weakened or stressed loblolly (*Pinus taeda*) and shortleaf pine (*P. echinata*) trees.

We established nine research sites in the Ozark Mountains, Ouachita Mountains, and southern Arkansas Gulf Coastal Plain, near Crossett Experimental Forest. We trapped at these sites in 2009 and 2010. Our collection of siricid adults yielded adults of both *Sirex edwardsii* and *S. nigricornis* from all of the general regions of Arkansas. Of the 278 adults captured, *S. nigricornis* was about twice as abundant. In 2009, most adults were trapped in the Ouachitas, and in 2010, more were captured in the Ozarks. Trapping was initiated too late in 2009, likely missing adults from October. Phenology of both species appears to be similar, with the more complete data from 2010 indicating peak flight in mid to late October and continuing through November.

We dissected female *S. edwardsii* and *S. nigricornis* collected from 2009 adult trapping studies in three regions of Arkansas. Adult females contained nematodes in 27 of 164 adults dissected. Nematodes were found in both *Sirex* species. Dissections from 2010 trapping are currently underway.

We reared field collected adult native *Sirex* and dissected their mycangia with the intent of confirming that, similar to other North American collections, our Arkansas species do in fact harbor *Amylostereum chailletii*. PCR on mycangia dissected from *S. edwardsii* and *S. nigricornis* resulted in a positive identification of *A. chailletii*. Mycangia were also plated on PDA medium to isolate fungus.

Our observations suggest that insects which inhabit dead and dying pine sapwood, such as *Monochamus* spp. and *Xylotrechus sagittatus*, may compete with or prey upon native siricids. During laboratory dissection of field collected logs, we found cerambycid larvae preying on siricid larvae within their galleries. To test whether potential predators affect siricid oviposition behavior, we created artificial cerambycid oviposition pits (resembling those of *Monochamus* species) and provided choice tests to ovipositing siricid females. No avoidance of logs or areas in which *Monochamus* had oviposited was detected.

POPULATION SUPPRESSION OF *TETROPIUM FUSCUM* (F.) BY PHEROMONE-MEDIATED MATING DISRUPTION

Jon Sweeney, Peter Silk, J. Edward Hurley, and Ed Kettela

Natural Resources Canada, Canadian Forest Service, Fredericton, NB

ABSTRACT

The brown spruce longhorn beetle, *Tetropium fuscum* (F.), is a quarantine pest that has been established in Nova Scotia, Canada, since at least 1990. Initially discovered in Halifax in 1999, it has now been detected in nine counties of Nova Scotia. Practical means of population suppression are needed in an effort to slow the beetle's spread. We present results of field trials testing broadcast applications of fuscumol (the beetle's aggregation pheromone) in Hercon® flakes for mating disruption and population suppression of *T. fuscum.*

Fuscumol was formulated at 10 percent concentration in Hercon® flakes and applied twice per season (at the onset and peak of adult emergence) at a rate of 2.75 kg/ha. In 2008, pvc flakes were applied from the ground using modified leaf blowers; in 2009, Hercon Bioflakes® were aerially applied from a hopper suspended beneath a helicopter. Plots were 4 ha in size and were replicated twice in 2008 and four times in 2009; equal numbers of untreated plots served as controls. Response variables were: (1) percentage of female *T. fuscum* mated (2009 plots only); (2) mean percentage of spruce bait logs infested in each year; (3) mean density of *T. fuscum* larvae in bait logs; and (4) mean numbers of *T. fuscum* captured per trap baited with pheromone plus host volatiles. Data were pooled from 2008 and 2009 and one-tailed t-tests used to determine if mating success or infestation was lower in treated than control plots.

Broadcast application of fuscumol-impregnated Hercon® flakes significantly reduced: (1) the percentage of *T. fuscum* females that were mated; (2) the percentage of bait logs infested with *T. fuscum*; and (3) the density of *T. fuscum* larvae per m^2 in bait logs, but did not reduce the mean numbers of *T. fuscum* captured in pheromone-baited traps. These results are promising and further trials are planned for 2010 and 2011 to confirm efficacy.

MASS TRAPPING FOR POPULATION SUPPRESSION OF AN INVASIVE LONGHORN BEETLE, *TETROPIUM FUSCUM* (F.) (COLEOPTERA: CERAMBYCIDAE)

Jon Sweeney, Peter Silk, Marc Rhainds, J. Edward Hurley, and Wayne MacKay

Natural Resources Canada, Canadian Forest Service, Fredericton, NB

ABSTRACT

The brown spruce longhorn beetle, *Tetropium fuscum* (F.), is an invasive pest of spruce that has been established in Nova Scotia, Canada, since at least 1990, but was not discovered until 1999. Attempts to eradicate the beetle were discontinued in 2006. Although the beetle is under regulatory control, direct control methods are needed to suppress individuals in outlier populations and to slow spread along the leading edge. Black panel intercept traps baited with synthetic aggregation pheromone (fuscumol) and host volatiles have been used successfully in *T. fuscum* surveys since 2007. Because these traps capture both sexes of the beetle, mass trapping offers a potential means of suppressing the population of egg laying females at targeted sites. We present results of Nova Scotia field trials testing mass trapping for *T. fuscum* population suppression in 2008 and 2009.

High densities (100 per ha) of traps baited with synthetic fuscumol and host volatiles were set out in a 10 by 10 m grid and replicated in four 1 ha plots in each year (2008 and 2009). Each mass trapping plot was paired with a 1 ha untreated plot located 200-100 m away. Three decks of spruce bait logs were set out in each treated and control plot along a diagonal transect. Data from 2008 and 2009 were pooled (n=8) and one-tailed paired t-tests used to test whether infestation was lower in treated plots than control plots.

The percentage of spruce bait logs infested with *T. fuscum* and the mean density larvae per m^2 were significantly lower in mass trapped plots than untreated control plots. Future work will test the efficacy of fewer traps per ha (e.g. 25 traps per ha on a 20 by 20 m spacing) and methods of increase trapping efficacy (e.g., fluon).

