Movement of mature trees by man, an unexpected pathway for invasion of the urticating pine processionary moth

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24th Edition

New for 2013!

USDA INTERAGENCY RESEARCH FORUM on INVASIVE SPECIES

January 8-11, 2013 at the Loews Annapolis!

"Urban crawl"

USDA United States Department of Agriculture

Forest Service

FHTET-13-01
March 2013

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24TH
USDA Interagency Research Forum on Invasive Species

January 8-11, 2013
Annapolis, Maryland

Compiled by:

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FOREWORD

This meeting was the 24th 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 proceeding 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

International in scope, this meeting attracts a diverse audience of scientists and managers from North America and abroad because we are sharing invasive species issues that are common to all of us. In addition to presentations by scientists from agencies within USDA, presentations were made by scientists from France, Italy, Great Britain, Austria, Russia, and Canada and included representatives from the following:

- Food and Agriculture Organization (FAO), Rome
- European & Mediterranean Plant Protection Organization (EPPO), Paris
- Foreign Agricultural Service
- National Research Council, Board on Agriculture and Natural Resources
- Russian Forestry, Siberian Branch
- FS-International Forestry
- Embassy of Peru, Agricultural Attaché
- Costa Rica
- Tribal Enterprises, WI
- Canadian Food & Inspection Service, Natural Resources Canada, Canadian Forest Service

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PRESENTATIONS

Keynote Address

AMERICAN CHESTNUT: ITS PAST, PRESENT, AND FUTURE

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ABSTRACT

The loss of the American chestnut (*Castanea dentata*) to chestnut blight in the early half of the 20th century had monumental impacts to the ecosystems the species inhabited. The tree's range extended from Maine and Ontario in the north to Georgia, Alabama and Mississippi in the south, an almost contiguous area, especially through the central Appalachians. The tree comprised almost 25% of the eastern forest providing wood and yearly crops of nuts for man and animals alike. The species was without rival in growth, especially on poorer sites where other species struggled. American chestnut was such a significant part of people's lives that numerous songs and poems were written about it. Who hasn't sung the lyrics of Mel Torme's "*Chestnuts roasting on an open fire*"? Of course, the fate of the American chestnut changed rapidly with the introduction of *Cryphonectria parasitica*, a fungus of Asiatic origin that rapidly spread through eastern North America, all but eliminating chestnut as an eastern forest tree. Unfortunately, *C. parasitica* was introduced before there was an appreciation of the damage that could result from microorganisms of exotic origin. Despite early attempts to slow the invading fungus, only a few years passed before scientists acknowledged that the spread of the fungus could not be halted. Early research by scientists with the USDA and The Connecticut Agricultural Experiment Station attempted to breed blight resistant trees by crossing the American chestnut with its Asiatic relatives. This work too was abandoned as many of the offspring were more Asiatic in their growth than that of the more desirable American species.

This initial period of discouraging results was followed by decades where little attention was given to the species. Most individuals assumed chestnut's future already had been cast and that the species would remain as an understory shrub surviving only by the perpetually sprouting stems that too would succumb to the blight. However, the sense that the species was lost would change in the 1970s with the report that European chestnut (*Castanea sativa*) growing in areas of Italy were recovering from blight. While the disease was not as devastating in Europe as North America, it also took a significant toll on their chestnut resource. Apparently, recovery in Italy was due to a disease of the blight fungus, a disease now known to be caused by a fungal virus. The recovery phenomenon in Italy, termed "hypovirulence," has become pervasive in much of the chestnut growing regions of Italy. The causal virus has its origin in the native Asiatic range of the fungus. Since the initial findings of this unique biological control phenomenon, considerable research has lead to the discovery of additional viruses, including several within the North American chestnut range, particularly Michigan. Numerous viruses capable of reducing the virulence of the blight fungus have been sequenced and their biology recorded including the effects they have when infecting the blight fungus. Unfortunately, none of the attempts to establish the phenomenon of hypovirulence in North America by artificially introducing the viruses have resulted in biological control of the blight on a large scale as has occurred naturally in Italy. Considerable coordinated research by several institutions continues in an effort to utilize this unique biological control mechanism.

The interest spurred by the discovery of European chestnuts that were recovering from blight prompted other scientists to again consider if breeding blight resistant chestnuts was a viable option. Many decades had passed since the initial blight epidemic and the early 20th century breeding efforts, and significant advances in plant breeding have occurred since those initial breeding trials . This renewed interest resulted in a reevaluation of the early breeding work in light of contemporary genetics. The method of backcross breeding that had been developed and utilized for most crop plants was considered. This methodology subsequently was employed to reestablish a breeding program with the goal of developing blight resistant chestnuts with the characteristics of the American chestnut. The program led to formation of The American

Chestnut Foundation. This non-profit organization is now over 25 years old and has chapters in many states that contribute to the breeding and evaluation process. The backcross breeding approach involves crossing blight resistant Asiatic species with American chestnut to produce a hybrid. Another American tree then is crossed to the hybrid resulting in a "backcross" tree that is three-quarters American. By repeatedly backcrossing to an American parent, the offspring become increasingly American in the traits they possess. The goal is to produce trees that contain the genes for resistance from the Asiatic species but the timber-like growth form of the American. The difficulty is that the process requires each generation of offspring be screened for their resistance to blight; many are not resistant. The time to produce successive generations and to screen each of hundreds offspring can be significant and laborious. Current research includes development and utilization of molecular procedures that would permit the identification of progeny with appropriate resistance genes when the seedlings first emerge from the nut. This would shorten the screening time significantly by eliminating the need to grow trees for several years before they can be tested for resistance by inoculation with the blight fungus.

Unfortunately, chestnut is not without other problems. When chestnut blight was taking its toll, another non-native pathogen also was active in the southern Appalachians. This soil-borne, root-invading pathogen, *Phytophthora cinnamoni*, has a wide range of hosts, including the very susceptible American chestnut. Warm southern climates seem to favor its activity. This organism has been so damaging that it too must be evaluated with respect to the breeding program for blight resistance. Fortunately, there appears to be resistance to *P. cinnamoni* associated with the Asiatic species. A third non-native organism also has entered the picture. The Asian chestnut gall wasp, *Dryocosmus kuriphilus*, is rapidly spreading throughout the range of American chestnut. The infesting insect damages foliage, twigs and the developing fruit. Control of this insect pest may be through the use of gall wasp parasites. This insect also is damaging large acreages of chestnut in Europe.

Two very useful chestnut references that contain both general historic information and specific technical discussions are listed below.

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PRESENTATIONS

Research Reports

DEVELOPING A SYSTEMS APPROACH TO REDUCING THE RISKS OF INTRODUCTION OF INVASIVE ALIEN SPECIES

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ABSTRACT

The international movement of pests occurs overwhelmingly via trade pathways, almost regardless of the goods being traded. This fact is recognized in the phytosanitary measures applied through national and regional legislation worldwide. To prevent the entry of quarantine pests, potential pathways may be prohibited or require particular measures to ensure pest freedom. For example, wood packaging used to transport goods is regulated under the International Standard for Phytosanitary Measures (ISPM) No 15 in which heat treatment or fumigation is approved to ensure freedom from pests. Whilst such procedures are generally applied to eliminate certain known pests, they also have the advantage of killing other unknown pest organisms that might be present.

Other pathways, notably plants for planting, generally cannot be treated using one direct intervention to remove all pests. Indeed, there is increasing evidence that the plants for planting pathway, in which living plants often with soil still attached to the roots are moved globally, is responsible for many of the recent establishments of high-impact pests around the world. This is particularly apparent for ash dieback, caused by the fungus *Chalara fraxinea* where movement of infected nursery plants is the principal known pathway; the fungus has spread rapidly across Europe with devastating effects on native ash species. Clearly application of treatments such as heat or fumigation to living plant material is generally not possible and therefore, in such cases, two or more independent risk management measures need to be applied to reduce pest prevalence. These measures will be most effective if they follow a Systems Approach (SA) based on ISPM 14 and the recently published ISPM 36 that deals specifically with plants for planting. There are currently no internationally accepted SAs for specific pathways for forest pests, but ISPM 35 - Systems approach for pest risk management of fruit flies (Tephritidae) – provides a useful exemplar of how an SA has been developed for a well characterized pest.

SAs tend to be created on a bilateral basis so they can take into account all the factors specific to the pathway at origin, in transit and in the importing country. SAs are developed by first evaluating how pests become associated with the pathway and, in a sequential approach, how a combination of different direct processes, inspection regimes at critical control points and indirect processes can reduce risk to acceptable levels by the time the pathway reaches its end point and associated goods are delivered to end users. SAs that strictly or loosely follow ISPM 14 are already in place or are being developed in countries such as Australia, Canada, New Zealand, the USA and the EU, but there is still a pressing need to link the ecology of pest invasion to the practicalities of risk reduction. The SA is proposed as the most effective way forward of ensuring pest freedom with minimal impact on trade. We will present an overview of measures that can be applied within a SA for the regulation/prevention of movement of pests associated with live plants that are traded internationally.

UPDATE OF EPPO ACTIVITIES IN FOREST QUARANTINE IN 2012

Andrei D. Orlinski

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ABSTRACT

In 2012, the revision of the EPPO Standard PM 9/1 "Official control of Pine Wood Nematode" was accepted by the EPPO Working Party for Phytosanitary Regulations and adopted by the EPPO Council. In EPPO countries (there are 50 member countries in the organization), Pine Wood Nematode (PWN) *Bursaphelenchus xylophilus* is established in Portugal, and several incursions observed in Spain and are under eradication. PWN is sometimes intercepted in imported consignments.

The draft EPPO standards of series PM 9 "National Regulatory Control System for *Agrilus planipennis*", "National Regulatory Control System for *Anoplophora glabripennis*" and "National Regulatory Control System for *Anoplophora chinensis*" were revised in 2012 and sent to country consultation. The country comments will be discussed by the EPPO Panel on Quarantine Pests for Forestry and then presented to the EPPO Working Party for Phytosanitary Regulations. The aim of these standards is to provide guidance to EPPO countries on measures at the detection of the pests: clear-cut zones, detection and delimiting surveys, sampling, etc.

EAB (*Agrilus planipennis*) is continuing to spread in Russia from its original introduction in Moscow. Two new parasitoid species of EAB have been described: *Spathius galinae* and *Sclerodermus pupariae*. CLB (*Anoplophora chinensis*) was eradicated in Croatia, France, Germany and Netherlands; is under eradication in Denmark, Guernsey, Italy, Switzerland and the United Kingdom. CLB is regularly intercepted in imported plants for planting (originating mainly from China). ALB (*Anoplophora glabripennis*) was eradicated in Belgium and Netherlands; is under eradication in Austria, France, Germany, Italy, Switzerland and the United Kingdom. ALB is regularly intercepted in wood packaging material accompanying imported consignments (originating mainly from China). An International Symposium: '*Anoplophora chinensis & Anoplophora glabripennis*; new tools for predicting, detecting and fighting - how to save our forests and our urban spaces' was organized by EPPO in Italy in May 2012; the main topics were the prediction/detection tools and the control strategies developed against these two *Anoplophora* species.

In 2012, a number of forest pests (*Ips amitinus, Ophiostoma ulmi, Pseudomonas syringae pv. aesculi, Paysandisia archon, Blueberry scorch virus, Colletrotricum acutatum, Cryphonectria parasitica, Glycaspis brimblecombei, Phytophthora austrocedrae, Phytophthora ramorum, Cylindrocladium buxicola, Cameraria ohridella, Mycosphaerella pini, Chalara fraxinea, Aromia bungii, Dryocosmus kuriphilus, Ips cembrae, Mycosphaerella dearnessii, Phytophthora ramorum, Aproceros leucopoda* and *Thaumastocoris peregrinus*) were reported in 2012 in new areas or on new hosts.

Pest Risk Analysis was performed in 2012 by EPPO Expert Working Groups for *Oemona hirta* (Lemon Tree Borer intercepted in the United Kingdom on plants for planting imported from New Zealand) and *Polygraphus proximus* (Four-eyed Fir Bark Beetle recently spread to Western Siberia and European Russia from the Far East).

INTERACTIONS BETWEEN INVASIVE TAMARISK, INTRODUCED TAMARISK BEETLES, ENDANGERED SPECIES PROTECTION, AND RIPARIAN RESTORATION IN THE CHIHUAHUAN AND SONORAN DESERTS

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ABSTRACT

Four species of tamarisk beetles (*Diorhabda* spp.) introduced for biological control of tamarisk (*Tamarix ramosissima/T. chinensis*) in arid and semiarid riparian habitats of western North America are dispersing and producing widespread defoliation and dieback of tamarisk. Active riparian restoration may be needed in some tamarisk woodlands to ensure return of native plant biodiversity providing desired ecosystem services, including mitigation for loss of services provided by tamarisk. Restoration of cottonwoods and willows is planned to replace bird nesting habitat lost to tamarisk biological control and protect the endangered southwestern willow flycatcher (*Empidonax traillii extimus*, flycatcher). Projections of the timing and locations of interactions between the four species of tamarisk beetles and flycatchers are being developed through tamarisk beetle continental species distribution models linked to cost-distance connectivity dispersal models. At selected sites where tamarisk beetles and flycatchers should interact, patch-level flycatcher Habitat Suitability Index (HSI) models are being developed to simulate impacts of tamarisk beetles on flycatcher habitat, and plan the restoration of cottonwood/willow woodland needed to mitigate habitat loss. Patch-level HSI models for cottonwoods, willows and other native riparian plants are proposed to facilitate site specific restoration planning to follow up tamarisk biological control.

HOW PATTERNS OF MOVEMENT, REFUGIA, AND WOODY HOSTS CREATE THE PERFECT STORM FOR THE INVASIVE BROWN MARMORATED STINK BUG, *HALYOMORPHA HALYS*

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ABSTRACT

The Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål) (BMSB)(Hemiptera: Pentatomidae), is an exotic invasive stink bug native to China, Japan, Korea, and Taiwan that was first observed in the US in the mid-1990s. BMSB is an occasional pest of soybeans and many fruit trees in Asia; however, in North America it was first recognized as a nuisance pest when it entered homes in large numbers in the autumn. In 2001 BMSB was identified as a new invasive species by E. Richard Hoebeke, entomologist, taxonomist and assistant curator of the Cornell University Insect Collection. Hoebeke found a match for the new stink bug among specimens collected from Harima, Japan, in 1916 (Hoebeke and Carter 2003). Reports soon followed that BMSB was feeding on ornamental plants, vegetables in residential and commercial gardens, fruit and shade trees in suburban and urban landscapes, and in vineyards. Initial fears that BMSB would become a significant agricultural pest in this country were confirmed when it appeared in field and row crops, vegetables, orchards, vineyards, and ornamental plant nurseries in many states. In 2010, crop losses due to BMSB feeding damage and management costs were in the tens of millions of dollars (Leskey et al. 2012). Since confirmation of BMSB as a resident in Pennsylvania, the insect has spread to 39 states in the US and several provinces in Canada. BMSB is a strong flyer and opportunistically hitchhikes on or in vehicles. This likely contributes to the rapid spread of this pest (Sargent et al. 2012).

The association between forests, trees in managed landscapes, and BMSB is yet to be fully understood. However, since its discovery in the U.S. more than a decade ago, much has been learned about the relationships between woody plants and BMSB. The proclivity for BMSB to overwinter inside homes and other manmade structures is well known (Inkley 2012). However, manmade structures clearly were not a historical factor in shaping the overwintering behavior of BMSB. The quest to discover the natural hibernacula of BMSB has been spearheaded by a research group at USDA-ARS led by Tracy Leskey. By sampling hundreds of trees as well as rocky outcroppings and plots of leaf-litter in forests, they evaluated potential winter refuges for BMSB. They discovered the prime hideout for BMSB populations were large (> 19 cm dbh), dry dead standing trees with loose and peeling bark. BMSB wedged their bodies beneath the bark of these porous trees or inside the decomposed woody tissue. Favored host genera included species of *Quercus* (oak), *Robinia* (locust), *Ailanthus* (tree of heaven), and *Ulmus* (elm). Rocky outcroppings are also likely to provide refuges for BMSB; however, the contribution of this resource to BMSB's overwintering sites is yet unknown. Surprisingly, while several species of native stink bugs were found in leaf litter, BMSB was not one.