NATIVE SPECIES ON A NONNATIVE HOST: POTENTIAL BIODIVERSITY MAINTENANCE FOR EASTERN HEMLOCK FORESTS

R. Talbot Trotter III[1] and Alexander Evans[2]

[1]U.S. Forest Service, Northern Research Station, Hamden, CT 06514
[2] Forest Guild, Santa Fe, NM 87501

ABSTRACT

The hemlock woolly adelgid (*Adelges tsugae*) is a small, piercing-sucking herbivore native to Asia and western North America (Havill et al. 2006). This herbivore, which feeds on tigertail spruce (*Picea torano*) and members of the genus *Tsuga* in its native habitat (Havill et al. 2006), was unintentionally introduced to the eastern United States sometime before 1951 when it was first documented on eastern hemlock (*Tsuga canadensis*) near Richmond, VA (Gouger 1971). Since its introduction, the hemlock woolly adelgid has spread to infest hemlock in at least 18 states, and now occupies roughly half of the distribution of hemlock in eastern North America. Infestation by the hemlock woolly adelgid can lead to needle loss, bud kill, and ultimately, tree mortality. Infestations in southern regions, where warm winter temperatures exert little control of adelgid populations (Trotter III and Shields 2009), can lead to the mortality of trees in as little as 2 years, and rates of mortality within stands can approach 95 percent. Evergreens provide key ecosystem functions in eastern hardwood forests (Kizlinski et al. 2002, Orwig 2002, Orwig et al. 2008), and in many stands, hemlock represent either the only softwood component, or are one of only two conifers within stands (the other conifer is often white pine, *Pinus strobus*). The loss of eastern hemlock, therefore, also means the loss of a major ecological and structural component in eastern forests.

Although the loss of hemlock poses obvious threats to aesthetic, cultural, and commercial aspects of eastern forests, the impacts of this loss on associated species may be less apparent. Past work has shown that hemlocks are associated with more than 300 species of arthropods (Dilling et al. 2009), and with the removal of hemlock, many of these species may be lost from stands and regions. Here, we seek to expand on these data by addressing three key questions: First, what is the seasonal composition of arthropod species found in direct association with eastern hemlock? Second, what species cooccur on white pine, and might white pine provide a buffer for the loss of biodiversity in eastern stands impacted by the hemlock woolly adelgid? Third, do arthropods found on eastern hemlock colonize Chinese hemlock (*T. chinensis*) in forested settings, and what role might replacement with this nonnative species play in buffering against the loss of biodiversity resulting from eastern hemlock mortality?

Arthropods were collected from eastern hemlock, white pine, and Chinese hemlock growing in the Yale-Myers Forest, a working forest owned and managed by the Yale School of Forestry and Environmental Studies. Surveys of these hemlocks have yielded four key patterns: First, arthropod diversity and abundance varies through the year, with eastern hemlock supporting higher numbers of species in the growing season than its cooccurring native softwood, white pine. Second, the composition of these communities differs among eastern hemlock, white pine, and Chinese hemlock. Third, 756 morphospecies of arthropods were detected on eastern hemlock, indicating these softwoods host a large component of forest biodiversity. Fourth, when white pine and eastern hemlock were surveyed in combination, more than half of the species detected on eastern hemlock were found only on eastern hemlock. Adding Chinese hemlock to the survey reduced the number of species found only on hemlock by about half, suggesting that there is potential for Chinese hemlock to buffer arthropod biodiversity in eastern hemlock forests.

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EFFECT OF EXPOSURE TO IMIDACLOPRID ON ASIAN LONGHORNED BEETLE SURVIVAL AND REPRODUCTION

Todd A. Ugine and Ann E. Hajek

Cornell University, Department of Entomology, Ithaca, NY 14853-2601

ABSTRACT

Asian longhorned beetles (ALB), *Anoplophora glabripennis* (Motschulsky), are an invasive species in North America and Europe that threatens urban and natural forests. ALB is currently under federal quarantine in the United States, and eradication programs are underway in several cities. As a preventive measure, the USDA APHIS injects the trunks of known host tree species within quarantine zones with the systemic pesticide imidacloprid. Our laboratory has been developing a biological control method that is synergistic with imidacloprid, so we wanted to understand more about the effect of imidacloprid on ALB adults. A laboratory study was conducted to determine the effect of imidacloprid concentration and delivery method (oral versus contact) on adult beetle survival times. Additional studies were conducted to determine the effect of number of consecutive days of exposure to high doses of imidacloprid on adult ALB survival and the effect of imidacloprid concentration on ALB reproductive parameters.

ALB survival times in the test of exposure method decreased with increasing concentration of imidacloprid when delivered either orally (1µl of 0, 2, 10 or 50 ppm/day for 25 days) or via contact exposure (beetles exposed continuously to 90 mm diam. filter paper treated one time with1.25 ml of 0, 2, 10 or 50 ppm). In the second test, when beetles were given daily oral doses of 1 µl of 50 ppm imidacloprid, the number of beetles surviving for 20 days decreased as the number of consecutive days of treatment was increased from 0 to 2, 3, 4 or 5. The maximum time survived for beetles treated for 5 consecutive days was 13.5 days, assuming the midpoint of an interval as the time of death. In the reproduction test, for female beetles that were mated and fed 1µl imidacloprid solutions of 0, 2, 10, 20, 30, 40 or 50 ppm imidacloprid daily for six weeks, egg production decreased from a mean total of 110 eggs per female at 2 ppm imidacloprid to 70 eggs total at 30 ppm. This was followed by a steep decline in oviposition at 40 and 50 ppm (medians 0-1 eggs per female). Mortality of females treated daily with 0-30 ppm imidacloprid did not vary significantly, whereas most beetles treated with 40 and 50 ppm died within two weeks. This indicates that the reduction in egg production that occurred between 2-30 ppm imidacloprid was the result of sublethal effects of intoxication, whereas the reduced egg production at 40-50 ppm resulted from both lethal and sublethal effects. These results suggest that beetles in the field may be able to recover from imidacloprid intoxication (within-tree concentrations are variable) and contribute to population growth, although at a significantly reduced rate, thus sublethal concentrations of imidacloprid can still contribute to population suppression.