With the return of warm weather in spring, BMSB emerges from overwintering sites and moves to vegetation to feed prior to laying eggs of the first summer generation. This highly mobile pest readily moves between trees, shrubs, and herbaceous plants to feed on leaves, stems, and developing seeds and fruits (Funayama 2004, Leskey et al. 2012). One important aspect of assessing risks to agronomic crops related to

attack by BMSB is to establish patterns of early season host use in trees and shrubs surrounding agricultural fields. Kathy Kamminga and her colleagues at Virginia Tech conducted timed surveys of woody plants adjacent to field and row crops in several locations in Virginia. They found BMSB utilized seven genera of trees native to North America: *Acer* (maple), *Catalpa* (catalpa), *Cercis* (redbud), *Juglans* (walnut), *Magnolia* (magnolia), *Morus* (mulberry), *Prunus* (cherry), *Robinia* (black locust), and three exotic genera of trees *Ailanthus* (tree of heaven), *Albizzia* (mimosa), and *Paulownia* (empress tree) and one exotic genus of shrub, *Lagerstroemia* (crepe myrtle) as sources of food and oviposition sites. Clearly, woodlots populated with these trees and shrubs increase the likelihood of subsequent infestations of BMSM to adjacent field and row crops.

Recently, we observed large numbers of BMSB amassing on the trunks of landscape trees in autumn just prior to their entry into homes. In addition to resting on tree trunks, BMSB probed and consumed carbohydrate rich tissues beneath the bark (Martinson et al. 2013). In wholesale plant nurseries and managed landscapes we noticed wide variation in the types of trees on which BMSB aggregated. This variation prompted us to investigate patterns of host utilization by different life stages of BMSB using trees commonly grown and installed by the landscape industry. Our goal was to identify species and cultivars of trees used and not used by BMSB for feeding and oviposition. Ultimately, we will make recommendations to nursery growers, landscapers, and citizens as to which plants do not support populations of BMSB. By reducing the number of susceptible hosts in the landscape we can reduce the numbers of BMSB invading homes and thereby reduce its nuisance potential. Moreover, we hope to greatly reduce the need or desire to treat BMSB infested trees in autumn with pesticides. By reducing these inputs, we hope to avoid disruptive effects associated with pesticide use and create more sustainable urban forests.

To these ends, we conducted surveys of juvenile and adult BMSB across a wide range of potential landscape tree hosts planted at a large (300 acre) wholesale nursery in Maryland. Between 2 June and 10 October 2011, we sampled 178 cultivars of trees (2006 trees total) on nine sampling dates. During this interval more than 13,400 stink bugs and egg masses were observed. Sampling was spatially stratified to quantify edge effects and the dynamics of movement into the nurseries. We found marked variation in stink bug counts among tree species and cultivars, as a function of time, and based on location within the nursery. BMSB reached higher densities on plants at the edges of fields and planting blocks compared to those in the centers of the fields and planting blocks. The strength of the edge effect depended on the type of habitat nearby. Trees closest to a soybean field infested with BMSB housed greater numbers of stink bugs than those adjacent to a turf farm where BMSB was uncommon. In 2011, abundance of BMSB was greatest early in the season from June to early August but declined dramatically thereafter. Most importantly we observed dramatic differences in the abundance of BMSB on different types of host plants. These differences were life stage dependent. BMSB was found on 150 (84%) of the plants studied. Adults showed the greatest breadth of host use with occupation of 145 cultivars. Nymphs were somewhat less catholic and found on 112 varieties. The placement of eggs by females was most restrictive with only 63 varieties of trees serving as oviposition sites. All life stages of BMSB were absent during the entire course of study for 28 varieties of woody plants. This list was included several varieties on native and non-native trees including , *Picea* (spruce), *Acer* (maples), *Thuja* (arborvitae), and *Cedrus* (cedars), and representatives from other genera many of which were gymnosperms. Among the favored varieties used by nymphs and adults for feeding and adults for egg-laying were cultivars in the genera *Syringa* (lilac), *Acer* (maple), *Cercis* (redbud), *Cladrastis* (yellowwood) , *Plantanus* (sycamore), *Prunus* (cherry), *Nyssa* (black gum), *Evodia* (bee tree), and *Tilia* (linden) (Bergmann in prep.)

FUTURE PLANS

In 2012, we expanded our research to a second wholesale nursery with 183 cultivars and we are now in the process of analyzing these data. We will conclude our field studies after the 2013 growing season. We have also gathered data on tissue specific patterns of host use within plants and will correlate patterns of abundance with those of plant phenology such as bud break and fruit production. Future studies will elucidate the strength of the match between patterns of abundance observed in the field and host suitability. We will also evaluate the influence of host origin (native vs. non-native) on the preference and performance of BMSB.

SUMMARY

These studies revealed several factors important to the life history of BMSB. First, we know that large, dry, standing dead trees provide a critical overwintering resource for BMSB. As they move from the forest, a diverse collection of native and non-native trees and shrubs serve as early season hosts for overwintered adults. Homesites adjacent to agricultural fields that support populations of BMSB may be the ones most heavily colonized by BMSB. Finally, landscape trees vary greatly in their utility as food sources and oviposition sites for BMSB. Several common genera including *Syringa, Acer, Cercis*, and *Prunus* are heavily used by feeding and non-feeding stages and others such as *Picea* and *Cedrus* are used infrequently or not at all. This information identifies some of the risk factors associated with BMSB infestations and will provide guidance for designing landscapes refractory to this invasive pest.

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IMPROVEMENTS IN THE TRAPPING SYSTEM TO DETECT ASIAN LONGHORNED BEETLES USING A COMBINATION OF PHEROMONES AND PLANT VOLATILES

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ABSTRACT

Development of an effective trapping system to detect *Anoplophora glabripennis* (Motschulsky), the Asian longhorned beetle (ALB), has been a goal of the USDA eradication program since beetles were discovered in New York City in 1996. ALB is under eradication where it has been found in the United States and any tree found to have signs of the beetle is cut and chipped. To detect infested trees, the program uses ground surveyors and tree climbers to search for oviposition pits and exit holes. However, ground surveys are 30% effective at best and climbing is labor intensive and expensive. Trapping will provide a cost effective, efficient means to guide surveys to infested trees within large geographic areas.

A previously identified two-component male-produced pheromone (MP) that consists of an aldehyde, 4-(n-heptyloxy)butanal, and an alcohol, , 4-(n-heptyloxy)butan-1-ol, in a 1:1 ratio is used in combination with a mixture of five plant volatiles, (±)-linalool, *cis*-3-hexen-1-ol, linalool oxide, *β*-caryophyllene, and *trans*-pinocarveol to bait Intercept ™ panel traps. Previous work in both China and the quarantine zone around a relatively large ALB infestation in Worcester, MA had shown that the trap and lure combination was effective at detecting ALB, primarily by trapping virgin females. The goal of our 2011-12 research was to test low and high release rates of MP and 3-4 plant volatile combinations in a large-scale deployment of traps across previously infested, surveyed and unsurveyed areas throughout the Greater Worcester, MA (Worcester, Boylston, West Boylston, Holden, Shrewsbury, Auburn) quarantine area to detect ALB. Traps were hung in trees by throwing a paracord line over a limb and using cable ties to attach the line to the trunk or a branch. Lures for baiting the traps were prepared by either Synergy or Chem Tica and were replaced every 4-6 weeks based on component elution rates. Treatments were laid out in a sequence that was repeated that ensured that every lure treatment would be equally represented and adjacent to all possible lure combinations. Traps were coated with 10% Fluon ® making them more slippery which has been shown to enhance trap captures and a saturated salt solution was used as a killing and preserving agent in the collection. Captured insects were collected every two weeks from early July through September. When an ALB was trapped, the eradication program staff was informed and a survey team was deployed to check for infested hosts near the positive trap. In 2011, hosts within an 80 m radius of each positive trap were checked but the search ended when a tree with an exit hole was found. In 2012, hosts within a 200 m radius of positive traps were checked for signs of ALB infestation.

In 2011, a total of 500 traps were deployed 100 m apart in a spatially balanced design that consisted of 10 lines (50 traps each); one line through the Worcester core area and the other 9 arrayed like spokes radiating out from the core area. A total of 23 beetles were caught in 2011, 21 females and 2 males. A total of 8 were captured in traps baited with a mixture of 3 plant volatiles, 6 were captured with a mixture of 4 plant

volatiles and MP at a low release rate, 4 were captured with a mixture of 4 plant volatiles and MP at a high release rate, 5 were captured with the original mixture of 5 plant volatiles and a low MP release rate, and none were captured in unbaited control traps. Males were caught only in traps baited with the first 2 lure types. Four of the beetles that were trapped were caught in traps hung on ALB non-host trees, including choke cherry, shagbark hickory and white oak. The high release rate of the male pheromone seemed to increase beetle catches in non-host trees even though the ratio of its aldehyde alcohol components was not 1:1. Removal of *trans*-pinocarveol and linalool oxide from the mixture of plant volatiles and altering ratios of the other 3 plant volatiles requires further evaluation to determine its effect on trap catches. In the core area, where several trees with up to 10 exit holes were found (either in the 80 m or subsequent surveys) within a 200 m radius of positive traps, beetles were only captured in traps baited with the mixture of 4 or 5 plant volatiles and MP released at the low rate even though traps baited with the other lures were also present within 200 m of the infested trees. No infested trees have since been found within a 200 m radius around 7 of the 21 positive traps and the average distance from the remaining positive traps to an infested tree with exit holes was ~100 m.

In 2012, a total of 392 traps were deployed in the greater Worcester area in locations where lingering ALB populations were suspected and where the risk of ALB infestation was higher. Trap line length was variable since they were deployed in a targeted design but they were still placed 100 m apart. A total of 8 beetles were caught in 2012, all were females and all were captured in traps hung on maple trees. Two beetles were captured in traps baited with MP alone released at the high rate used in 2011, 2 were captured with a mixture of 3 plant volatiles and MP released at the same high rate, 4 beetles were captured with a mixture of 3 plant volatiles and MP released at a higher rate (5x the high rate used in 2011), and none with MP alone at the higher rate or with the mixture of 3 plant volatiles alone. No infested trees were found within 200 m of 3 of the 9 positive traps and the average distance from the remaining positive traps to a tree with exit holes was ~140 m. The furthest distance from a positive trap to the closet tree with beetle signs (either with oviposition pits or exit holes) was ~120 m. In some locations where no infested trees were found it was more than 200 m in one direction from the trap to the nearest trees, so a wider radius may need to be surveyed in these cases. Increasing the release rate of MP to about 2 mg/day (higher rate in 2012) appears to improve trap catch, especially as the population of ALB continues to decline in the eradication zone (only 13 beetles were seen in the greater Worcester area in 2012, the majority caught in traps).

In 2012, traps were also deployed in Harbin, China to evaluate MP release rates and ratios of the two components in combination with the mixture of 3 plant volatiles. MP lure treatments (alcohol: aldehyde ratios) were: 1:1, 4:4, 8:8, 1:4, 1:8, 8:1, 4:1 and 0:0, where the number represents the mg/day. All MP lure treatments were combined with the mixture of 3 plant volatiles and compared to a control (no lure). There were 10 replicates of each treatment and traps were checked and treatment positions rotated every 3 days (27 days total). A total of 43 beetles were trapped, 25 females (mostly virgin) and 18 males. Nineteen of the beetles (4 males and 14 females) were trapped in treatments where the MP ratio was 1:1 (combining all MP release rates) and 6 males were caught in traps baited with the mixture of 3 plant volatiles alone. Two females were trapped in control traps in locations where the traps were spaced at 10-20 m apart, but none were captured in control traps at locations where the traps were spaced at 50 m apart, suggesting there could be some between lure interference when traps are too close together. Similar numbers of beetles were caught in traps baited with MP at the 1:1 and 4:4 ratios and only one was caught in traps with MP at the 8:8 ratio. This suggests that an MP release rate of 1-4 mg per day (1:1 ratio) is good but higher rates are not. Traps baited with the mixture of 3 plant volatiles alone captured males in China where ALB population levels are higher but plant volatiles alone has not caught males in Worcester where ALB population levels are lower.

A lure releasing MP at 2 mg/day in a 1:1 ratio with 3 or 4 plant volatiles appears to be the best current option for trapping ALB when it is present at low population levels. This trap and lure may be useful for presence/absence delimiting surveys, for checking quality control after removal of infested trees, and potentially for early detection around high risk sites. In 2012, traps baited with MP at 1 mg/day in a 1:1 ratio with 3 plant volatiles were also deployed in 15 states by federal or state agencies or by the Sentinel Plant Network arboreta and by groups in 3 other countries. The only other ALB captured were in known infested

areas. Additional research needs to be done to further refine the lures (particularly the plant volatiles used) and the companies that produce the emitters are still working to improve the elution rates and longevity of some lure components. In 2012 beetles were observed walking on some traps; therefore, alternative trap designs and the optimal Fluon® coating for traps will be evaluated.

FIELD TRAPPING OF EUROPEAN OAK *AGRILUS* SPECIES USING NANOFABRICATED BEETLE DECOYS

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ABSTRACT

An overview of recent progress in developing trapping approaches for several European oak buprestid species is provided. In field observations it had been determined that males of three such species, *Agrilus biguttatus* Fabricius, *Agrilus angustulus* Illiger, and *Agrilus sulcicollis* Lacordaire, are visually attracted to pinned dead *Agrilus* specimens placed on leaves (Domingue et al., 2011). Furthermore there was substantial attraction to pinned specimens of several different species, including the three species described above, as well as *Agrilus planipennis* Fairmaire (EAB) and *Agrilus cyanescens* Ratzeburg. *A. biguttatus* males were most aggressive in such approaches and were also observed attempting to copulate with the pinned dead females, but only conspecifics or EAB.

Given this demonstrated attraction of European oak buprestids to pinned EAB "decoys", in 2011 field trapping was performed incorporating such decoys into the trap design. The most effective trap devised was a cardboard structure wrapped around the leaves of a branch, with two green plastic surfaces (5×9 cm^2), each presented at a 45 degree angle (Domingue et al., 2013). In the trapping experiments for this year, 1962 buprestid specimens, from14 species, and 9 from the genus *Agrilus,* were caught on 178 traps in a 22-day time-span. EAB decoys significantly increased captures of total numbers of buprestids, and many of the individual species. Semiochemical lures, including manuka oil, (Z)-3-hexen-1-ol, and (Z)-9-tricosene also led to greater numbers of buprestids trapped. The visual decoy was particularly important for the most serious pest detected, *Agrilus biguttatus* Fabricius, which was captured 13 times on traps with decoys, but only once without a decoy. Surprisingly females of this species were also frequently caught on such decoy traps.

In 2012, through a bioreplication approach, we fabricated artificial visual beetle decoys. We used a dead female EAB to make a negative die of nickel and a positive die of epoxy. Decoys were then completed by first depositing a quarter-wave-stack Bragg reflector on a polymer sheet and then stamping it with a pair of matched negative and positive dies to take the shape of the upper surface of the EAB female (Pulsifer, 2013). A field experiment was conducted where decoys were placed on the same green branch traps as described above, and compared to similar traps with EAB decoys and blank traps. Trap captures were much greater than the previous year with 9872 buprestids found in 54 traps, including 83 *A. biguttatus*. Severe drought in Hungary may have caused an overall increase in buprestid populations. The data suggests that decoys are as effective as real EAB in attracting beetles to traps.

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TACKLING BORDERS, BUDGETS, AND BAD BUGS: INVASIVE SPECIES OUTREACH WITH EMERALD ASH BORER UNIVERSITY

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ABSTRACT

As emerald ash borer (EAB) spread through the Midwest and Canada, leaving millions of dead trees in its wake, there was a catastrophic disconnect between research findings and the public's understanding of the destructive capacity of this pest. Discovery of new EAB-infested areas was occurring faster than information about it disseminated, and communities were being overwhelmed with dead trees before they knew what hit them. Tragically, they were even less informed about how they could act to mitigate tree loss.

The idea for the Emerald Ash Borer University (EABU) project was born in 2009 out of the necessity to deliver accurate, timely information about EAB and other regionally important forest pests to a large number of relevant stakeholders in the face of a national economic downturn. The capacity of arborists, forest managers, green industry workers, and stakeholders to conduct and travel to educational events has been greatly constrained by dwindling budgets. EABU, a Web-based educational initiative housed at www.emerald ashborer.info, brings the information to them, directly from nationally recognized experts, in a format that requires nothing more than an internet connection, computer speakers, and an hour of their time. At no cost to the participant, webinars allow live chatting among viewers and presenters, and all webinars are recorded and archived for access on demand. More than 2,100 live participants have accessed the 30 EABU webinars to date, and archived presentations have been downloaded more than 10,000 times.