ATTENDEES

22ND USDA INTERAGENCY RESEARCH FORUM ON INVASIVE SPECIES

January 11-14, 2011
Annapolis, Maryland

Christopher Abell
University of Massachusetts
Fernald Hall
Amherst, MA 01003
kabell@psis.umass.edu

Robert Acciavatti
2111 Cherry Street
Morgantown, WV 26505
USDA-FS, Retired
auriebob@comcast.net

Judy Adams
U.S. Forest Service
2650 Centre Ave., Bldg. A
Fort Collins, CO 80526
jadams04@fs.fed.us

Gillian Allard
FAO of the United Nations
Viale Deue Terme di Caracallia
Rome, Italy
gillian.allard@fao.org

Don Allen
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401

Judith Antipin
U.S. Forest Service, Northeastern Area
11 Campus Blvd., Ste. 200
Newtown Square, PA 19073
jantipin@fs.fed.us

Carissa Aoki
Dartmouth College
Gilman Life Sciences Bldg.
Hanover, NH 03766
carissa.f.aoki@dartmouth.edu

Ellen Aparicio
USDA ARS
501 S. Chapel St.
Newark, DE 19713
ellen.aparicio@ars.usda.gov

Matt Ayres
Dartmouth College
Hanover, NH 03766
matt.ayres@dartmouth.edu

Thomas Baker
Pennsylvania State University
105 Chemical Ecology Lab.
University Park, PA 16802
tcb10@psu.edu

Yilmaz Balci
University of Maryland
2114 Plant Sciences
College Park, MD 20742
ybalci@umd.edu

Jay Bancroft
USDA ARS
501 S. Chapel St.
Newark, DE 19713
jay.bancroft@ars.usda.gov

Yuri Baranchikov
V.N. Sukachev Institute
Academogorsk 50/28
Krasnoyarsk, 660036 Russia
baranchikov_yuri@yahoo.com

Roger Barnes
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401

Leah Bauer
U.S. Forest Service
1407 S. Harrison Rd.
East Lansing, MI 48823
lbauer@fs.fed.us

Dick Bean
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
beanra@mda.state.md.us

Jeanna Beard
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401

Joe Beckwith
USDA APHIS
920 Main Campus Dr.
Raleigh, NC 27606
joseph.beckwith@aphis.usda.gov

John Bedford
PO Box 30017
Lansing, MI 48909
bedfordj@michigan.gov

Philip Bell
USDA, APHIS, PPQ
920 Main Campus Dr., Ste. 200
Raleigh, NC 27606
philip.d.bell@aphis.usda.gov

Steve Bell
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401

Bob Bennett
USDA ARS
10300 Baltimore Avenue
Beltsville, MD 20705
robert.bennett@ars.usda.gov

Ken Bloem
USDA APHIS
1730 Varsity Dr.
Raleigh, NC 27606
kenneth.bloem@aphis.usda.gov

Joyce Bolton
USDA ARS, NAL
10301 Baltimore Avenue
Beltsville, MD 20705
joyce.bolton@ars.usda.gov

Jerry Boughton
U.S. Forest Service
11 Campus Blvd., Ste. 200
Newtown Square, PA 19073
jboughton@fs.fed.us

Katalin Boroczky
North Carolina State University
PO Box 7613
Raleigh, NC 27695
katalin.by@gmail.com

Kerry Britton
U.S. Forest Service
1601 North Kent, RPF-7
Arlington, VA 22209
kbritton01@fs.fed.us

Mark Buccowich
U.S. Forest Service
11 Campus Blvd.
Newtown Square, PA 19073
mbucchowich@fs.fed.us

Jim Buck
USDA APHIS
5936 Ford Ct.
Brighton, MI 48116
james.h.buck@aphis.usda.gov

Rose Buckner
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
bucknerm@mda.state.md.us

Russ Bulluck
USDA APHIS
1730 Varsity Dr., Ste. 400
Raleigh, NC 27606
Russ.bulluck@aphis.usda.gov

Stas Burgiel
National Invasive Species Cncl.
1201 Eye St., NW
Washington, DC 20005
stas_burgiel@ios.doi.gov

Susan Burks
Minnesota Dept. Natural Resources
500 Lafayette Rd.
St. Paul, MN 55406
susan.burks@state.mn.us

Barbara Burns
Dept. Forests, Park & Rec.
100 Mineral Street, Suite 304.
Springfield, VT 05156-3168
barbara.burns@state.vt.us

Robert Cain
U.S. Forest Service
740 Simms Street
Golden, CO 80401
rjcain@fs.fed.us

Faith Campbell
The Nature Conservancy
4245 North Fairfax Drive
Arlington, VA 22203
fcampbell@tnc.org

Lidia Carrera
USDA APHIS
4700 River Rd., Unit 160
Beltsville, MD 20737
lidia.carrera@aphis.usda.gov

Sourav Chakraborty
The Ohio State University
2021 Coffey Rd.
Columbus, OH 43210
chakrabouty.27@osu.edu

Paul Chaloux
USDA APHIS
4700 River Rd., Unit 137
Riverdale, MD 20737
paul.chaloux@aphis.usda.gov

Maria Lourdes Chamorro
USDA ARS
PO Box 39012
Washington, DC 20013-9012

Jonathan Chase
National Park Service
7206 National Seashore Lane
Berlin, MD 21811
Jonathan_chase@nps.gov

Robert Chastain
U.S. Forest Service
2222 W 2300 S
Salt Lake City, UT 84119
rchastain@fs.fed.us

Ed Clark
USDA ARS
10300 Baltimore Avenue
Beltsville, MD 20705
ed.clark@ars.usda.gov

Allen Cohen
c/o Dept. Entomology
North Carolina State University
Raleigh, NC 27695
accohen@ncsu.edu

Robert Collignon
State University of New York
1 Forestry Drive
Syracuse, NY 13210
rmcollig@syr.edu

Amanda Conover
University of Delaware
531 S. College Ave.
Newark, DE 19716
aconover@udel.edu

Miriam Cooperband
USDA APHIS
1398 W. Truck Rd.
Buzzards Bay, MA 02542
miriam.f.cooperband@aphis.usda.gov

Robert Coulson
Texas A&M University
HEEP Center, Room 408
College Station, TX 77843
r-coulson@tamu.edu

Damon Crook
USDA APHIS
13978 West Truck Rd.
Buzzards Bay, MA 02542
damon.j.crook@aphis.usda.gov

Donna Crouch
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
crouchdl@mda.state.md.us

John Crowe
USDA APHIS
4700 River Road
Riverdale, MD 20737
john.f.crowe@aphis.usda.gov

Daniel Cucora
State University of New York
1 Forestry Drive
Syracuse, NY 13210
dmcucora@syr.edu

Vince D'Amico, III
U.S. Forest Service
c/o University of Delaware
Newark, DE 19717
vincentdamico@yahoo.com

Kimberly Mae Dean
State University of New York
1 Forestry Drive
Syracuse, NY 13210
kim_mae@hotemail.com

Christopher Dionigi
National Invasive Species Cncl.
1201 Eye Street, NW
Washington, DC 20005
chris_dionigi@ios.doi.gov

Heather Disque
Delaware Dept. Agriculture
2320 S. DuPont Highway
Dover, DE 19901
heather.harmon@state.de.us

Andrea Diss-Torrance
WI Dept. Natural Resources
101 S. Webster St., GEF2 FR-4
Madison, WI 53703
andrea.disstorrance@wi.gov

Mary Ellen Dix
U.S. Forest Service
1601 North Kent St.
Arlington, VA 22209
mdix@fs.fed.us

Kevin Dodds
U.S. Forest Service
271 Mast Road
Durham, NH 03824
kdodds@fs.fed.us

Nate Dodds
Mauget Company
5435 Peck Rd.
Arcadia, CA 91006
nate@mauget.com

Michael Domingue
Pennsylvania State University
119 Chemical Ecology Lab.
University Park, PA 16802
mjd29@psu.edu

Dave Donnelly
36 Brentwood Ave.
Freehold, NJ 07728

Leo Donovall
2301 N. Cameron St.
Harrisburg, PA 17110
ldonovall@state.pa.us

Marla Downing
U.S. Forest Service
2150 Centre Ave., Bldg. A, Ste. 331
Fort Collins, CO 80526
mdowning@fs.fed.us

Jian Duan
USDA ARS, BIIRL
501 S. Chapel St.
Newark, DE 19713
Jian.duan@ars.usda.gov

Don Eggen
Pennsylvania Bureau of Forestry
208 Airport Drive
Middletown, PA 17057
deggen@state.pa.us

Joseph Elkinton
University of Massachusetts
PSIS, Fernald Hall
Amherst, MA 01003
elkinton@ent.umass.edu

Roeland Elliston
USDA APHIS
2150 Vista Ave.
Fort Collins, CO 80506
roeland.j.elliston@aphis.usda.gov

Armando Equihua-Martínez
Colegio de Postfraduados/Monteallo
Edo de Mexico, Mexico
equihua@colpos.mx

Bob Farrar
USDA ARS
10300 Baltimore Ave.
Beltsville, MD 20705
robert.farrar@ars.usda.gov

Heather Finch
Fairfax County Urban Forest Mgmt.
12055 Government Center Pkwy.
Fairfax, VA 22035
heather.finch@fairfaxcounty.gov

Joanna Fisher
Cornell University
6119 Comstock Hall
Ithaca, NY 14850
jjf236@cornell.edu

Joel Floyd
USDA APHIS
4700 River Road
Riverdale, MD 20737
joel.p.floyd@aphis.usda.gov

Joe Francese
USDA APHIS
1398 W. Truck Road
Buzzards Bay, MA 02542
joe.francese@aphis.usda.gov

Ivich Fraser
USDA APHIS
5936 Ford Ct.
Brighton, MI 48116
ivich.fraser@aphis.usda.gov

Kevin Freeman
USDA APHIS
151 West Boylston Dr.
Worcester, MA 01518
kevin.j.freeman@aphis.usda.gov

Roger W. Fuester
USDA-ARS, BIIRL
501 S. Chapel Street
Newark, DE 19713
roger.fuester@ars.usda.gov

Lucie Gagné
Canadian Food Inspection Svc.
7101 rue Jean-Talon Est
Anjou, Québec, Canada H1M 3N7
lucie.gagne@inspection.gc.ca

Lynn Garrett
USDA APHIS
1720 Varsity Drive
Raleigh, NC 27606
lynn.j.garrett@aphis.usda.gov

Ken Gooch
Massachusetts Dept. Cons. & Rec.
99 Cummington Rd.
Worthington, MA 01098
Ken.gooch@state.ma.us

Kurt Gottschalk
U.S. Forest Service
180 Canfield Street
Morgantown, WV 26505
kgottschalk@fs.fed.us

Juli Gould
USDA APHIS
1398 W. Truck Road
Buzzards Bay, MA 02542
juli.r.gould@aphis.usda.gov

William Graham
NASA Applied Sciences
Bldg. 1100, Room 2005G
Stennis Space Center, MS 39529
william.d.graham@nasa.gov

Jerome Grant
University of Tennessee
205 Ellington Plant Sciences Bldg.
Knoxville, TN 37996
jgrant@utk.edu

Matthew Greenstone
USDA ARS
BARC-West, Bldg. 011A
Beltsville, MD 20705
matt.greenstone@ars.usda.gov

Dawn Gunderson-Rindal
USDA ARS
10300 Baltimore Ave.
Beltsville, MD 20705
dawn.gundersen-rindal@ars.usda.gov

Ann Hajek
Cornell University
Dept. Entomology
Ithaca, NY 14853
aeh4@cornell.edu

Jason Hansen
Michigan State University
1407 S. Harrison Rd.
East Lansing, MI 48823
hansen98@msu.edu