The webinar model used by EABU is highly collaborative, drawing communications, research, and management expertise from multiple agencies and institutions from across the U.S. It was designed with a forward-thinking, dynamic framework, making the program easily modified to communicate evolving management strategies and scientific advancements in dealing with emerging invasive forest pests, such as Asian longhorned beetle and thousand cankers disease of walnut. The capacity of the webinar technology to handle high numbers of participants spanning large geographical areas is a unique feature.

The adaptability of the webinar platform has enhanced communication as various aspects of the EAB situation have evolved. At the outset of the project in 2009, webinars primarily focused on delivering basic information about EAB biology, impacts, and ash utilization. Over the past four years, efficacy and economic feasibility of chemical controls have been elucidated and new decision making tools for citizens and municipalities have been developed. It has also become clear that planning in advance of EAB's arrival can save communities money and allows options for canopy conservation. The focus of EABU webinars has therefore shifted to providing viewers with a management "toolbox" and motivating them towards planning and early action.

The primary goals of EABU are to (1) enhance participant knowledge and (2) enact a change in participant behavior. In April 2012, in order to better assess the impacts of EABU webinars and tailor content to address participant needs, we designed a short online post-webinar survey to collect directed feedback and pertinent demographic information.

In the six webinars held since survey implementation, there have been 101 survey respondents from 22 US states, representing 46% of live viewership. The majority of respondents resided in IN, OH, MI, NY, and MD – states currently experiencing heavy EAB impacts. Interestingly, there were also respondents from CO, ND, SD and other states without known EAB infestations. When queried regarding their primary perspective when viewing the webinar, 24% of respondents self-identified as tree care professionals (foresters or arborists), and another 28% self-identified as professional or para-professional educators. Seventy three percent of respondents reported a "high degree of change" in their knowledge of webinar subject matter. Similarly, 84% self-reported a "high degree of change" in their level of preparation to manage the pest. Ninety eight percent of respondents indicated they would share webinar information with others.

Of particular importance to impact assessment were the "outcomes," or what participants intended to do with knowledge acquired in the webinars. When asked to list one specific action they planned to take as a result of viewing the webinar, 82% of survey-takers responded. Individual responses varied widely but, for analyses, were placed into two broad categories: implementation of concepts in a field setting ("Action") and dissemination of acquired knowledge ("Education"). Forty seven percent of respondents proposed Action (such as conducting tree inventories, neighborhood outreach events and tree taggings, developing EAB management plans, implementing plans already in place). Forty five percent of respondents proposed Education (such as incorporating new concepts into existing educational programs, increasing amount of outreach, educating specific groups), and 8% of respondents proposed both Action and Education.

Overall, according to the results of the 2012 webinar participant survey, EABU appears to be increasing the knowledge and preparation level of our participants in the face of EAB, both in the U.S. and Canada. The outreach message is also reaching key audiences (tree care professionals and educators), who are positioned to implement and share the latest developments in the EAB situation. Participation from states that do not yet have active EAB infestations is perhaps indicative that the "plan ahead" message is being heard. These encouraging survey results are a testament to the quality of information delivered by webinar presenters and of the communications networks established as the project has progressed. Participant surveys will continue through project end in 2014.

EABU is an example of a functioning communications system that is addressing a need to bring together many sources to communicate about a complex invasive insect problem. The collaborative nature of the project strengthens its relevancy and reach. With Asian longhorned beetle and thousand cankers disease of walnut emerging as important invasive pest issues, the adaptability, as well as the financial and physical accessibility of webinar technology, makes it a sound choice for tackling communications needs of the future.

MOVEMENT OF MATURE TREES BY MAN, AN UNEXPECTED PATHWAY FOR INVASION OF THE URTICATING PINE PROCESSIONARY MOTH

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ABSTRACT

The pine processionary moth (PPM), *Thaumetopoea pityocampa*, is a forest insect of Mediterranean origin which is extending its distribution northwards and upwards in Europe in response to climate warming. Because the gregarious larvae develop during winter, a warming up, even limited, is significantly increasing their survival. Barriers for PPM range are thus delimited by thermal thresholds such as a lethal temperature of -16°C and a nocturnal feeding needing a positive air temperature preceded by a day temperature higher than 9°C in the nest built by the larvae. Such conditions were rarely observed before the 1990s in most areas of Western Europe and in the mountainous regions. These thermal constraints were largely released from the mid-1990s on. Climate models showed that a large part of Western, Central and Eastern Europe then became favourable to PPM establishment. For example, PPM naturally progressed by more than 100 km in the Paris Basin between 1972 and 2012, with a significant acceleration during the last 10 years. This expansion led the moths to enter highly-populated semi-urban areas, impacting not only forest trees but also human and animal health because the mature larvae are highly urticating.

In order to be able of measuring precisely the speed of expansion in the future we mapped, with the help of a large number of European colleagues, the 2011-2012 front of natural expansion all over the European range from French Brittany to the West to Bulgaria to the East, using a 16km-cell grid. During this survey, a total of 9 pioneer colonies were detected in France far beyond the main range, especially in the vicinity of downtown Paris but also in Northern and Eastern (Obernai nr. Strasbourg) France. These colonies were located from 40 to 190km to the nearest point of the front, and some of them apparently existed since the early 2000s.

Our study therefore aimed at understanding the origin and possible pathways corresponding to these pioneer colonies. Flight mill experiments confirmed that female moths fly only a few kilometers on the average, even if some of the individuals, especially these originating from the newly-colonized areas, can fly up to 11km. These limited flight capabilities would not have allowed females to come from the nearest established colonies. In addition, DNA microsatellite analyses excluded that most colonies originated from the front populations but indicated for three of them at least a link with remote areas of Southern France. A parallel analysis of their parasitoid complex did not show any egg nor larval parasitoids but the presence of specific pupa parasitoids. Moreover, all the pioneer colonies were detected near human dwellings (highways, runabouts, urban buildings, swimming pools, university and factory parks, Disneyland Paris) which have been recently planted with pines.

Combining these results tends to indicate that most pioneer colonies correspond to long-distance jumps, the likely pathway being the trade of mature pine trees for ornament, the moth being moved as pupae with the accompanying soil. A similar pathway has been hypothesized for the introduction of oak processionary moth in England. Such transportations may have existed for a long time although they probably recently increased with the 'green' fashion. However, if PPM was transported before the late 1990s, it is likely that the moth offspring could not have survived the harsh winter conditions in northern areas. At present, once PPM is introduced in urban and semi-urban areas, the combination of global warming and urban microclimate is favoring its survival. A comparative survey of the colonies during two recent, severe cold periods having occurred in 2009 and 2010 in the Paris Basin revealed that the larvae survived significantly more in urban areas, which behave like heated islands, than in the main range.

In conclusion, although a large part of western and central Europe is at present favorable to establishment, PPM is colonizing it slowly (ca. 5km/ year) due to the limited flight capabilities of females. However, aaccidentally- introduced colonies are likely to establish in a large part of the yet uninfested regions, especially in urban areas. A human-mediated dispersal was largely unexpected because of the sanitary risks. It may have also concerned the endemic area and could have led to a genetic admixture in some regions.

This work was funded by the French National Research Agency through the project URTICLIM (*Anticipation of the response of processionary moths to climate change and its biodiversity and sanitary impacts*), and by INRA with the project PCLIM (*International research network about the adaptive response of processionary moths and their associated organisms to global change*).

ASIAN GYPSY MOTH: IT'S BACK!!!

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ABSTRACT

The presentation was introduced with a short YouTube® video documenting a mass flight of Asian gypsy moth (*Lymantria dispar*) in the fishing port of Kuji, Iwate Prefecture in northwestern Honshu, Japan during 2008.

Asian gypsy moth (AGM) was introduced into the west coast of Canada (Vancouver, BC), and the United States (Seattle-Tacoma, WA, and Portland, OR) in the spring of 1991 when large numbers of egg masses laid the previous year on the hulls and superstructures of vessels in the Russian Far East hatched on vessels at anchor or in berths in the west coast ports. The larvae from hatching egg masses ballooned ashore. In the fall of 1991, male gypsy moths were recovered from monitoring traps placed in each port and conclusively identified as Asian origin using newly developed DNA diagnostic tools. As these recoveries indicated that AGM had established on the west coast, three treatment programs using BTK were undertaken in Vancouver (18,800 ha), Seattle-Tacoma (1608 ha) and Portland (228 ha) in the spring of 1992. No male moths exhibiting the Asian genotype were recovered from pheromone traps in the treatment areas in the fall of 1992, indicating that eradication efforts were successful. Since these introductions and subsequent establishment of AGM in North America, efforts have been undertaken to mitigate any further introductions on vessels from Asia. We briefly summarize the history and current status of these mitigation efforts.

Early efforts to prevent the establishment of Asian genotypes of *Lymantria dispar* in North America focussed on the Russian Far East. In 1993 annual pheromone trap monitoring of AGM populations in ports and nearby forests was initiated. This program was funded by USDA-APHIS & Forest Service, Forest Health Protection (FHP) and implemented by the Russian Center of Forest Health and the Federal Service for Veterinary and Phytosanitary Surveillance (FSVPS) of the Russian Federation. This trapping program has run continuously since 1993, providing early warning of impending outbreaks of AGM in the Russian Far East. Trap data has been used to designate high risk periods associated with adult flight and oviposition for each monitored port. FSVPS also inspects and cleans egg masses from vessels departing the Russian Far East for North American ports and issues phytosanitary certificates certifying ship inspection and cleaning. This program has been used as a model to establish certification programs for the other Asian countries within the range of AGM.

The National Plant Protection Organizations of Canada (Canadian Food Inspection Agency [CFIA]) and the United States (USDA-Animal and Plant Health Inspection Service [APHIS]) currently regulate all vessels that have visited Asian ports within the native range of AGM during the flight and oviposition period of *L. dispar* that will arrive in ports under their respective jurisdictions during the spring and summer risk periods for egg hatch in the subsequent year. The area regulated roughly corresponds to all ports in Asia between 30º and 50ºN latitude. This region encompasses: all Russian ports in Krasnoyarsk Krai, Primorsky Krai and Sakhalin Oblast; all Japanese ports on Hokkaidō, Honshū, Shikoku and Kyūshū and associated islands; all ports in South Korea; and all ports in China north of Shanghai.

In August of 2009, the North American Plant Protection Organization adopted the Regional Standard for Phytosanitary Measures (RSPM) No. 33 – "Guidelines for Regulating the Movement of Ships and Cargo from Areas Infested with the Asian Gypsy Moth" which outlines the requirements for vessels and cargo moving from Asian to North American ports to prevent any further introductions of AGM into Canada, the United States or Mexico. Since the adoption of RSPM No. 33, USDA-APHIS and the CFIA, supported by USDA-FS, FHP and Natural Resources Canada-Canadian Forest Service (NRCan-CFS) respectively have worked with the National Plant Protection Organizations (NPPOs) of China, Japan and Korea to establish offshore vessel certification and monitoring programs for AGM.

Efforts to develop an offshore mitigation program in Japan were initiated in 2004 with pheromone trap monitoring of AGM males in port areas. Traps and lures were supplied by USDA-APHIS and monitoring was conducted by the Ministry of Agriculture, Forestry and Fisheries (MAFF), the NPPO of Japan. This monitoring program was used to determine the high risk period for AGM flight and egg mass deposition in Japanese ports. Peak monitoring activities occurred in 2009 within 53 Japanese ports. By 2011, only one port (Yokohama) was monitored and the program has now been abandoned. With the exception of the 2011 trapping in Yokohama, all monitoring was conducted within the confines of each port. No data on AGM population levels in forested areas adjacent to the ports is available. MAFF has also established a program of vessel inspection, cleaning and certification with twenty private inspection companies operating the program. There is no direct oversight of the inspection companies by MAFF and no direct communication between companies of inspection and certification results.

Both China and Korea began implementing vessel certification programs in 2010. In both countries, a single certification body operating under the oversight of their respective NPPO's has been established. In China, inspection and certification of vessels is delivered by the China Certification & Inspection Co., Ltd. (CCIC) while in Korea it is delivered by the International Plant-quarantine Accreditation Board (IPAB). As creation of the IPAB required changes to National Legislation, certifications in Korea were performed by the NPPO until March 1, 2012 when IPAB assumed jurisdiction. In 2008, USDA-APHIS supplied pheromone traps to China and Korea to begin flight monitoring programs in each country. In 2008, pheromone trapping of AGM males was established in 8 Korean port areas by the NPPO. The port monitoring program was expanded to 11 ports in 2009 and is currently ongoing at each of the 11 ports. No data is currently available on the status of AGM in port areas monitored in China.

The first year of full implementation of the vessel certification program in Canada and the United States was 2012. Specifically vessels that had visited regulated ports during the 2011 AGM flight season in Asia and the Russian Far East were required to obtain Certificates of Inspection for AGM life stages from the last Asian or Russian Far East port visited prior to departure to North American ports. This resulted in a significant increase in vessel arrivals from regulated ports in Asia during 2012. During 2011, Canada had 499 arrivals from regulated ports in Asia (10 Japanese ports and all ports in the Russian Far East). In 2012 with the expanded regulated area encompassing all ports in Japan, Korea, ports north of Shanghai (~30° N) in China and all ports in the Russian Far East south of 60° N, the number of vessel visits from regulated areas increased to 1,623 vessels in Canada alone. Additionally, the proportion of vessels arriving without inspection certificates increased from 5% to 13% between 2011 and 2012, putting increased demands for vessel inspections on regulatory staff. Greater than 95 percent of the vessels arriving without certification (205) were inspected. An additional 27% of the vessels with valid certificates were audited for the presence of AGM egg masses in Canada. Egg masses were detected on 32 of the vessels inspected in 2012, a 10-fold increase in detections from 2011. Surprisingly, egg masses were detected on only 4 of the vessels that arrived without certification, the other 28 infested vessels all held valid inspection certificates.

Many of the infested vessels arriving from the regulated areas of Asia have visited multiple ports in one or more countries during the AGM flight season making it difficult to determine where the infestation occurred. One infested vessel detected in 2012 visited five regulated ports in three countries during an eleven day period within the AGM flight season. Examination of ports documented in vessel itineraries coupled with the number of egg masses recovered on each vessel or off-loaded cargo suggest that populations of AGM have increased markedly in the Seto Naikai region that includes the inland sea between the islands of Honshu

and Kyushu of southern Japan. AGM populations continue to remain high around a number of ports on Hokkaido in northern Japan. Other detections on vessels that visited ports only in China or Korea indicate that populations may be increasing in other regions of the regulated area.

Regulatory missions have occurred or are currently planned by the Canadian and US NPPOs to discuss the outcomes of the 2012 Certification Programs and the plans for the 2013 programs with the NPPOs of Korea, China and Japan. Each country will be encouraged to adopt additional mitigation measures in and around their ports during the AGM flight season to reduce vessel and cargo egg mass infestations. These include: cessation or limited night loading (with closing of hatch covers); minimizing vessel and port lighting during periods when active flight of AGM is evident; protection of cargo from ovipositing females; and changing port and vessel lighting to wavelengths that are less attractive to adult moths (eliminate mercury vapour lights). Establishing long-term AGM monitoring programs in forested areas in and around port areas could provide advance warning of increasing populations and allow adoption of additional mitigation measures.

The increase of vessels infested with AGM egg masses arriving in Canada and the United States and the high numbers of egg masses detected on individual vessels suggest that populations are increasing in regulated areas of Asia. During 2013, the NPPOs of Canada and the US will continue to inspect non-certified vessels for AGM egg masses; audit certified vessels to evaluate the off-shore mitigation measures undertaken by each country; and to work with the respective NPPOs of each country to enhance off-shore mitigation measures.

EUWALLACEA SP./*FUSARIUM* SP.: A NEW AMBROSIA BEETLE/FUNGUS THREAT TO CALIFORNIA TREES

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ABSTRACT

Bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are one of the most important groups of insects affecting forests and trees worldwide. There are approximately 560 species currently reported in the United States, and 62 of these are non-native species. Since the 1980's, the rate of introduction and detection of these non-native species has been steadily increasing. Of the 62 species, 28 are considered ambrosia beetles, most of which belong to the tribe xyleborini. These beetles are successful invaders because they are cryptic, have a skewed sex-ratio with a sib-mating system and have a broad host range. All ambrosia beetles carry with them symbiotic fungi which serve as a food source. Several of the bark and ambrosia beetles that have had the greatest impact on North American forests are ambrosia beetles, such as the red bay ambrosia beetle, which vectors the laurel wilt fungus, or bark beetles, such as the European elm bark beetle, which vectors the Dutch elm disease fungus.