Stephen Hauss
Delaware Dept. Agriculture
2320 S. Dupont Highway
Dover, DE 19901
stephen.hauss@state.de.us

Nathan Havill
Yale University-USDA NRI Fellow
51 Mill Pond Rd.
Hamden, CT 06514
Nathan.havill@yale.edu

Dan Herms
The Ohio State University, OARDC
1680 Madison Ave.
Wooster, OH 44691
herms.2@osu.edu

Robert Heyd
Michigan Dept. Natural Resources
1990 US 41 South
Marquette, MI 49855
heydr@michigan.gov

Shelly Hicks
MD Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
hickssd@mda.state.us

E. Richard Hoebeke
Cornell University
Comstock Hall, Rm 2144
Ithaca, NY 14853
Erh2@cornell.edu

Carol Holko
Maryland Department of Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
holkoca@mda.state.md.us

Anne Hoover
U.S. Forest Service
1601 N. Kent St.
Arlington, VA 22209
ahoover@fs.fed.us

Kelli Hoover
Dept. Entomology, 501 ASI Bldg.
Pennsylvania State University
University Park, PA 16802
kxh25@psu.edu

Jing Hu
USDA ARS
10300 Baltimore Ave.
Beltsville, MD 20841
jing.hu@ars.usda.gov

Jiri Hulcr
North Carolina State University
127 Clark Labs.
Raleigh, NC 27695
jhulcr@ncsu.edu

Leland Humble
Canadian Forest Service, NRC
506 W. Burnside Road
Victoria, British Columbia V8Z 1M5
Leland.humble@nrcan.gc.ca

Ren Iwaizumi
1-16-10 Shin-Yamashita, Naka-ku
Yokohama City, Kanagawa-ken, Japan
iwaizumir@pps.maff.go.jp

Bill Jacobi
Colorado State University
Dept. Bioagric. Sci. & Mgmt.
Fort Collins, CO 80523
william.jacobi@colostate.edu

Lisa Jackson
USDA APHIS
1720 Varsity Dr.
Raleigh, NC 27695

Nina Jenkins
Pennsylvania State University
Dept. Entomology
University Park, PA 16802
nej2@psu.edu

Wood Johnson
U.S. Forest Service
2500 Shreveport Highway
Pineville, LA 71360
woodjohnson@fs.fed.us

Derek Johnson
University of Louisiana
PO Box 42451, Dept. Biology
Lafayette, LA 70504
derekjohnson@louisiana.edu

Tappey Jones
Dept. Chemistry
Virginia Military Institute
Lexington, VA 24450
jonesth@vmi.edu

Melody Keena
U.S. Forest Service
51 Mill Pond Rd.
Hamden, CT 06514
mkeena@fs.fed.us

Patrick Kelly
301 Hope Road
Centrevillem MD 21617
pkelly1001@verizon.net

Marc Kenis
CABI
Rue des Grillons 1 CH-2800
Delémont, Switzerland
m.kenis@cabi.org

Ashot Khrimian
USDA ARS
10300 Baltimore Avenue
Beltsville, MD 20705
ashot.khrimian@ars.usda.gov

Natalia Kirichenko
V.N. Sukachev Institute
Academogorsk 50/28
Krasnoyarsk, 660036 Russia
nkirichenko@yahoo.com

Mary Jo Klovensky
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401

Jennifer Koch
U.S. Forest Service
359 Main Road
Delaware, OH 43015
jkoch@fs.fed.us

Jimmy Kroon
Delaware Dept. Agriculture
2320 S. DuPont Highway
Dover, DE 19901
jimmy.kroons@state.de.us

Daniel Kuhar
USDA ARS
10300 Baltimore Avenue
Beltsville, MD 20705
Daniel.kuhar@ars.usda.gov

Ken Kukorowski
Bayer Environmental Science
2 TW Alexander Dr.
Research Triangle Park, NC 27709
ken.kukorowski@bayer.com

Paul Kurtz
NJ Department of Agriculture
692 Maple Ave.
Brick, NJ 08724
pjkurtz@ag.state.nj.us

Bill Laubscher
Pennsylvania Bureau of Forestry
One Nessmuk Lane
Wellsboro, PA 16901
wlaubscher@state.pa.us

Douglas LeDoux
Missouri Department of Agriculture
1616 Missouri Blvd., PO Box 630
Jefferson City, MO 65102
douglas.ledoux@mda.mo.gov

Jonathan Lelito
USDA APHIS
5936 Ford Ct.
Brighton, MI 48116
jonathan.lelito@aphis.usda.gov

Mike Leventry
Longwood Gardens
PO Box 501
Kennett Square, PA 19348
mleventry@longwoodgardens.org

Phil Lewis
USDA APHIS
1398 W. Truck Road
Buzzards Bay, MA 02542
phillip.a.lewis@aphis.usda.gov

Andrew Liebhold
U.S. Forest Service
180 Canfield Street
Morgantown, WV 26505
aliebhold@fs.fed.us

Steve Lingafelter
USDA ARS SEL
PO Box 37012
Washington, DC 20013
steve.lingafelter@ars.usda.gov

Houping Liu
PA Bureau of Forestry
208 Airport Drive
Middletown, PA 17057
hliu@state.pa.us

Jeff Lombardo
Dartmouth College
54 W. College Street
Hanover, NH 03785
jeffrey.a.lombardo@dartmouth.edu

Douglas Luster
USDA ARS
1301 Ditto Avenue
Ft. Detrick, MD
doug.luster@ars.usda.gov

Priscilla MacLean
Hercon Environmental
PO Box 435, Aberdeen Rd.
Emigsville, PA 17318
pmaclean@herconenviron.com

Steve Malan
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
malansc@mda.state.md.us

Mary Kay Malinoski
Maryland Coop. Ext. Svc.
12005 Homewood Road
Ellicott City, MD 21042
mkmal@umd.edu

Robert Mangold
U.S. Forest Service
1601 N. Kent St.
Arlington, VA 2209
rmangold@fs.fed.us