In the Long Beach area of Los Angles County, California, a large number of box elder (*Acer negundo*) trees were found dying in 2010. By 2012, backyard avocado trees (*Persea americana*) were also found dying in the Los Angeles area. The beetles associated with these dying trees were identified as *Euwallacea fornicatus*, a non-native ambrosia beetle first reported in the continental US in 2002 in Florida and 2003 at Whittier Narrows, Los Angeles County. Also associated with this beetle in California was an undescribed species of fungus in the genus *Fusarium*. By the summer of 2012, the beetle and fungus were found associated with dying trees in Los Angeles and Orange Counties, California.

Studies conducted at the Los Angeles Arboretum and Huntington Botanical Gardens, found the beetle attacking more than 200 species of trees in 59 different families. The fungus was able to colonize more than 100 of these species, and the beetle was able to produce a brood in 19 species, most notably box elder, coast live oak (*Quercus agrifolia*), avocado and California sycamore (*Platanus racemosa*).

In 2009, there were reports of similar symptoms and mortality of avocado trees in Israel. The same beetle and fungus were found associated with this mortality in Israel.

Although the beetles in both California and Israel were identified morphologically as *E. fornicatus*, commonly known as the tea shot-hole borer from southern Asia, large differences in DNA sequences between these beetles and those from tea in Sri Lanka and other parts of Asia, indicate that the beetles in California and Israel may be a different species. In addition, the *Fusarium* associate of the beetles in California and Israel is the same, but different from the *Fusarium* associated with beetles from other parts of its range.

Additional work is needed to identify the beetle species found in California and Israel. It is still unclear if this is a new species, a cryptic sibling species or a species currently recognized as a synonym of *E. fornicatus*. Work also needs to be completed on the identity of the *Fusarium* species associated with the beetles and its role in tree mortality. Additional work on the host ranges of the beetle and fungus, potential geographic range and basic biology and host-beetle-fungal interactions needs to be addressed. Finally, surveys need to be expanded to determine the area affected and the impacts to forest, shade trees and orchards.

THE EFFECTS OF GYPSY MOTH DEFOLIATION AND BT SUPPRESSION ON FOREST LEPIDOPTERA DIVERSITY AND ABUNDANCE

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ABSTRACT

Since its introduction to North America, the gypsy moth (*Lymantria dispar* L.) has become a major defoliator of eastern hardwood forests. While there has always been interest in understanding the impact of these defoliation events on native arthropods, studies have met with limited success due to the unpredictability of gypsy moth outbreaks. Shenandoah National Park experienced a major defoliation event in June 2008, in addition to treatment of some defoliated areas with the Lepidopteran-specific insecticide *Bacillus thuringiensis* var. *kurstaki* (Btk) in May of the same year. The impacts of gypsy moth defoliation and Btk on native forest night-flying Lepidoptera diversity and abundance were evaluated over four months in summer 2011 to determine any long-term differences between defoliated regions treated with Btk, untreated defoliated regions, and regions that have not experienced defoliation since 1995. Fifteen sites – five of each treatment type – were selected across a 30-km range. Trapping using a standard blacklight trap was conducted every four weeks (May – September) during optimal trapping conditions. Preliminary analysis of the May and June trapping sessions suggested that 2011 early summer species diversity was not affected by the 2008 defoliation event and Btk treatment. Randomization tests found no significant effects of treatments on species diversity within (α diversity) or among (β diversity) locations in either May or June. Similarly, there were no effects of treatments on total Lepidoptera abundance. Such results are not unexpected due to the phenology of the insects captured in May and June: Blacklight traps only sample adult moths, and only species that were larvae during the same period of gypsy moth defoliation (May and June) would be affected by Btk or competition with the gypsy moth. Adults flying in the early summer are most likely larvae during periods where they face no BTK treatment or competition from gypsy moth. The processing of specimens captured in late summer is ongoing. When the processing is complete, I will examine how the phenology and diet of the moth species influences their susceptibility to gypsy moth defoliation and Btk. This research builds on our understanding of the long-term impacts of an invasive defoliator on forest communities.

MAKING TOUGH DECISIONS WHEN LITTLE IS KNOWN ABOUT AN INVASIVE THREAT: HOW TO INCORPORATE UNCERTAINTY AND DECISION-MAKER PREFERENCES INTO PEST RISK MAPS

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ABSTRACT

Pest risk maps, which estimate the likelihood that a pest will be introduced or established in geographic locations of interest, can serve as important decision support tools when devising strategies to limit successful introductions of invasive species and mitigate their impacts. For example, these maps may be used to prioritize locations for regulation, surveillance or implementation of preventive measures. When possible management responses to an invader include costly or socially sensitive activities, decision-makers tend to choose management options that are more certain to slow the pest's spread or prevent further incursions. We present a new concept for application in pest risk mapping that assesses risk from the perspective of a risk-averse decision-maker. Formally, we approach the mapping of risk as a portfolio allocation problem, applying techniques from financial asset allocation theory to build a map that incorporates both the uncertainty in the risk predictions and the perception of them by a decision-maker.

We demonstrate this new approach by analyzing pathways of human-assisted spread (i.e., with infested firewood that may be carried by campground visitors) of the emerald ash borer (*Agrilus planipennis* Fairmaire), a major pest of ash trees in North America. We used a spatial pathway-based model to generate distributions of plausible invasion outcomes over a target geographic region (i.e., eastern North America). We then applied a portfolio-based allocation technique that uses partial stochastic ordering, the second-degree stochastic dominance rule to prioritize the locations where the introduction of the pest with the transport of firewood by campers is most likely.

Overall, the new methodology offers a workable strategy for dealing with the typical lack of knowledge in pest risk assessments and provides a way to incorporate decision-making preferences into final estimates of pest invasion risk. Thus, the approach better incorporates decision-makers' risk preferences and also helps to outline the geographic areas that need to be targeted by costly surveillance or public outreach activities.

AN OVERVIEW OF OUR QUEST FOR ASH RESISTANCE TO EMERALD ASH BORER

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ABSTRACT

Since emerald ash borer (EAB), *Agrilus planipennis*, was discovered in North America in 2002, it has killed millions of ash trees in North America, and mortality of ash now exceeds 99% in southeast Michigan (Herms et al. 2009). The development of EAB resistant ash trees will be critical for restoration of ash in natural and urban forests. The overarching goals of our multidisciplinary collaboration are (1) to identify, breed and screen ash germplasm for EAB resistance and silvicultural traits; (2) identify mechanisms of EAB resistance to facilitate breeding and screening; and (3) conduct functional genomic studies of ash and EAB to accelerate development of resistant ash trees, including sequencing and functional characterization of resistance genes in ash, as well EAB genes that detoxify defenses.

In Asia, EAB does not devastate its endemic hosts, which suggests that Asian ashes are inherently resistant by virtue of their coevolutionary history with EAB. Consistent with this hypothesis, we confirmed in our common garden study that Manchurian ash is much more resistant to EAB than North American species, and thus is a source of resistance genes (Rebek et al. 2008). We subsequently identified phenolic compounds and defense proteins in phloem tissue that may provide the mechanistic basis for this resistance (Eyles et al. 2007; Cipollini et al. 2011; Whitehill et al. 2011, 2012). *In vitro* bioassays with artificial diets have confirmed antibiotic properties of some candidate compounds to EAB larvae, and testing of others is underway. We have also characterized the preferences and response of EAB adults to foliar characteristics of North American and Asian ash (Chen et al. 2011, in press).

We have initiated a breeding program based on hybridization of resistant Asian ash and susceptible North American ash (following the successful chestnut blight breeding program) (Koch et al. 2012), and have begun screening rare surviving native trees for resistance (Knight et al. 2012). We have made progress in overcoming barriers to hybridization of Asian and North American ash (Koch et al. 2012), and an extensive common garden was established at OARDC in 2012 to evaluate additional Asian species for EAB-resistance for use as parental lines to expand the breeding program.

We have established a strong molecular foundation for this research through gene sequencing and gene expression analysis of EAB (Mitapalli et al. 2010), as well as Asian and North American ash species, which is providing a foundation for targeted breeding and identification of genetic markers to screen ash (Bai et al. 2011; Rivera-Vega et al. 2012). We have also identified EAB genes important for counteracting defenses of ash (Rajarapu et al. 2011), thus revealing potential chinks in the armor of EAB that could be exploited as breeding targets.

EMERALD ASH BORER BIOLOGICAL CONTROL: A DECADE OF PROGRESS

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Buprestidae), is an invasive beetle that kills even healthy ash trees. Mortality results when larvae feed on the phloem, cutting off the flow of nutrients within the tree. Tens of millions of ash trees have died, and economic and environmental losses are considerable. Mechanical control, chemical control, and regulatory strategies were put in place to slow the spread of the EAB, but the beetle often becomes established in new areas before it is discovered and control measures can be implemented. As a consequence, EAB infestations are now known in 18 northeastern states. In Asia, parasitoids are the most important mortality factors affecting EAB infesting introduced North American ash tree species, and these parasitoids have now become a critical component of EAB management in the U.S.

When research on EAB natural enemies began in Michigan and China in 2003, little was known about their biology or population dynamics. In the past decade, however, considerable progress has been made, including the discovery of several important parasitoids that attack EAB eggs and larvae in northeast Asia. The first three parasitoid species discovered were from China: the egg parasitoid, *Oobius agrili* Zhang & Huang (Encyrtidae) and two larval parasitoids, *Spathius agrili* Yang (Braconidae) and *Tetrastichus planipennisi* Yang (Eulophidae). These parasitoid species were studied in the field in China and in U.S. quarantine laboratories for several years to determine their biology, rearing methods, and host ranges. In 2007, approval was granted for their release at field sites in Michigan, and reproduction and overwintering was confirmed the following year. By 2008, scientists and managers with APHIS, FS, and ARS worked together to initiate the USDA Emerald Ash Borer Biocontrol Program. Methods for rearing a wood-boring beetle had to be developed to rear parasitoids, and rearing had to be done efficiently for mass rearing purposes. Release methods were also optimized to reduce labor and allow the parasitoids to emerge naturally in the field rather than rapidly dispersing when released as adults. A mass rearing facility started operation in 2009, and the numbers of each parasitoid species released increased from a few hundred to tens of thousands of females per year. Parasitoids have now been released in 14 of the 18 infested states, with more states set to start releasing in 2013. Methods to efficiently and effectively sample all three parasitoid species have been developed, resulting in the recovery of parasitoid progeny in seven states. Researchers in MI have documented increasing EAB mortality due to *T. planipennisi* and *O. agrili*, as well as dispersal away from the release sites; however *S. agrili* does not seem to persist after the first year or two after release in the more northern release sites. Climatic variables may be partly responsible for the poor persistence of *S. agrili* since Tianjin China, where *S. agrili* was collected, and the northern United States are not well matched climatically. *Spathius agrili*, with its long ovipositor, is an important biocontrol agent because *T. planipennisi* has a short ovipositor and cannot attack EAB through thick bark. *Spathius galinae*, which was collected in Vladivostok, Russia, has a very long ovipositor and climate matching indicates it may be better-suited for establishment in the more northern parts of the U.S. Host specificity testing is complete, and an application to release *S. galinae* will soon be submitted.

PRESENTATIONS

Non-Native Plant Invasions: Repercussions and Species Interactions

RESPONSE OF THREE NON-NATIVE SPECIES AND A NATIVE OAK TO FIVE DIFFERENT MANAGEMENT REGIMES ACROSS LOCAL AND REGIONAL MOISTURE GRADIENTS: CAN WE REDUCE NON-NATIVE PLANT SPECIES IMPACTS ON OAK REGENERATION?

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ABSTRACT

Oak regeneration in eastern U.S. forests is dependent on management that increases light to the forest floor, i.e., harvesting and fires to increase canopy openings. Forest canopy openings of any kind may lead to invasions by non-native invasive plant species, creating a dilemma for forest managers. Invasive plant establishment and oak regeneration are also dependent on available resources as defined by site topography and regional gradients. We compared germination, survival, growth, and productivity of three invasive plants – garlic mustard (*Alliaria petiolata*), Japanese stiltgrass (*Microstegium vimineum*), and tree of heaven (*Ailanthus altissima*) as well as the native Northern red oak (*Quercus rubra*) at 56 sites in the Allegheny Plateau and Ridge and Valley Provinces, across northeastern and southwestern slopes. Five management regimes in these regions and local gradients were evaluated: control, single prescribed burn, repeat prescribed burn, diameter-limit cutting, and shelterwood harvests. The first-year shelterwood harvest was the most aggressive management regime of the five studied, in terms of the amount of light (approximately 400 $umolm^{-2}s^{-1}$ on average) reaching the forest floor.

Percent germination of the three invasive species was highest on the northeastern slopes, the Ridge and Valley, and the shelterwood sites. Thus, these species have a higher probability of germinating under light conditions 200 $umolm^{-2}s^{-1}$ (10% full sun light) or greater, if moisture is adequate (i.e., on the northeastern slopes). Related on-going seed dormancy and mortality data support these findings. Once germinated, tree of heaven and Japanese stiltgrass are likely to survive equally well under the tested site conditions and management regimes. Garlic mustard may be more sensitive and may survive better on burn sites, though more research is needed. Northern red oak survived best in the Ridge and Valley, but management regime had no effect on its survival. Moreover, while the three invasive species showed greater productivity in the shelterwoods, Northern red oak showed no positive response in shoot growth to this management regime. The two herbaceous plants (Japanese stiltgrass and garlic mustard) showed greater growth in the Ridge and Valley, while the two woody species (tree of heaven and Northern red oak) showed greater growth in the Allegheny Plateau. The root biomass data for tree of heaven suggests that this species, and perhaps Northern red oak, is putting more energy into root growth on the drier sites (with higher light levels). The difference in productivity for Northern red oak may also be a factor of higher deer herbivory in the Ridge and Valley.

Under current dominant management regimes and conditions (including deer herbivory) of the study area, management techniques used for Northern red oak are not promoting its survival or growth. Unfortunately, such aggressive management does promote the productivity of invasive plant species. Thus, based on our preliminary results, we could expect equal oak productivity under 10% full sun light as compared with more aggressive management (a heavy shelterwood removal). In contrast, keeping light levels below 10% full sun light would significantly reduce the probability of invasion by nonnative plants. However, our longer-term measurements of the planted oaks and a comparison of girth measurement (in addition to shoot growth) may complicate these findings.

Our next steps on this project include: (1) model forest stand growth and incorporate probability of invasion based on these data and propagule spread data; (2) using controlled growth chamber tests and plant biochemical analysis, determine the optimal light level which allows for oak survival and growth but does not promote survival and growth of invasive plant species (based on these results, the optimal light level is below 200 $umolm^{-2}s^{-1}$); and (3) determine if initial harvests (i.e., the first cut of a shelterwood or thinning conducted using the determined optimal light level) would be economically viable, with and without the cost of invasion included.

INSECT NATURAL ENEMIES IN ORNAMENTAL URBAN LANDSCAPES: PARASITIC HYMENOPTERA IN NATIVE AND EXOTIC RESIDENTIAL-SCALE PLOTS

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ABSTRACT

There is heightened interest in effects that the provenance of plants in the landscape has on animals inhabiting them. This question is of great interest for urban ornamental landscapes, which tend to be mosaics of native and exotic plants. There is a considerable body of research on insect herbivores, but little attention has been paid to arthropod natural enemies. In eastern North America, many commonly grown exotic woody plants were missing from the continent for millions of years prior to the arrival of Europeans. We present the hypothesis that due to the lack of a recent co-evolutionary history with these plants, native natural enemies will be less well able to utilize the resources – architectural features and nutritional supplements – provided by exotic plants than they will those of native plants, and hence be less numerous and diverse in landscapes dominated by exotic plants. We test this in a replicated experiment with residential size plots planted to congeners of 15 genera of woody plants from either Eurasia or North America, grown in a turf of tall fescue. Adult hymenopterous parasitoids collected by vacuum sampling from the turf were statistically less abundant, and exhibited statistically lower species richness, in the exotic plots, supporting the hypothesis. The parasitoid complex comprised 43 taxa of broad taxonomic and host affinities. At least some of these animals parasitize herbivores that feed on woody plants in the landscape, and probably visit the turf to take advantage of plant-provided nutritional supplements.

PRESENTATIONS

Gypsy Moth Population Dynamics Revisited

THE IMPACT OF *ENTOMOPHAGA MAIMAIGA* ON OUTBREAK GYPSY MOTH POPULATIONS: THE ROLE OF WEATHER

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ABSTRACT

The fungal pathogen *Entomophaga maimaiga* has become arguably the most important pathogen infecting gypsy moth (*Lymantria dispar*) in North America, since its appearance in 1989. However, unlike other pathogens such as the *Lymantria dispar* nucleopolyhedrovirus (LdNPV), the fungus has been suggested to be highly dependent on weather conditions for successful transmission. In the lab, the production of conidia, the germination of resting spores, and the infection of larvae all require high humidity or moisture and fungal development has been shown to be hindered by prolonged high temperatures. In order to understand how changes in weather-related variables over space and time might influence host-pathogen interactions, we measured fungal infection rates over three years concurrently with rainfall, relative humidity, air and soil temperatures, and soil moisture at a number of sites in central Pennsylvania. Fungal mortality was assessed using both field-collected larvae as well as larvae caged at the sites. Weather variables were measured with portable weather stations installed at each location. We found high variability in rates of larval mortality due to *E. maimaiga* between sites and across time, with individual samples ranging from 0 to 100% mortality. Overall, most sites showed high levels of mortality especially toward the end of the larval season. The effect of weather variables on fungal mortality was assessed using a mixed effects repeated measures model in R. After model-selection by Akaike Information Criterion, we found significant positive effect of soil moisture on mortality, and significant negative effect of soil temperature on mortality. The strongest results were obtained from the field-collected larvae, but data from the caged larvae were consistent. These results demonstrate the importance of moisture and temperature to predicting the prevalence of *E. maimiaga* infections in gypsy moth populations. These relationships may be helpful in understanding how gypsy moth dynamics vary across space and time, and in forecasting how the gypsy moth and fungus will interact as they move into warmer or drier areas, or as warmer and drier conditions are brought on as a result of climate change.

HOW HAS THE EMERGENCE OF *ENTOMOPHAGA MAIMAIGA* ALTERED THE DYNAMICS OF NORTH AMERICAN GYPSY MOTH POPULATIONS?

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ABSTRACT

Historically, North American gypsy moth, *Lymantria dispar*, populations have exhibited periodic oscillations with moderate outbreaks in some regions recurring every ca. 5 years and large regional outbreaks every ca. 10 years. The largest outbreaks occurred in the early 1980's and early 1990's. More recent outbreaks have been smaller but the pattern of periodicity has remained approximately constant. The fungal pathogen *Entomophaga maimaiga* was initially discovered in N. America in 1989. Since then, this pathogen has become ubiquitous in N. American gypsy moth populations with large numbers of larvae dying from this fungus in most gypsy moth populations. Considerable evidence has indicated that the oscillatory dynamics of gypsy moth populations have primarily been driven by epizootics of a nucleopolyhedrovirus but this work was largely conducted prior to *E. maimaga*'s arrival. Little is known about how the emergence of this fungal pathogen has altered multi-generational interactions of the gypsy moth with its virus. For example, it is not clear if the fungus has replaced the virus as the principle driver of population cycles or if it has stopped cyclic population cycles altogether. Here we compare detailed demographic data collected in PA gypsy moth populations prior to vs. after *E. maimaiga*'s invasion of N. America. We found that *E. maimaiga* virtually always causes greater levels of mortality in hosts than does the virus, but fungal mortality was largely density-independent. Moreover, the presence of the fungus has apparently not altered the gypsy moth/virus density dependent interactions that were shown to drive periodic oscillations in hosts before the arrival of the fungus. Taken together, these results indicate that gypsy moth population cycles are most likely still being driven by the moth's interaction with it's virus, but in the presence of *E. maimaiga*, the amplitude of these oscillations may be greatly dampened.

TOPOGRAPHY INFLUENCES ALLEE EFFECTS AT THE INVASION FRONT OF THE GYPSY MOTH

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ABSTRACT

Reproductive asynchrony leading to mate-finding failure is known to contribute to Allee effects, which can slow the establishment and spread of invasive species. We examined whether local topographic variation, via effects of elevation and aspect on thermal conditions, increases reproductive asynchrony and slows the establishment of an invasive insect, the gypsy moth (*Lymantria dispar* L.) (Lepidoptera: Lymantriidae). Using output from a phenology model, we found that temporal differences in reproductive maturation are correlated with differences in elevation. We then analyzed 22 years of data from pheromone-baited gypsy moth traps placed along the invasion front and used establishment time, defined as time from initial occurrence to exceeding a regional mean Allee threshold density, to test for variability in Allee effect strength.

Consistent with our expectation, we found that shorter establishment times occurred in areas of low topographic variability. In addition, establishment times were shortest at mid to high elevations, possibly due to an elevation gradient in densities of generalist predators thought to produces an Allee effect in gypsy moth populations. We conclude that, all else being equal, the rate of gypsy moth spread is likely to be reduced in regions of North America with high topographic variability.

PRESENTATIONS

Current Status, Mechanisms of Spread, and Management of Invasive Pathogens

THOUSAND CANKERS DISEASE: WHAT WE KNOW AND WHERE WE GO FROM HERE

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ABSTRACT

The fungus, *Geosmithia morbida,* vectored by the walnut twig beetle (WTB), *Pityophthorus juglandis*, has been associated with devastating disease outbreaks in black walnut *(Juglans nigra)*. In 2010, the disease, known as Thousand Cankers Disease (TCD), was discovered within the native range of black walnut in Tennessee, followed by additional infestations in Virginia and Pennsylvania in 2011 and North Carolina in 2012. To date, all confirmed cases of TCD in the native range of black walnut have been in urban areas, along rural roadsides and/or fence rows. The report in North Carolina is within Great Smoky Mountains National Park, but the trees are not in a deep forest setting.

For symptomatic trees, numerous cankers have been observed, but *G. morbida* has been isolated in low frequency (8%). However, other fungal pathogens, i.e., *Fusarium* sp., have been associated with cankers as well, but their potential role in the TCD complex is unknown. Using isolates from the collection of *G. morbida* maintained at the University of Tennessee, 16 *G. morbida* microsatellite loci have been identified and characterized. Most of the genetic variation was attributed to individual variation rather than divergence across populations and the introduction into Tennessee appears to be a point introduction. Furthermore, both principal coordinate and STRUCTURE analyses indicated lack of genetic structure among 54 *G. morbida* isolates.

Our findings further indicated that the life cycle of the WTB takes approximately four to five weeks to develop from egg to adult. Also, two parasitic wasps and several predaceous beetles of WTB have been identified. Although some of these predators can consume several WTBs in laboratory settings, little is known of their predation levels in the field. In examinations of bark from field collections, mycelia of *G. morbida* were observed in galleries of WTB during the pupal and adult stages, but the association between this fungus and the beetle beyond their co-occurrence in walnut species remains unclear. Future research concerning the role of other fungi in the TCD complex, understanding the relationship between WTB and *G. morbida*, genetic relationships between WTB and *G. morbida* isolated from other areas within the native range of black walnut, and the role that natural enemies of the WTB may play in managing TCD, are critically needed.

EXOTIC PATHOGENS OR SHIFTS IN CLIMATE? LAUREL WILT AND BUR OAK BLIGHT

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ABSTRACT

Two new diseases in eastern USA, laurel wilt (caused by *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva) and bur oak blight (BOB, caused by *Tubakia iowensis* T.C. Harr. & D. McNew), recently have appeared in eastern USA. A new disease on a native host suggests that the pathogen is exotic or that there has been a substantial change in the environment that favors pathogen over host. A few principles help distinguish between the two. Introduced pathogens generally go through severe genetic bottlenecks and show limited diversity compared to native pathogens. Also, in native pathosystems, especially with foliage diseases, susceptible host populations typically show substantial variation in resistance, with resistant and susceptible individuals in proportion to the disease pressure, often determined in part by rainfall and humidity.

Mortality of redbay (*Persea borbonia* (L.) Spreng.) on Hilton Head Island, South Carolina, was first noticed in 2003. Although initially attributed to drought, Steve Fraedrich of the US Forest Service found that the affected trees had vascular streaking typical of a wilt disease. True vascular wilt diseases (systemic colonization of the host through non-living vessels in the early stages) are generally rare on native trees.

Cultures of an unusual fungus with a slimy yeast phase were isolated from the xylem discoloration and sent to Iowa for identification. Analysis of rDNA sequences showed that the fungus was related to *Ophiostoma* spp., a genus that includes numerous saprophytic bark beetle associates but also the causal agents of two wilt diseases, Dutch elm disease and black stain root disease on conifers, both of which involve bark beetle vectors. The redbay pathogen was most closely related to Raffaelea spp., which are fungal symbionts of ambrosia beetles, close relatives of bark beetles.

The pathogen was named *R. lauricola* and the disease was named laurel wilt because all tested American members of the family Lauraceae appeared to be susceptible to systemic colonization. These hosts include sassafras (*Sassafras albidum* (Nutt.) Nees, pondspice (*Litsea aestivalis* (L.) Fern.), pondberry (*Lindera mellisifolia* (Walter) Blume), California bay (*Umbellularia californica* (Hook. & Arn.) Nutt.), and avocado (*Persea americana* Mill.). Redbay and swampbay (*Persea palustris* (Raf.) Sarg.) appear to be uniformly susceptible, all cultures look identical, and there is no genetic variation in the pathogen.

Several ambrosia beetle species were associated with wilted redbay, and one of them, the redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff, was a recent arrival. A specimen had been indentified from near Savannah, Georgia in 2002, and the beetle was likely introduced in solid wood packing material. This Asian ambrosia beetle proved to be the vector of the pathoge. Adult ambrosia beetles generally produce brood galleries in the sapwood of dead and dying trees and inoculate the walls of their tunnels with symbiotic fungi carried in special sacs called mycangia. Larvae and adults of ambrosia beetles feed primarily or exclusively on fungal growth in the galleries. Most ambrosia beetles have *Raffaelea* species as their fungal symbionts. Isolations from *X. glabratus* collected in the Southeast and in its native range in Taiwan and Japan found *R. lauricola* to be the primary inhabitant of the mandibular mycangia, but five other *Raffaelea* species have also been isolated from samples in the Southeast. The mycangial flora of the Southeast populations of *X. glabratus* was very similar to that of the Japanese and Taiwanese populations, but another part of Asia was a likely source for the Savannah introduction.

Only *X. glabratus* has been shown to regularly carry a lethal vascular wilt pathogen in its mycangium. Some exotic ambrosia beetles have proven to be pests when they have built up large populations and have

attacked and killed horticulturally important trees or stressed forest trees. Generally, ambrosia beetles are attracted to ethanol and other "stress compounds" emanating from dead and dying trees. But there appears to be a unique relationship among *X. glabratus*, *R. lauricola* and their aromatic hosts in that the beetle is attracted to host volatiles from living trees. The beetle will bore into xylem of healthy branches and stems of Lauraceae but abort the galleries and not lay eggs. It is during this unique tunneling phase that the pathogen is introduced into the host, and after systemic colonization, wilting occurs and another generation of *X. glabratus* attacks the weakened or killed tree for egg-laying. In this way, laurel wilt is similar to Dutch elm disease, in which the young bark beetle vectors go through a period of maturation feeding and inoculate the pathogen into the twigs of healthy elms during maturation feeding, and a later generation of beetles breed in the wilted trees.

Redbay and the closely related swampbay are highly susceptible to laurel wilt and are good hosts for *X. glabratu*s. The epidemic has spread rapidly on these hosts, which are native to the coastal plains of the Atlantic and Gulf Coasts, but spread has been slower on sassafras. The epidemic has moved along fronts at more than 20 miles per year, and there have been numerous satellite centers that likely were initiated by movement of infested wood, such as firewood or craftwood. The disease is known from North Carolina south to the commercial avocado production area of Miami-Dade County, Florida and west to Mississippi. Only the smallest redbay and swampbay survive the advancing fronts. To the west, the Mississippi Delta area has little native Lauraceae, so there should be some effort to prevent movement of the vector beyond this line. California bay is susceptible, and it is believed that many important tropical and subtropical Lauraceae native to Mexico, Central and South America could be severely affected by the disease.

The second, less dramatic disease emerging in the eastern USA is a late-season leaf blight on bur oak, *Quercus macrocarpa* Michx. It was first noted in southwest Wisconsin and southern Minnesota in the late 1990s, with later reports in Iowa and eastern Nebraska. It was thought that the leaf pathogen *Tubakia dryina* (Sacc.) Sutton was responsible, but we looked closer and found that there are a number of undescribed species of *Tubakia* on oaks and chestnut in the eastern USA. We described the bur oak pathogen as a new species, *T. iowensis*, and the disease was named bur oak blight, or BOB for short.

Variation in culture morphology and in rDNA sequences suggested that the pathogen was native to Iowa. The close relatives of the BOB pathogen also seemed to be native to eastern USA. The disease was found to be most severe on mature bur oak on former savanna sites and more severe on upland sites than on bottomland sites. But even on the most severely affected sites, most of the trees appeared to be relatively resistant. Severely diseased and apparently disease-free trees were side-by-side, with the same susceptible trees showing symptoms each year. Variation in the pathogen and some resistance in natural host populations suggested that this was a native disease.

With the help of many cooperators and funding from the Northeastern Area of the US Forest Service, Doug McNew and I established that *T. iowensis* was relatively common on former upland savannah sites across most of Iowa, northern Illinois, southern Wisconsin, much of Minnesota, eastern South Dakota and eastern Nebraska (http://www.public.iastate.edu/~tcharrin/BOB.html). Scattered sites in northeastern Kansas, northern Missouri and western Illinois also had BOB. The distribution of the fungus appears to coincide with the distribution of a poorly studied variety of bur oak, *Q. macrocarpa* var. *oliviformis* (Michx. f.) A. Gray, which is an upland variety with relatively small acorns. The olive-shaped acorn of var. *oliviformis* is a fraction of the size of the acorns of var. *macrocarpa*, which is primarily a bottomland species that is eastern and southern to the distribution of var. *oliviformis*. A third variety, var. *depressa* (Nutt.) Engelm., also has small acorns and is native to the Great Plains, but it may not be susceptible to BOB. Although other species of *Tubakia* may cause veinal necrosis on var. *oliviformis* and var. *macrocarpa*, *T. iowensis* appears to be specific to var. *oliviformis*. However, oaks, including bur oak, hybridize readily, many populations may be mixtures of two species or varieties, and oak taxonomy is controversial.

All *Tubakia* species form pycnothyria on necrotic areas on leaves. These asexual fruiting bodies produce rainsplashed conidia on the underside of a shield-like structure. A unique feature of *T. iowensis* is a second asexual fruiting body, a crustose layer enclosing conidia that develop slowly over the winter. Leaves

killed by *T. iowensis* in late summer first show small areas of necrosis at the petiole base, then the necrosis girdles the petiole and the leaf dies. Many of these killed leaves hang on the branches through the winter, and the crustose fruiting bodies form on the petioles and release their spores for rain dispersal in the spring. If there is abundant rainfall as the new shoots expand in spring, then conidia from the crustose fruiting bodies may infect the undifferentiated shoots. Infection of expanding shoots apparently leads to a prolonged endophytic or latent phase, lasting for two months or more. Eventually the petioles become necrotic at their attachment point to the twigs, and the leaves are cast off or remain attached on the branches through the next spring. Fully expanded leaves infected by the primary inoculum may develop necrotic spots or veinal necrosis, in which case conidia soon form along the veins, thus producing a secondary inoculum. However, the primary inoculum and infection of expanding shoots in the spring appears to be critical to the dramatic blight symptoms seen on some trees, where every leaf on the tree is symptomatic by August.

If BOB is a native disease (variation in the pathogen and host resistance), it is surprising that it went unrecognized until recently. One theory is that a shift in climate is responsible for the current epidemic. One of the most significant changes in Iowa's climate has been increased rainfall, especially in the spring, the period when bur oak breaks bud and new shoots are expanding, when the host appears to be most susceptible to endophytic infections. Rainfall during this critical period of shoot expansion leads to spore release, dispersal of the spores, and leaf wetness for germination and infection.

A fundamental principle of native forest tree diseases, especially for foliar diseases, is that there is variation in resistance, i.e., there are resistant and susceptible individuals in host populations. If the environment is conducive and disease pressure high, a high amount of resistance is expected in the host population. If the environment is not conducive, then little resistance is expected because there is a "cost to resistance." Bur oak blight is primarily a disease in relic savanna forests that are often dominated by bur oak of 125 years or more. In the previous century, there may have been little disease pressure because spring rainfall was low, or a least there occasionally were consecutive years of low spring rainfall and probably little disease, so even susceptible individuals would likely produce good acorn crops and pass on susceptibility. However, those relic trees are now in a wetter climate. Iowa has not had consecutive dry springs since the 1988/89 drought, and the last 12 years (including 2012) have had average or above spring rainfall. Predictions are for increased rainfall for much of the Midwest and Northeast.