Timothy Marasco
Pennsylvania Bureau of Forestry
208 Airport Drive
Middletown, PA 17057
tmarasco@state.pa.us

Mireille Marcotte
Canadian Food Inspection Agency
59 Camelot
Ottawa, Ontario, Canada K1A 0Y9
mireille.marcotte@inspection.gc.ca

Sarah Marnell
USDA APHIS
1730 Varsity Dr.
Raleigh, NC 27606
sarah.marnell@aphis.usda.gov

Jim Marra
Washington Dept. Agriculture
3939 Cleveland Ave., SE
Olympia, WA 98501
jmarra@agr.wa.gov

Jordan Marshall
Indiana-Purdue Fort Wayne
2101 E. Coliseum Blvd.
Fort Wayne, IN 46805
marshalj@ipfw.edu

Phillip Marshall
Vallonia State Nursery
2782 W. County Rd., 540 S.
Vallonia, IN 47281
pmarhsall@dnr.in.gov

Debra Martin
VA Dept. Agric. & Consumer Affairs
102 Governor St.
Richmond, VA 23219
debra.martin@vdacs.virginia.gov

Phyllis Martin
USDA ARS
10300 Baltimore Avenue
Beltsville, MD 20705
Phyllis.martin@ars.usda.gov

Timothy Martin
Towson University
8000 York Road
Towson, MD 21252-0001
tmarti7@students.towson.edu

Holly Martinson
University of Maryland
4112 Plant Sciences Bldg.
College Park, MD 20742
hmartins@umd.edu

Laura Marx
The Nature Conservancy
25 Main St., Ste. 214
Northampton, MA 01060
lmarx@tnc.org

Victor C. Mastro
USDA, APHIS, PPQ
Bldg. 1398, Otis ANGB
Buzzards Bay, MA 02542
vic.mastro@aphis.usda.gov

Mamoru Matsui
CIQ Government Office
Senshu-Kuko Naka 1
Tajiri, Japan
matsuim@pps.maff.go.jp

Tiffany Rose Mauro
USDA APHIS
1447 Pinewood St.
Rahway, NJ 07065
tiffany.r.mauro@aphis.usda.gov

Albert Mayfield
U.S. Forest Service
1577 Brevard Rd.
Asheville, NC 28806
amayfield@fs.fed.us

Beth McClelland
Virginia Dept. Agriculture
234 W. Shirley Avenue
Warrenton, VA 20186
beth.mcclelland@vadcs.virginia.gov

Deborah McCullough
Michigan State University
1407 S. Harrison Dr.
East Lansing, MI 48823
mccullo6@msu.edu

Richard McDonald
Symbiont, Inc.
194 Shull's Hollar
Sugar Grove, NC 28679
the_edge@goboone.net

Max W. McFadden
The Heron Group, LLC
PO Box 339
Ribera, NM 87560
mcfadden@dca.net

Michael McManus
U.S. Forest Service, Retired
51 Mill Pond Road
Hamden, CT 06514
mlmcmanus@fs.fed.us

Joel McMillin
U.S. Forest Service
2500 S. Pine Knoll Dr.
Flagstaff, AZ 86001
jmcmillin@fs.fed.us

Charles Michler
U.S. Forest Service
715 W. State Street
West Lafayette, IN 47907
cmichler@fs.fed.us

Manfred Mielke
USDA Forest Sevice
1992 Folwell Avenue
St. Paul, MN 55108
mmielke@fs.fed.us

Jocelyn Miller
University of California
900 University Avenue
Riverside, CA 92521
miller@ucr.edu

Bruce Moltzan
U.S. Forest Service
1601 N. Kent Street
Arlington, VA 22209
bmoltzan@fs.fed.us

Erin Morris
Cornell University
6119 Comstock Hall
Ithaca, NY 14853
eem62@cornell.edu

Isabel Munck
U.S. Forest Service
271 Mast Rd.
Durham, NH 03824
imunck@fs.fed.us

Steve Munson
U.S. Forest Service
4746 S. 1900 E.
Ogden, UT 84403
smunson@fs.fed.us

Scott Myers
USDA APHIS
1398 W. Truck Road
Buzzards Bay, MA 02542
scott.w.myers@aphis.usda.gov

Hannah Nadel
USDA APHIS
1398 W. Truck Rd.
Buzzards Bay, MA 02542
hannah.nadel@aphis.usda.gov

Larry Nichols
VA Dept. Agric. & Consumer Affairs
102 Governor St.
Richmond, VA 23219
larry.nichols@vdacs.virginia.gov.

Stephen Nicholson
Valent Biosciences
2704 OrserRd.
Elginburg, Ontario, Canada
K0H 1M0
Stephen.nicholson@valent.com

William Oldland
U.S. Forest Service
180 Canfield Street
Morgantonw, WV 26505
woldland@fs.fed.us

Hideki Okada
1-2-1 Kasumigaseki
Chiyoda-ku,Japan
hideki_okada@nm.maff.go.jp

Jason Oliver
Tennessee State University
472 Cadillac Lane
McMinnville, TN 37110
joliver@tnstate.edu

Forrest Oliveria
U.S. Forest Service
2500 Shreveport Highway
Pineville, LA 71360
foliveria@fs.fed.us

Brad Onken
U.S. Forest Service
180 Canfield Street
Morgantown, WV 26505
bonken@fs.fed.us

Craig Oppel
USDA ARS
501 S. Chapel Street
Newark, DE 19713
craig.oppel@ars.usda.gov

Andrei Orlinski
EPPO
21 boulevard Richard Lenoir
75011 Paris, FRANCE
orlinski@eppo.fr

Mary Orr
Canadian Food Inspection Agency
1124 Finch Avenue, West
Toronto, Ontario, Canada M3J 2E2
mary.orr@inspection.gc.ca

Ronald Overton
U.S. Forest Service
715 West State St.
West Lafayette, IN 47907
roverton@fs.fed.us

J. Patrick Parkman
University of Tennessee
2431 Joe Johnson Dr.
Knoxville, TN 37996
jparkman@utk.edu

Gregory Parra
USDA APHIS
1730 Varsity Drive
Raleigh, NC 27606
greg.parra@aphis.usda.gov

Joshua Pezet
University of Massachusetts
250 Natural Resources Rd.
Amherst, MA 01003
jpezet@cns.umass.edu

Scott Pfister
USDA APHIS
4700 River Road
Riverdale, MD 20737
scott.e.pfister@aphis.usda.gov

Charles Pickett
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401

Sarah Pocock
State University of New York
1 Forestry Drive
Syracuse, NY 13210
srpocock@syr.edu

John Podgwaite
U.S. Forest Service
51 Mill Pond Road
Hamden, CT 06514
jpodgwaite@fs.fed.us

Therese Poland
U.S. Forest Service
1407 S. Harrison Rd.
East Lansing, MI 48823
tpoland@fs.fed.us

Talitha Price
USDA APHIS
1730 Varsity Drive
Raleigh, NC 27606
talitha.price@aphis.usda.gov

Martin Proctor
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401

Derek Puckett
U.S. Forest Service
200 WT Weaver Blvd.
Asheville, NC 28804
dlpuckett@fs.fed.us

Robert Rabaglia
U.S. Forest Service
1601 N. Kent St., RPC-7
Arlington, VA 22209
brabaglia@fs.fed.us

Iral Ragenovich
U.S. Forest Service
PO Box 3623
Portland, OR 97208
iragenovich@fs.fed.us

Karen Rane
University of Maryland
Dept. Entomology
College Park, MD 20742
rane@umd.edu

Brendon Reardon
USDA APHIS
4700 River Road
Riverdale, MD 20737
brendon.reardon@aphis.usda.gov

Richard Reardon
U.S. Forest Service, FHTET
180 Canfield Street
Morgantown, WV 26505
rreardon@fs.fed.us

Christine Rega
University of Delaware
531 S. College Avenue
Newark, DE 19716
christinerega@gmail.com

Brian Rehill
US Naval Academy
121 Blake Road
Annapolis, MD 21402
rehill@usna.edu

James Rhea
U.S. Forest Service
200 W.T. Weaver Blvd.
Asheville, NS 28804
rrhea@fs.fed.us

Kim Rice
MD Department of Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
riceka@mda.state.md.us

Stefan Richard
Sylvar Technologies, Inc.
921 College Hill Rd.
Fredericton, New Brunswick E3B 5A6
srichard@sylvar.ca

John Riggins
Mississippi State University
Box 9775
Mississippi State, MS 39762-9775
johnjriggins@gmail.com

Carlos Rodriguez-Franco
U.S. Forest Service
1601 N. Kent Street
Arlington, VA 22209
crodriguezfranco@fs.fed.us

Jim Rollins
Mauget Company
5435 Peck Rd.
Arcadia, CA 91006
jim@mauget.com

Kristian Rondeau
USDA APHIS
2150 Centre Avenue
Fort Collins, CO 80506
kristian.c.rondeau@aphis.usda.gov

Alain Roques
INRA, 2163 avenue de la Pomme de Pin
CS 40001 - Ardon, 45075 Orleans, CEDEX 2, France
alain.roques@orleans.inra.fr

Krista Ryall
Canadian Forest Service
1219 Queen Street E
Sault Ste. Marie, Ontario, Canada
kryall@nrcan.gc.ca

Cliff Sadof
Purdue University
901 W. State Street
West Lafayette, IN 47907
csadof@purdue.edu

Scott Salom
Virginia Tech
216 Price Hall, Dept. Entomology
Blacksburg, VA 24016
salom@vt.edu

Laura Samson
Alphawood Foundation
2451 N. Lincoln Avenue
Chicago, IL 60614
lsamson@alphawoodfoundation.org

Elisandra Sanchez
USDA APHIS
1447 Pinewood St.
Rahway, NJ 07065
elisandra.sanchez@aphis.usda.gov

Rhonda Santos
USDA APHIS
151 West Boylston Drive.
Worcester, MA 01606
rhonda.j.santos@aphis.usda.gov

Chris Sargent
University of Maryland
4112 Plant Sciences Bldg.
College Park, MD 20742
csargen1@umd.edu

Linda Saunders
USDA ARS
501 S. Chapel Street
Newark, DE 19713
linda.saunders@ars.usda.gov

Taylor Scarr
Ministry of Natural Resources
70 Foster Drive, Ste. 400
Sault Ste. Marie, Ontario P6A 6V5
taylor.scarr@ontario.ca

David Schmit
Pennsylvania Bureau of Forestry
192 North Valley Rd.
Harrisonville, PA 17228
dschmit@state.pa.us

Noel Schneeberger
U.S. Forest Service
11 Campus Blvd., Ste. 200
Newtown Square, PA 19073
nschneeberger@fs.fed.us

Dave Schumacher
Hercon Environmental
PO Box 435, Aberdeen Rd.
Emigsville, PA 17318
dschumacher@herconenviron.com

Keely Shaughnessy
Longwood Gardens
PO Box 501
Kennett Square, PA 19348
kshaughnessy@longwoodgardens.org

Vonnie Shields
Towson University
8000 York Road
Towson, Maryland • 21252-0001
vshields@towson.edu

Greg Shriver
University of Delaware
257 Townsend Hall
Newark, DE 19716
gshriver@udel.edu

Michael Simon
USDA APHIS
4700 River Road, Unit 60
Riverdale, MD 20737
michael.simon@aphis.usda.gov

Nathan Siegert
U.S. Forest Service
271 Mast Road
Durham, NH 03824
nwsiegert@fs.fed.us

Branislav Simic
USDA APHIS
8811 165 Street, 7th Floor
Jamaica, NY 11432
bransilav.v.simic@usda.gov

Dominick Skabeikis
State University of New York
1 Forestry Drive
Syracuse, NY 13210
ddskabeki@syr.edu

James Slavicek
U.S. Forest Service
359 Main Road
Delaware, OH 43015
jslavicek@fs.fed.us

Michael Smith
USDA ARS
501 S. Chapel St.
Newark, DE 19713
michael.smith@ars.usda.gov