American forests are seeing increased disease pressure from exotic pathogens and shifts in climate. Not all of these threats are against commercially important tree species, but their ecological importance should warrant further investigation and mitigation.

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ROOT DISEASE PATHOGEN *HETEROBASIDION IRREGULARE*: INVADER, PROLIFERATOR, OR JUST IMPORTANT?

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ABSTRACT

The conifer root disease pathogen *Heterobasidion irregulare*, first detected in Wisconsin in 1993, has now been found in 60 forest stands in 23 counties in this state. Root disease foci in Wisconsin's infested stands are sometimes numerous, especially those in red pine (*Pinus resinosa*) plantations that have been thinned multiple times. Compared to its wide distribution the southeastern USA and New England, however, known occurrence of *H. irregulare* is more limited and discontinuous in coniferous forests of Wisconsin, other north-central states, and the provinces of Ontario, and Quebec.

Invasion of the north-central USA by *H. irregulare* may have occurred centuries ago, subsequent to glacial recession and the northward migration of pines. But discovery of Heterobasidion root disease in Wisconsin only 20 years ago could suggest more recent establishment following arrival of windblown basidiospores from sources in southern portions of Iowa, Illinois, Indiana, Ohio, and Michigan, or even more distant sources. Additionally, Heterobasidion root disease has yet to be detected in large areas of northern Wisconsin, Minnesota, and northern Michigan where pines have predominated since times prior to European settlement. Conversely, many red pine plantations infested by *H. irregulare* in southern Wisconsin are established on former agricultural lands at or beyond the natural range of this tree.

Regardless of time of arrival and establishment, management of red pine in highly productive red pine plantations facilitates proliferation of *H. irregulare*. Red pine is genetically very uniform, and red pine plantation trees are highly root grafted. Thinning at regular intervals results in fresh stump surfaces that are infected by the pathogen. Basidiocarp production on dead standing or fallen red pine trees and on stumps can be prolific and perennial, at least to some degree.

The importance of *H. irregulare* is linked to the value of the threatened resource, consequences of disease to red pine trees, and knowledge of the practices that can prevent losses. A majority of the red pine type in the north-central region is comprised of plantations established at considerable cost. Because most of these plantations are in age classes in which thinning occurs, infestation by Heterobasidion root disease is projected to become more common. Red pines appear to be highly susceptible, with root rot foci expanding radially as trees die. Loss in value due to mortality accelerates as trees grow from pulpwood into pole and saw log sizes. Importantly, however, it has long been known that stump infection, number of foci, and thus damage, can be minimized if protective chemical or biological treatments are applied to freshly cut stumps.

RESISTANCE BREEDING TO MITIGATE BUTTERNUT CANKER DISEASE

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ABSTRACT

Butternut (*Juglans cinerea* L.) is being killed throughout its native range by an exotic fungus *Ophiognomonia clavigignenti-juglandacearum* (*Ocj*), the causal organism of butternut canker disease. *Ocj* has not been reported as a pathogen outside North America, but the fungus has proven highly invasive and is thought to be an exotic pathogen since Asian *Juglans* species are highly resistant and most native butternuts have died. *Ocj* is an Ascomycete that only reproduces asexually, but despite its low levels of genetic diversity based on differences in DNA sequence (single nucleotide polymorphisms), there is evidence that there may have been three or four independent introductions of *Ocj* into North America.

Butternut is a valuable native tree in the eastern deciduous forest of North America. The range is similar to the black walnut (*J. nigra*) but shifts northward. The seeds provide nutritious mast for wildlife. Human uses include foods, medicines, dyes, and syrup. The lumber is of high-value used for veneer, paneling, and furniture. Butternut wood looks similar to black walnut except it has lighter colored heartwood and the density of the wood is half that of walnut making it excellent for carving.

Butternut is a relatively short lived tree species, ranging from 75-100 years, and requires full sunlight to regenerate. It can occupy sandier and rockier sites than black walnut. Historically, butternut has been less prevalent than black walnut except in the northern part of its range where black walnut drops out.

Butternut canker disease first became a concern in surveys from Wisconsin foresters in the early 1970's. Later that decade, tree dissections in the southern portion of butternut's range showed the disease was present 30 to 40 years earlier. Scientists and foresters in Wisconsin were the first to identify the fungus and to measure the impact of *Ocj* in natural stands. By the late 1990's, the disease had become endemic throughout butternut's entire range. The few trees that remain are not regenerating in natural forests. Artificial regeneration with resistant material is the only long-term means to mitigate butternut canker disease and to save the species from extinction.

The epidemiology of the disease consists of sticky conidia spores transported via insects, birds, and small mammals, into the upper crown of the trees where they germinate and infect branches. Branch cankers produce new conidia spores that move via rain splash down the stem and infect larger branches and the trunk. Susceptible trees are killed as numerous cankers coalesce and girdle the bole. Screening in the 1980's found that black walnut (*J. nigra*) could be artificially inoculated even though walnut is rarely affected by the disease in nature, and that the Japanese walnut (*J. ailantifolia*), was much more resistant to *Ocj* than butternut.

As natural stands with butternut canker disease were surveyed, healthy trees were identified. In stands where many trees had died or were dying, scion wood from healthy trees (phenotypically resistant) were grafted into clone banks beginning in the early 1990's at St. Paul, MN. These grafted selections were used to test different inoculation methods and the time of year to inoculate to develop an *Ocj* resistance screening technique. Branch inoculations were made in the field, and more work was conducted in the greenhouse with seedlings of butternut, other *Juglans* species, and other sympatric species such as oaks, ash, pecan and hickories to try and determine the host range of *Ocj*. This work showed that *Ocj* is capable of surviving and colonizing on many other tree species without causing lethal cankers. A mixture of fungal

mycelium and spores of *Ocj* was most effective to cause infection, and fall inoculations were better than spring or summer inoculations to produce disease.

To support breeding and conservation of butternut from different geographic regions, with the help of various state and private cooperators, we have now collected scion wood and seed accessions from over 600 different butternut and hybrid butternut trees. Accessions are grown in the field at several sites at the University of Minnesota, Purdue University, Indiana State Forests, and National Forests as clone banks, seed orchards, germplasm collections, progeny tests, silviculture tests, and screening trials.

The Northern Research Station's Hardwood Tree Improvement and Regeneration Center (HTIRC) began breeding resistant butternut a decade ago with the establishment of three plantings. The first planting includes thirty open-pollinated half-sibling families from clone banks in St. Paul, MN, comprised of phenotypically resistant selections. Seedlings from these clone banks were planted into a screening block along with seven half-sib seed families from northern Indiana in a bottomland field – Martell Forest B-Nut 1 Block, Purdue University, West Lafayette, IN. The second planting resulted from a large seed crop in the fall of 2002 that we harvested in a natural stand with hundreds of butternut trees. This stand had naturally regenerated from seven progenitors around an old pasture ~50 years earlier in southern WI. A dozen phenotypically resistant trees had been selected from this stand and we harvested seed from resistant, moderately resistant, and susceptible trees. Four families of each group were planted at the Southeastern Purdue Agricultural Center (SEPAC), Butlerville, IN, in an upland field. We added a known hybrid family from a commercial nursery, and included some black walnut. The third planting contains resistant and moderately resistant butternut families from the same WI stand which were planted one-year later in the Martell B-Nut 1 Block. These three plantings, established in 2003 and 2004 were inoculated five years later in the fall of 2008 and 2009 respectively. Resulting cankers were measured annually for three years; the same trees were rated for natural disease incidence. In 2012, five years after inoculating, all trees were examined and evaluated with a 5-point subjective canker rating system.

To further investigate the genetic resistance of different *Juglans* species and hybrids to *Ocj*, we inoculated grafted trees of four different *Juglans* species and a number of complex hybrids (*J. Xcinerea*) in our upland site at the Martell Germplasm Block in 2011.

Several years ago, we discovered that hybrid butternuts were much more extensive in the landscape, across the range, than was previously known. F1 hybrids (*J.* × *bixbyi*) were easily distinguished and had been reported for a long time. What was not known was that so many F1 hybrids from the past had back-crossed to butternut or inter-pollinated among themselves creating complex hybrid progeny, hence the designation *J. Xcinerea*. Several papers have now been published documenting this hybridization along with guides for distinguishing between hybrids and butternuts.

While many *Juglans* species can be infected with *Ocj*, the cankers are not always lethal and do not persist. Canker incidence the first year after artificial inoculation alone reveals little difference among resistant black walnut and susceptible butternut clones (Figure 1).

We measured the incidence and size of artificially induced cankers annually for three years after inoculating. In addition, at our bottomland site, natural *Ocj* infection began three years after planting and by the tenth year, 99% of all trees had developed natural cankers. The correlation between artificial canker incidence and natural disease incidence was low (18%). Other host-plant traits such as vigor, height, diameter, or timber quality, i.e., branchy or apically dominant, were not correlated with resistance.

By 2012, as the trees began their tenth year of growth, natural infection had become widespread in the two bottomland plantings at Martell, and 10-20% of susceptible trees were dead or dying from butternut canker disease. In contrast, less than 3% natural disease incidence was recorded in the two upland sites - the Germplasm Block at Martell or the SEPAC resistance test.

In 2012, given high *Ocj* pressure in the bottom land site, we rated each tree using a 5-point subjective scale similar to the method employed by the American Chestnut Foundation. The rating is based on the number and size of cankers along with the callus reaction of the host-tree. A rating of 1= highly resistant with

few small and well callused cankers; 2= resistant, 3= moderately resistant to moderately susceptible, 4= susceptible, and 5= highly susceptible where numerous cankers coalesce with little callus. Overall, hybrids averaged a canker rating of 3.1 and butternuts averaged 4.0 (Figure 2).

The fact that most butternut families derived from a seed orchard of phenotypically resistant selections, proved to be mostly susceptible indicates that healthy surviving trees in nature, while phenotypically resistant, are either not genetically resistant, or that resistance to *Ocj* in butternut has a low heritability. Hybrid families exhibit a wider range of resistance than do butternut families. The few butternut families with a canker rating of 3.5 or less are promising sources of resistance (Figure 3).

Our conclusions after the first decade of screening are: Hybrid butternut families have wide variation in resistance. Butternut families have less variation in resistance, most being susceptible and a few moderately resistant. Canker ratings after five years of *Ocj* pressure in the field reflect genetic resistance among families much better than short-term artificial inoculations. Natural disease incidence differs between bottomland sites and upland sites.

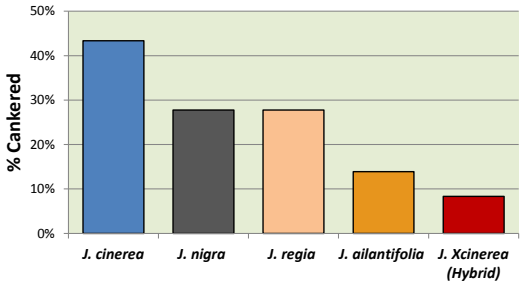

Figure 1. Canker incidence in Martell Germplasm Block nine months after fall, 2011 inoculations.

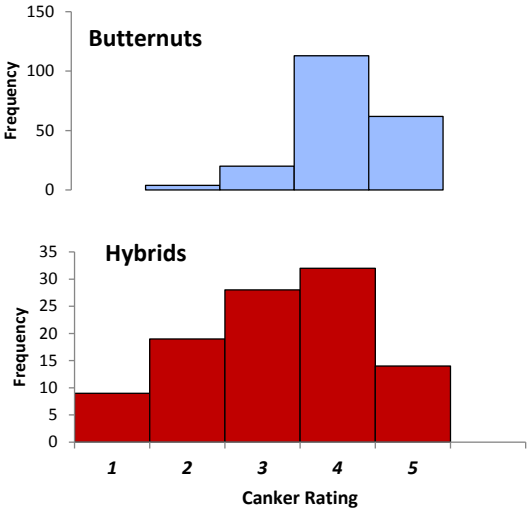

Figure 2. Histograms of the canker rating (1=highly resistant - 5= highly susceptible) for all butternut seedlings (n=199) and hybrid seedlings (n= 105) 10th growing season at Martell Forest B-Nut Block 1.

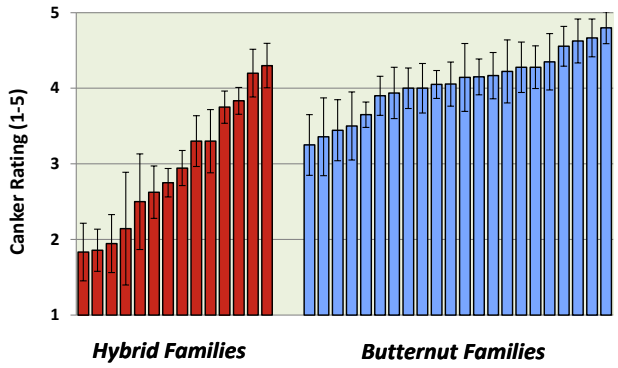

Figure 3. Average family canker rating values ± one-standard deviation for hybrid families (n=14) and butternut families (n=23) ten years after planting, Martell Forest B-Nut Block 1.

We now have additional screening blocks with more germplasm to evaluate. Inoculating seedlings at 5-years to supplement high natural *Oc* pressure, and rating cankers at 10-years should provide an efficient and robust method to select more resistant germplasm to support future restoration efforts.

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PRESENTATIONS

Ecology and Management of *Sirex* Woodwasps in Eastern North America

ATTRACTING *SIREX NOCTILIO* (FABRICIUS) USING VISUAL AND OLFACTORY SIGNALS

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ABSTRACT

The European woodwasp *Sirex noctilio* (Fabricius) (Hymenoptera: Siricidae) is a relatively recent invasive pest in North American pine forests. The objective of this study was to improve the methods used to detect *S. noctilio* adults. An aggregation pheromone has been recently discovered that attracts both male and female *S. noctilio*. The aggregation pheromone (100:1:1 ratios of Z-3-decenol, Z-4-decenol, and E,E-2,4-decadienal) was tested in a 2m x 2m x 4m wind tunnel with three different trap types (intercept panel traps and two newly designed traps) and with two different pheromone concentrations (0.1 and 1mg). Wasps were reared from logs collected in New York State and Pennsylvania and experiments were conducted with exposure to outdoor temperature and humidity, but with controlled light conditions. Trap designs were tested using no-choice flight tests in the wind tunnel, and the higher concentration pheromone (1mg) resulted in higher trap catch for all three traps, but did not increase trap efficacy in males. Females were caught in large proportions in the black intercept panel traps without the pheromone, suggesting that visual cues play a role in attracting female *S. noctilio*. This phenomenon was tested in a choice test in the wind tunnel with black and clear intercept traps being offered to the wasps at the same time, and the black traps had a greater success of catching *S. noctilio* females. When lights were added to the traps, regardless of the color of the trap, almost 100% of the wasps were captured, of both sexes.

During oviposition, female *Sirex noctilio* deposit a symbiotic fungus, *Amylostereum areolatum* (Fr.) Boid. That, along with a mucous toxin, helps to kill the tree. Attraction of *S. noctilio* to two symbiotic *Amylostereum* species was tested. A native member of Siricidae, *S. nigricornis* (Fabricius) has symbiotic associations with the native *A. chailletii* (Pers. : Fr.) Boid. and with *A. areolatum* (although the latter seems to be less frequent). When female *S. noctilio* were offered *A. areolatum* and *A. chailletii* in a choice test, mated *S. noctilio* were more attracted to their own symbiont, *A. areolatum,* but unmated females did not show the same pattern. When the native *S. nigricornis* mated females were tested in the same choice experiment, they chose *A. chailletii* over the strains of *A. areolatum* that were introduced to North America with *S. noctilio*. This suggested that these wasps are able to recognize the volatiles from their own symbiotic fungi, which may be important in species specific monitoring of Siricidae. Chemical analysis of these fungus volatiles showed many sesquiterpenes (C15) in *A. areolatum,* which were absent in *A. chailletii*. The detailed analysis of these fungus volatiles is not completed yet.

In summary, our study showed that commercially produced black intercept panel traps were more efficient tools to trap *S. noctilio* than other traps tested, and both visual and olfactory cues may play an important role in monitoring. These wasps are positively phototaxic, therefore the addition of a light source may increase trap efficacy, and volatiles from the symbiotic *A. areolatum* may also be useful as species-specific attractants in *S. noctilio* monitoring programs.

IDENTIFYING MOLECULAR PLAYERS IN PATHOLOGY PRECIPITATED BY *SIREX NOCTILIO* VENOM

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ABSTRACT

The woodwasp, *Sirex noctilio*, has devastated stands of North American pines grown in commercial plantations in the southern hemisphere. This invasive woodwasp was recently introduced to North America, and there are concerns it could lead to serious ecological, environmental, and economic losses here. A better understanding of how *S. noctilio* attacks and kills host trees will be part of any strategy to moderate and limit damage from this pest in North American forests.

During oviposition, female *S. noctilio* woodwasps introduce a secretion from the venom glands into pine hosts. Among other host responses, the venom affects photosynthetic tissues at sites distant from the site of oviposition. We have obtained evidence that the diffusible and heat-stable venom component responsible for drooping needles, the so-called wilt-inducing factor (WIF), is likely a small peptide. However, characterization of WIF requires complete purification of the factor.

To follow WIF activity during purification we have developed a biomarker assay for venom activity in pine tissues. Using a loblolly pine cDNA microarray, we identified a collection of pine genes whose transcription responded to venom application, and two of these genes, specifically encoding a pathogenesis-response class IV protein (PR4) and a thaumatin-like protein (TLP), have been used as biomarkers. The biomarker assay is substantially faster as well as more consistent, convenient, and quantitative, than the phenotypic assay described in previous literature. Using this assay, we have fractionated whole venom to a pool of heat-stable biomolecules containing a handful of small (1-3 kDa) sticky peptides.

In parallel with our venom fractionation efforts, we used Illumina DNA sequencing to characterize the transcriptome of *S. noctilio* venom gland tissue. We recovered 21.8 Gb of sequence information from which 68,887 unique clusters were assembled. Of the assembled clusters, 24,628 returned annotation from various BLAST searches. However, clusters corresponding to the five most abundant transcripts in these tissues returned no annotation information. The most abundant transcript returning an annotation most closely matched an insect laccase. Laccase activity in *S. noctilio* venom was confirmed experimentally using 1,8-diaminonaphthalene-staining of an SDS-PAGE zymogram. Sequences suggestive of the presence of *Deladenus siricidicola* in the *S. noctilio* donor tissues were also found in the RNA-Seq data. PCR analyses using DS-1 microsatellite primers for *D. siricidicola* were used to probe pools of *S. noctilio* females collected from New York, and all pools yielded amplimers matching a positive control of *D. siricidicola* (Kamona strain) genomic DNA.

This sequence data is the first generated for a member of the Symphyta, a basal suborder of the Hymenoptera. As such this data should find wide utility in comparative genomic and phylogenetic studies of the Hymenoptera. The sequence data from this study will be deposited in GenBank, and a database housing the assembly will be made publicly accessible upon acceptance of a manuscript describing this work in greater detail.

FUNGAL FIDELITY: NATIVE SYMBIONTS MEET INVASIVES

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ABSTRACT

The woodwasp *Sirex noctilio* is native to Europe and has been introduced to pine plantations in numerous countries in the Southern Hemisphere. In 2004 a specimen from an established population was collected in North America, constituting the first time that *S. noctilio* had been introduced to an area where pines, other species of *Sirex* and associates of this community are native. In eastern North America three *Sirex* species are native: *Sirex nigricornis* which prefers pines, *S. nitidus* which prefers spruce and *S. cyaneus* which prefers firs. Relatively little is known about these native *Sirex* species and we have been particularly interested in their associations with fungal symbionts. All *Sirex* are associated with white rot fungi belonging to the genus *Amylostereum*. *Amylostereum* arthrospores are carried by adult females, inserted into trees during oviposition and required by *Sirex* larvae for survival. These symbionts are thought to perform numerous functions including decreasing tree defensive reactions and wood water content. They are eaten by early instar larvae and later instars use the fungal enzymes to help degrade wood which aids in digestion. From this symbiotic association, the fungus derives transportation to new trees and it therefore rarely produces basidiocarps (i.e., external fruiting bodies on trees) that would produce dispersive sexual spores. Before the introduction of *S. noctilio* to northeastern North America previous reports stated that all North American *Sirex* were associated with *Amylostereum chailletii*. From Europe and the Southern Hemisphere S. *noctilio* was known to be associated with *A. areolatum*. Worldwide, it was assumed that each *Sirex* species was only ever associated with one species of *Amylostereum*.

We collected adult females of these four *Sirex* species from 2007-2012 from six eastern states (NY, PA, ME, WV, GA, and LA) by live-trapping with attractants or by rearing from infested wood. Fungal species and strains were evaluated using molecular techniques, with emphasis on the intergenic spacer region (IGS).. This region had been used in previous studies demonstrating differentiation between *A. areolatu* and *A. chailletii* and variability within *A. areolatum* (Slippers et al. 2002; Nielsen et al. 2009). *Amylostereum areolatum* can have multiple different IGS strains within one isolate.

We found three different IGS strains carried by *Sirex* in eastern North America: BD, D and BE. *S. noctilio* and *S. cyaneus* each carried only one *Amylostereum* species: *A. areolatum* and *A. chailletii*, respectively. *Sirex noctilio* was associated with either IGS-BD or D, which agrees with our previous work and with findings by Bergeron et al. (2011) of two variants of *A. areolatum* associated with *S. noctilio* in Ontario.

In contrast, the two other *Sirex* species, *S. nigricornis* and *S. nitidus*, each carried either of the two *Amylostereum* species. This was found for samples collected in areas outside of the distribution of *S. noctilio* (i.e., Maine, West Virginia, Pennsylvania, and Louisiana). The *A. areolatum* strain recovered from these hosts in these areas was the BE strain. These results demonstrated that the IGS-BE strain of *A. areolatum* is native to eastern North America and that a *Sirex* species is not always associated with only one fungal species.

Through this work, we also found three instances where fungi were swapped, from among 91 identified fungal samples from *S. nigricornis* or *S. noctilio* where these woodwasps occurred sympatrically. In these cases *S. nigricornis* emerging from pines co-infested with *S. noctilio* carried the IGS-D strain of *A. areolatum*, which is usually carried by *S. noctilio*. These three cases were among 52 *S. noctilio* or *S. nigricornis*

emerging from the same pines in our rearings for which we identified the fungal species and IGS strain. We never found *S. noctilio* carrying anything but the two strains of *A. areolatum* assumed to have been introduced with this species. Thus, the native *S. nigricornis* was found to occasionally carry a fungal strain introduced with *S. noctilio*.

We investigated further how fungal swapping can occur. First, *S. nigricornis* and *S. noctilio* occur in the same pines: from our 2007-2011 rearings, both of these species occurred in 25.9% of 58 pines. Next, *S. noctilio* usually oviposits before *S. nigricornis,* so its fungus is already established within trees when *S. nigricornis* oviposits: in our 2011 rearings in New York State, almost all *S. noctilio* emerged from pines in July and August while *S. nigricornis* predominantly emerged from pines in September. Behavioral choice tests being conducted in our laboratory have shown that *S. nigricornis* does not seem to avoid ovipositing into areas of pines near where *Amylostereum* strains carried by *S. noctilio* occur. Finally, *Sirex* eggs and larvae can occur very densely in trees. Therefore, we hypothesize that *S. noctilio* oviposits into pines first and if *S. nigricornis* oviposits into the same tree, near locations where *S. noctilio* has oviposited, developing *S. nigricornis* would be associated with the fungus originally inoculated into the wood by *S. noctilio*.

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NEW ASSOCIATIONS BETWEEN *DELADENUS* NEMATODES, THEIR *SIREX* HOSTS, AND FUNGAL SYMBIONTS

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ABSTRACT

Sirex noctilio, an invasive woodwasp that is a pest of pine (*Pinus* spp.), was collected in New York State in 2004 (Hoebeke et al. 2005). This pest is spreading and potentially threatens 200 million hectares of U.S. and Canadian pine forests. In the Southern Hemisphere, where *S. noctilio* has been an invasive pest of introduced pine plantations for many decades, it has been successfully controlled in biological control programs using the parasitic nematode *Deladenus siricidicola* (Hurley et al. 2007). This nematode is being explored for use in the North American invasion of *S. noctilio*; however, since this is the first introduction of the woodwasp to a native range of conifer hosts, developing a control program is more complicated (Williams et al. 2012).

Sirex noctilio is able to kill healthy pine trees through the combined use of phytotoxic venom and a symbiotic plant-pathogenic fungus, *Amylostereum areolatum*, which it injects into pine trees during oviposition. The life cycle of the parasitic nematode *D. siricidicola* also involves the symbiotic fungus. This nematode has a complex life cycle comprised of a fungal-feeding form, which lives in the tree and feeds on *A. areolatum*, and a parasitic form, which invades *S. noctilio* larvae. Upon pupation of a parasitized *S. noctilio* larva, nematodes migrate to the reproductive organs of the host, ultimately leading to the sterilization of adult female *S. noctilio* (Bedding 2009).

The presence of native species of *Sirex* in North America, which are not considered pests (Furniss and Carolin 1977; Madden 1988), makes it important to study possible non-target effects, should *D. siricidicola* be released for biological control. Native eastern *Sirex* species include *S. nigricornis*, *S. nitidus*, and *S. cyaneus* (Schiff et al. 2012). Of these, *S. nigricornis* is commonly found coinfesting pine trees along with invasive *S. noctilio*. The native *Sirex* species carry their own species and strains of *Amylostereum*. It was initially thought that North American *Sirex* only had a symbiotic relationship with *A. chailletii*, which would effectively shield them from *D. siricidicola* which only eats *A. areolatum*. However, more recently it was discovered that *S. nigricornis* and *S. nitidus* also can be associated with *A. areolatum* (Nielsen et al. 2009; Hajek et al. submitted) .This species of fungus has been identified according to IGS strain, showing that native *Sirex nigricornis* can be associated either with the strains of *A. areolatum* introduced to North America with the *S. noctilio* invasion, or they can be associated with a previously unknown strain of *A. areolatum* that is native to North America. Another complication is the existence of native *Deladenus* nematode parasites of North American *Sirex*, as well as a non-sterilizing strain of *D. siricidicola*, which was introduced with the invading *S. noctilio* (Yu et al. 2009).

In order to study the associations between the multiple species and strains of *Amylostereum* fungus and *Deladenus* nematodes in eastern North American *Sirex*, collections of the woodwasps were made in New York, Pennsylvania, Maine, and Louisiana. The *Sirex* were dissected to determine presence of nematodes. Mitochondrial and nuclear DNA was extracted from the nematodes, and the cytochrome oxidase I (mtCOI) gene and internal transcribed spacer (ITS) region were amplified and sequenced. Sequences were used to construct a phylogeny (maximum likelihood, Bayesian, and maximum parsimony) of *Deladenus* nematodes in eastern North American *Sirex*. It was found that each *Sirex* species has its own associated *Deladenus* nematode, and there were three incidences of nematodes parasitizing an unexpected *Sirex* host. In two cases, *S. noctilio* were found to be parasitized with *D. proximus*, the nematode associated with *S. nigricornis*, and in another case, a *S. nigricornis* was parasitized with the non-sterilizing strain of *D. siricidicola.*

To clarify the role that fungal species and strain may be playing in these interactions between woodwasp and fungus coinfesting trees, cultures of *D. siricidicola* and *D. proximus* were grown on various *Amylostereum* isolates. When *D. siricidicola* was grown on invasive strains of *A. areolatum*, (IGS-BD and IGS-D), as well as the native strain (IGS-BE), greater reproduction occurred when *D. siricidicola* fed on the native IGS-BE strain (Morris et al. 2012). This could mean that native siricids associated with the native IGS-BE strain can be in close proximity with *D. siricidicola*. This result of *D. siricidicola* feeding on the native strain of *A. areolatum* also could aid in the successful reproduction of *D. siricidicola*, should it be released. When the native nematode *D. proximus* was grown on different strains of *A. areolatum* as well as two isolates of *A. chailletii*, it was discovered that in addition to being able to reproduce on *A. chailletii*, the nematode is able to reproduce on the BE strain of *A. areolatum*. This is the first experiment to demonstrate that *D. proximus* can survive on a fungus other than *A. chailletii*. Interestingly, *D. proximus* was unable to reproduce on strains of *A. areolatum* originating from *S. noctilio*. These associations between *Sirex*, *Amylostereum*, and *Deladenus* may have impacts on the success of using *D. siricidicola* as part of a biological control program against *S. noctilio* in North America.

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LIFE HISTORY, OVIPOSITION PREFERENCES, AND INCIDENCE OF NEMATODES OF *SIREX NIGRICORNIS*

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ABSTRACT

Sirex nigricornis is a pine-inhabiting woodwasp native to the southeastern United States. Species in this genus are traditionally not considered pests, owing to their tendency to colonize dead, or dying, trees. However, in 2004, a non-native woodwasp, *S. noctilio*, was discovered in the northeastern US. This insect has successfully invaded exotic pine stands in the Southern Hemisphere where it has often caused severe damage. Its potential impact on pine forests in the southeastern United States is not yet known.

Our goals are to study phenology, oviposition behavior, mortality factors and interactions of our native *S. nigricornis* with their existing pine-inhabiting associates. We will examine these interactions to assess the biological similarities of our native species with *S. noctilio* in order to predict the likelihood of displacement, should *S. noctilio* reach the Southeast.

In order to establish phenology of adult flight, from 2009 through 2012 we placed baited panel traps across three regions of Arkansas. Females were collected in propylene glycol and held for dissection. We also felled trap trees in these locations, which were left intact for a year before being held in laboratory rearing containers, to obtain emergence data.

Each year of trap catch data showed a peak in emergence in mid-October with numbers dropping off by mid-December. However, 2012 showed an unexpected peak in mid- to late November in the Ouachita National Forest that was not seen any other year. Causes of this late peak have not been established.

Adult females from trap catches were dissected to examine ovaries for presence and abundance of the native nematode associated with *S. nigricornis, Deladenus proximus* Bedding. Nematodes were confirmed in all three sampled regions of Arkansas. Percent of parasitized females ranged from 3.6% (south Arkansas) to 43.2% (Ozark National Forest). Females contained nematodes in the haemocoel as well as in their eggs. Percent of eggs parasitized ranged from 0% (nematodes only present in the haemocoel) to 100%.

Live-trap containers, with exposed and mesh-covered bolts, were created to capture pine-associates in the field. Traps were baited with pine volatiles (ethanol and α-pinene) and *Ips* pheromones (ipsenol, ipsdienol, lanierone). This allowed for oviposition by any associates attracted to the trap. Bolts with and without field oviposition were exposed to siricid adult mating pairs and oviposition was examined. Oviposition by siricid females was greatest in bolts that did not have evidence of oviposition by other insects. Moisture content (MC) was also examined in relation to oviposition. Females preferred to oviposit in bolts that were left in the field for 15 days with an average of 70% MC. Development of siricids was only observed in bolts that were 15 days old.

COMMUNITY AND CHEMICAL ECOLOGY OF NATIVE SIRICIDS AND THEIR PARASITOIDS IN THE SOUTHEASTERN UNITED STATES

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ABSTRACT

Sirex noctilio Fabricius (Hymenoptera: Siricidae) is an introduced woodwasp present in the Great Lakes region of North America. This woodwasp is considered a secondary pest in its native range in Eurasia and North Africa, but a primary pest in its non-native range in southern hemisphere where pine trees (*Pinus* spp.) are planted. Mortality of pine trees has been attributed to *S. noctilio* in the southern hemisphere, especially in unthinned stands that are under drought-stress. *Sirex noctilio* may pose a threat to areas in the southern U.S. pine forests where similar stand and climatic conditions may exist. Our rearing studies of *S. noctilio* and subsequent hazard maps from host preferences studies on major southern pine species suggest that they may be susceptible to invasion by *S. noctilio*. Management techniques for *S. noctilio* include silvicultural practices, and use of entomophagous nematodes and hymenopteran parasitoids as biological control agents. In addition, native bark and woodboring insect communities that are species-rich and abundant in the southern forests may provide competitive pressure to *S. noctilio*. Our overarching goal for this project is to better understand the community and chemical ecology of native woodwasps and their hymenopteran parasitoids in the southern U.S. before *S. noctilio* is found in this conifer-dominated region.

Research Objective 1: To compare the species complex of native woodwasps and their hymenopteran parasitoids in the Piedmont and Coastal Plain regions of the southern region. We established sampling plots in three states: Georgia (Piedmont region), and Louisiana and Virginia (Coastal regions) in fall 2009. In each state, as depending upon the year of sampling (2009-2011), we tested various combinations of trap-types (santé, intercept panel, and funnel traps) and lure-types (ethanol, *Sirex* lure, Ipsenol, Ipsdienol, and host material) on the catches of wasps. Overall, we found that greater numbers of woodwasps were caught with baited traps with *Sirex* lure; addition of ethanol to the trap did not enhance catches. Host material in the form of small pine billets and needles placed in a mesh bag next to the intercept panel trap was more effective than the *Sirex* lure as it greatly increased catches of woodwasps. It is likely that certain compounds present in trees may be absent in the *Sirex* lure and that there may be differences in elution rates between two bait types.