Lee Solter
Illinois Natural History Survey
1101 W. Peabody Dr., Rm. 140
Urbana, IL 61801
lsolter@illinois.edu

Fraser Smith
Invading Species Awareness Program
4601 Guthrie Drive, P.O. Box 2800
Peterborough, Ontario Canada
K9J 8L5
fraser_smith@ofah.org

Michael Sparks
USDA ARS
10300 Baltimore Avenue
Beltsville, MD 20705
michael.sparks@ars.usda.gov

Julie Spaulding
USDA APHIS
4700 River Road
Riverdale, MD 20737
julie.s.sparks@aphis.usda.gov

Sven-Eric Spichiger
2301 N. Cameron Street
Harrisburg, PA 17110
sspichiger@state.pa.us

Joseph Spruce
Computer Sciences Corporation
Stennis Space Center
Stennis Space Center, MS 39529
joseph.p.spruce@nasa.gov

Chris Standley
State University of New York
1 Forestry Drive
Syracuse, NY 13210
crstandl@syr.edu

Michael Stefan
USDA APHIS
1730 Varsity Drive
Raleigh, NC 27606
michael.b.stefan@usda.gov

Jim Steinman
U.S. Forest Service
11 Campus Blvd., Ste. 200
Newtown Square, PA 19073
jsteinman@fs.fed.us

Fred Stephen
Dept. Entomology, AGRI 319
University of Arkansas
Fayetteville, AR 72701
fstephen@uark.edu

Sam Stokes
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401

Andrew Storer
Michigan Tech University
1400 Townsend Dr.
Houghton, MI 49931
storer@mtu.edu

Douglas Streett
U.S. Forest Service
2500 Shreveport Highway
Pineville, LA 71360
dastreett@fs.fed.us

Jil Swearingen
National Park Service
4598 MacArthur Blvd. NW
Washington, DC 20007
jil-swearingen@nps.gov

Jon Sweeney
Canadian Forest Service
PO Box 4000
Fredericton, New Brunswick E3B 5P7
jsweeney@nrcan.gc.ca

Daria Tatman
USDA ARS
501 S. Chapel Street
Newark, DE 19713
daria.tatman@ars.usda.gov

Mark Taylor
MD Department of Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
taylormc@mda.state.md.us

Philip Taylor
USDA ARS
501 S. Chapel St.
Newark, DE 19713
philip.taylor@ars.usda.gov

Steve Teale
SUNY ESF
1 Forestry Drive, 241 Illick Hall
Syracuse, NY 13210
sateale@esf.edu

Biff Thompson
Maryland Dept. Agriculture
51 Main Street
Lonaconing, MD 21539
bifft7@verizon.net

John Thompson
4400 Old Grain Highway
Upper Marlboro, MD 20772
ipetrus@msn.com

Christine Thurber
Maryland Dept. Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401

Robert Tichenor
USDA APHIS
4700 River Road, Unit 133
Rivedale, MD 20737
robert.h.tichenor@aphis.usda.gov

Ned Tisserat
Colorado State University
Plant Sciences 1177
Fort Collins, CO 80523
ned.tisserat@colostate.edu

Patrick Tobin
U.S. Forest Service
180 Canfield Street
Morgantown, WV 26505
ptobin@fs.fed.us

Timothy Tomon
West Virginia Dept. Agriculture
1900 Kanawha Boulevard, East
Charleston, WV 25305-0170
ttomon@wvda.us

Matthew Travis
USDA APHIS
2200 Broening Highway, Ste. 140
Baltimore, MD 21224
matthew.a.travis@aphis.usda.gov

Joe Tropp
USDA ARS
501 S. Chapel Street
Newark, DE 19713
tropp@udel.edu

R. Talbot Trotter
U.S. Forest Service
51 Mill Pond Road
Hamden, CT 06514
rttrotter@fs.fed.us

Robert Trumbule
MD Department of Agriculture
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
rtrumbule@erols.com

Todd Ugine
Cornell University
Comstock Hall
Ithaca, NY 14853505
todd.ugine@gmail.com

Roy VanDriesche
University of Massachusetts
Fernald Hall
Amherst, MA 01003
vandries@cns.umass.edu

Trang Vo
USDA APHIS
4700 River Road
Riverdale, MD 20737
trang.t.vo@aphis.usda.gov

Joseph Vukovich
USDA APHIS
50 Harry S. Truman Pkwy.
Annapolis, MD 21401
joseph.m.vukovich@aphis.usda.gov

David Wakarchuk
Synergy Semiochemicals Corporation
7061 Merritt Avenue
Burnaby, British Columbia V5J 4R7
david@semiochemical.com

Kimberly Wallin
University of Vermont
321 Aiken Center
Burlington, VT 05405
kwallin@uvm.edu

Ronald Weeks, Jr.
USDA APHIS
920 Main Campus Drive, Ste. 200
Raleigh, NC 27606
rdweeks@aphis.usda.gov

Geoff White
USDA ARS
10300 Baltmore Avenue
Beltsville, MD 20705
Geoffrey.white@ars.usda.gov

Mark Whitmore
Cornell University
Fernow Hall
Ithaca, NY 14853
mcw42@cornell.edu

Jeffrey Wildonger
USDA ARS
501 S. Chapel Street
Newark, DE 19713
jeffrey.wildonger@ars.usda.gov

Dave Williams
USDA APHIS
Bldg. 1398
Otis ANGB, MA 02542
david.w.williams@aphis.usda.gov

Jinquan Wu
USDA ARS
501 S. Chapel Street
Newark, DE 19713
jinquanw@udel.edu

Wei Xian
State University of New York
1 Forestry Drive
Syracuse, NY 13210
wxian04@syr.edu

James Young
USDA APHIS
2200 Broening Highway, Ste. 140
Baltimore, MD 21224
jim.d.young@aphis.usda.gov

Aijun Zhang
USDA ARS
10300 Baltimore Avenue
Beltsville, MD 20705
aijun.zhang@ars.usda.gov

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