During 2009-2011, we created trap-logs from 10-12 trees in each state to assess the phenology of woodwasps and their parasitoids. All trees were left in the field for a year, and were placed next fall in emergence cages to rear out insects. Emergence results suggest a fall emergence for *S. nigricornis* Fabricius (native woodwasp) and *Ibalia leucospoides ensiger* (Norton) (a parasitoid) with greatest emergence in mid- to late-

October depending upon the region. Male *S. nigricornis* emerged first followed by females, and then *I. l. ensiger*. We also placed emergence cages over cut stumps, but did not rear out any insects. In 2009, we tested the effects of different methods and timings of inducing tree mortality on the colonization activity of wasps. We girdled and applied herbicide (dicamba) to four loblolly pine trees (*P. taeda* L.) between 1 September and 1 October 2009; cut down four trees during the week of 2 November 2009; and another four trees during the week of 16 November 2009. Greater numbers of *S. nigricornis* and *I. l. ensiger* were reared from trees cut down in early November, suggesting that trap-logs created in the early part of the woodwasp flight season may be optimal.

Research Objective 2: To synthesize literature on ecology and biology of parasitoids of woodwasps in North America. We assessed the distribution range of parasitoids of woodwasps in the subfamily Siricinae (only on conifers), and found that four species including *Rhyssa persuasoria* (L.), *R. lineolata* (Kirby), *Megarhyssa nortoni* (Cresson), and *I. l. ensiger* are most widely distributed in North America. Similarly, we assessed numbers of parasitoid species on siricid and conifer hosts, and found that three species including *R. persuasoria*, *M. nortoni*, and *I. l. ensiger* have the greatest number of hosts. Hence, these three species along with *R. lineolata* may exert the greatest pressure on *S. noctilio* depending upon site, and abiotic and biotic conditions in North America.

Research Objective 3: To assess the chemical cues used by parasitoid species to locate native woodwasp hosts in pine trees. *Ibalia l. ensiger* is a koinobiont parasitoid that oviposits on either the eggs or first instar woodwasps. We therefore hypothesize that *I. l. ensiger* may be cuing to the actual oviposition site of woodwasps, and perhaps to the relatively large volume of phytotoxic mucus inoculated in trees by ovipositing woodwasps. We cut-down four trees at different times in fall in Louisiana. Each tree was cut into logs to which three treatments were each randomly assigned as follows: 1) sterile water drilled into log; 2) mucus taken from the venom glad of *S. nigricornis* and drilled into log; and 3) fresh oviposition site of *S. nigricornis*. SPME fibers were attached to each 4-5 treatment location on the log, and volatiles were collected every 2, 4, 8, and 16 days. GC-EAD was used to assess chemicals present in the volatiles and responses of antennae of female *I. l. ensiger* to these compounds. Preliminary results for the GC-trace from the oviposition site of *S. nigricornis* indicated that pine volatiles such as monoterpenes eluted first followed by oxygenated monoterpenes. Antennae of *I. l. ensiger* appeared to the responsive to both the kinds of terpenes. However, further work on synthetic compounds found in previous assays suggested that the antennae of *I. l. ensiger* were responsive to several oxygenated monoterpenes. Our future work will focus on the responses of female *I. l. ensiger* to mucus gland, and to further assess the importance of oxygenated monoterpenes to host location activities by *I. l. ensiger*.

POSTERS

ASSESSING PHENOLOGY AND RELATIVE ABUNDANCE OF *TETRASTICHUS PLANIPENNISIS*, AN INTRODUCED PARASITOID OF EMERALD ASH BORER

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ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive wood-boring beetle from Asia responsible for the mortality of millions of ash trees in North America. The larval parasitoid *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), native to northeast China and the Russian Far East, is being released in Michigan and other states as a biological control agent of EAB. Since it was first introduced in 2007, the establishment and spread of *T. planipennisi* in EAB populations has been confirmed at several study sites in Michigan and other states. Knowledge of the phenology of *T. planipennisi* adult flight, however, can be valuable in optimizing methods being used to evaluate its establishment, prevalence, and interactions with native parasitoids such as *Atanycolus* spp. (Hymenoptera: Braconidae). The objectives of this study were to monitor the phenology and relative abundance of the larval parasitoids *T. planipennisi* and *Atanycolus* spp. at Michigan study sites. Small ash logs, each containing three sentinel EAB larvae, were deployed biweekly at study sites where establishment of *T. planipennisi* had been confirmed since 2009. One study site was sampled in 2011 (Moore Park) and two study sites in 2012 (Moore and Burchfield Parks).

In 2011 at Moore Park, *Atanycolus* spp. were present from June to mid-September, parasitizing up to 80% of sentinel larvae and *T. planipennisi* parasitized <1%. In 2012 an additional site, Burchfield Park, was added to the study. Interestingly, *Atanycolus* spp. were much less abundant at Moore Park in 2012, and accounted for up to 20% larval parasitism while *T. planipennisi* parasitized up to 50% of the sentinel larvae. At Burchfield Park, larval parasitism by *Atanycolus* spp. was <1%, while *T. planipennisi* parasitized up to 70% throughout the sampling period (July to mid-October). These results showed that EAB larval parasitism by *Atancolus* spp. varied between sites and declined from 2011 to 2012, whereas parasitism by *T. planipennisi* increased. EAB larval parasitism by *T. planipennisi* occurred nearly continuously at high rates from July to early-October in 2012 at both study sites, completing several generations (the same as observed in its native range), with peak larval parasitism from mid- to late-August.

THE EFFECT OF BARK THICKNESS ON PARASITISM OF TWO EMERALD ASH BORER PARASITOIDS: *TETRASTICHUS PLANIPENNISI* AND *ATANYCOLUS* SPP.

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive wood-boring beetle from Asia killing ash trees (*Fraxinus* spp.) in North America. The larval parasitoid *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) is one of three EAB parasitoid species from China being released for biological control of EAB in the United States. Recent studies in Michigan show increasing parasitism of EAB larvae by species of *Atanycolus* (Hymenoptera: Braconidae), which are native ectoparasitoids of *Agrilus* larvae.

At EAB biocontrol study sites in central Michigan, EAB larval parasitism by *T. planipennisi* is more common in smaller than in larger diameter ash trees, whereas *Atanycolus* is unaffected by tree diameter. Since parasitoids of wood-boring insects must drill into tree trunks, ovipositor length, bark thickness, and host depth affect their ability to reach and successfully parasitize hosts. We evaluated the effect of bark thickness on EAB larval parasitism by *T. planipennisi* (ovipositor length range = 2.0 to 2.5 mm) and *Atanycolus* (ovipositor length range = 4 to 6 mm).

In spring 2011, we grafted EAB eggs onto trunks of small and large green ash trees (*F. pennsylvanica*). Later in the summer, we caged either *T. planipennisi* or *Atanycolus* adults on tree trunks where the EAB larvae were feeding. When the larvae were mature in the fall, we debarked the lower tree trunks, measured the thickness of the outer bark, and collected the larvae to determine parasitism. We found *T. planipennisi* parasitized EAB larvae in green ash trees up to 11.2-cm in diameter breast height (DBH), which correlates to outer bark thicknesses up to 3.2 mm. *Atanycolus* parasitized EAB larvae in ash trees up to 57.4-cm DBH, which correlates to outer bark thicknesses up to 8.8 mm. These results indicate that establishment and spread of *T. planipennisi* is more likely at release sites dominated by small, early successional or regenerating ash trees than at sites with only large, mature ash trees. Moreover, this study demonstrates that sustainable term management of EAB in North America will require a diverse natural enemy complex including native parasitoids, which may become important allies in controlling EAB.

DETERMINING ESTABLISHMENT AND PREVALENCE OF PARASITOIDS RELEASED FOR BIOLOGICAL CONTROL OF THE EMERALD ASH BORER

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ABSTRACT

The emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire), an invasive buprestid from Asia, is causing widespread mortality of ash trees in North America. Classical biological control of EAB in the United States started in 2007 with release of three hymentopteran parasitoids from China: *Oobius agrili* Zhang and Huang (Encyrtidae), *Tetrastichus planipennisi* Yang (Eulophidae), and *Spathius agrili* Yang (Braconidae). Release of these species started at study sites in Michigan, and has since expanded to the other EAB-infested states. Using destructive and non-destructive sampling methods, establishment of at least one of these parasitoid species is confirmed in Michigan, Ohio, Indiana, Illinois, and Maryland.

Different methods are needed for detection of egg vs. larval parasitoids. The egg parasitoid *O. agrili* can be detected in the field by 1) sampling EAB eggs from bark of EAB-infested ash trees and determining which eggs are parasitized, 2) rearing *O. agrili* from bark or log samples in the laboratory, and 3) hanging egg-sentinel logs (ESLs, small ash logs on which EAB eggs were laid) on ash trees. Using ESLs, we have tracked *O. agrili* parasitism seasonally and spatially since 2009 at higher prevalence than determined by other detection methods. The larval parasitoids *T. planipennisi* and *S. agrili* can be detected in the field by 1) debarking infested ash trees and assessing parasitism of each larva, 2) rearing adult wasps from ash logs in the laboratory, 3) hanging larval-sentinel logs (LSLs, small ash logs in which EAB larvae were inserted) on ash trees, and 4) placing yellow pan traps (YPTs) on ash trees in late summer or fall to catch adult parasitoids, which are later identified. Tree debarking provides the most data on parasitoid species attacking EAB larvae, however, LSLs and YPTs may be useful for detecting the presence of adult larval parasitoids if ash trees are too scarce to sample.

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

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ABSTRACT

The emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) is an invasive buprestid from Asia causing extensive mortality of ash trees (*Fraxinus* spp.) in areas of North America. For sustainable management of EAB, a classical biological control program began in Michigan in 2007 with the release of three hymenopteran parasitoids of EAB from China. Biocontrol has since expanded to other EAB-infested states. Given the high population densities of EAB and the limited resistance of North America ash species to it, more management methods are needed to assure survival and recovery of *Fraxinus* spp. To this end, we are working to develop a microbial insecticide made from the insect-pathogenic bacterium *Bacillus thuringiensis* (Bt) strain SDS-502, which is toxic to EAB adults. Due to the narrow host range of Bt, this bacterium is used worldwide to control specific insect pests in agricultural, riparian, and forested ecosystems (e.g. aerial sprays to control gypsy moth), and after decades of use it continues to have a good safety record with respect to human health and the environment.

Previously, we reported on the toxicity and mode of action of Bt SDS-502 in EAB adults, the lack of Bt toxicity in adult hymenopteran parasitoids, and the efficacy of Bt-test formulations sprayed ash leaves and fed to EAB adults. We now report on the mortality response of EAB adults to three concentrations of a wettable-dispersable granular (WDG) formulation of Bt SDS-502 (Bt-WDG contained 50% Bt-technical powder, Lot #PHY-3-11) sprayed using a rotary atomizer (Micronair ULVA+). One-mL aliquots of each Bt concentration (25-, 50-, 100-mg Bt-WDG /mL 10% sucrose solution) were pipetted into the sprayer reservoir, sprayed on greenhouse-grown ash leaves (*F. uhdei*), and fed to EAB adults. After 7 d, EAB mortality was 20, 43, and 50% at each concentration, respectively whereas control mortality was <3% for the WDG-blank formulation and 10% sucrose. Time to death averaged 4.3 d, and was similar at the three Bt-WDG concentrations. By increasing the concentration of Bt SDS-502 in the formulation and designing a formulation for aerial application, we anticipate higher levels of EAB-adult mortality in the laboratory. Once this is achieved, further testing of the formulation in the field will be needed.

ESTABLISHMENT OF *OOBIUS AGRILI*, AN INTRODUCED EGG PARASITOID OF THE EMERALD ASH BORER, IN THE UNITED STATES

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ABSTRACT

The emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) is an ash-killing beetle from Asia discovered in 2002 in Michigan and Ontario and now known in 18 states and two provinces. Foreign exploration, as part of a classical biological control effort for long-term management of EAB, began in 2003 when researchers sought out its natural enemies in China. One of three hymenopteran parasitoids found attacking EAB in northeast China was *Oobius agrili* Zhang & Huang (Encyrtidae), a solitary and parthenogenic egg parasitoid. After studying its biology and host specificity in the laboratory, permission was granted for release of *O. agrili* at field sites in Michigan's Lower Peninsula in 2007. Releases of *O. agrili* soon expanded to Ohio in 2008, Maryland in 2009, Michigan's Upper Peninsula in 2010, and Pennsylvania in 2011. A total of 100 to 700 adult females were released at sites in the summer when EAB eggs were being laid.

Methods used to determine *O. agrili* establishment and to study its prevalence, distribution, and phenology in the field included: 1) sampling EAB eggs from the bark of infested ash trees, 2) rearing adult parasitoids from log or bark samples, or 3) placing EAB eggs (sentinel eggs) on ash trees. As of fall 2012, the establishment of *O. agrili* was confirmed at study sites in Michigan's Lower and Upper Peninsulas (as far north as Houghton), Ohio, and Maryland, and overwintering was confirmed in Pennsylvania. At one release plot in Lower Michigan, where *O. agrili* was released 2007 and 2008, egg parasitism averaged ~25% by August 2011, as measured in sentinel eggs placed on ash trees. Although samples of naturally occurring EAB eggs recovered lower a lower prevalence of parasitized eggs, *O. agrili* was recovered on ~65% of sampled ash trees, indicating an important role for this parasitoid in managing EAB and ash health in the U.S.

POTENTIAL OF NURSERY STOCK REGULATORY TREATMENTS FOR ASIAN LONGHORNED BEETLE

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ABSTRACT

Introduction. Due to the wide scope of the ALB infestation in Worceser, MA (110 sq mi), commercial nurseries are now being impacted by quarantine restrictions on the movement and sale of nursery stock. Treatments were tailored to the needs and constraints of the nursery industry and were developed in order to better understand the dynamics of uptake and resultant residues from applied insecticides.

In 2011, elms, red maples and sugar maples averaging 2.0", 1.8" and 1.2" in DBH were randomly assigned to treatment groups consisting of four neonicotinoid insecticides applied by either basal soil injection at high and low rates or as a bark spray. Initially, the treatment groups contained 13, 14, 15 trees each for the elms, red maples and sugar maples, respectively. The trees were to receive two years of consecutive spring treatments.

Basal soil injection. In-ground trees were dosed with two rates of soil-applied neonicotinal products (imidacloprid, clothianidin, thiamethoxam, dinotefuran) for two years and the foliage was sampled twice during the summer of each treatment year to monitor tree uptake of chemical residue from the treatments.

Results after one year of treatment showed that the higher treatment rate of imidacloprid and dinotefuran produced sufficient pesticide residue in all three tree species to kill adult ALB. Results for the other two chemicals were poor to marginal and residue tended to decrease in those treatments between the June and August foliage collections. Significant increases in residue levels in the sugar maples for both the imidacloprid high and low rates were observed when the first to second year August residue levels are compared, with elms displaying a similar trend. The second year of treatments may also elevate subpar residues of the other chemicals to acceptable levels, and that data analysis is on-going.

Bark spray applications. A concentrated mix of the chemicals was applied to the lower 4 feet of trunk with a split sprayer oriented such that chemical could be applied to each side of the trunk.

Imidacloprid produced excellent residue levels in elm and red maples but substantially lower values in sugar maples. Dinotefuran had adequate levels of residue for the season in the three species, however August residue for elm and sugar maples was subpar. Clothianidin achieved adequate levels of residue in the June sampling of elm and red maples, other values were well below desirable levels. Thiamethoxam produced adequate levels of residue in the elms but failed to produce much above detectable levels for the red and sugar maples.

MOBILE APPLICATIONS THAT ENGAGE USERS WITH INVASIVE SPECIES, FOREST HEALTH, NATURAL RESOURCE AND AGRICULTURAL MANAGEMENT

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ABSTRACT

Bugwood Apps are available for iPhone, iPad and Android systems and enable users to have access to identification information and to report sightings of invasive species from their smartphone. Reports include images, are georeferenced and are incorporated into the EDDMapS mapping system (*www.eddmaps.org*). Established EDDMapS protocols are used for verification of reports, follow-ups as necessary and subsequent access/viewing of report data by the public.

EDDMapS combines data from other databases and organizations as well as volunteer observations to create a national network of shared invasive species distribution data.

EDDMapS has over 1.9 million records and is being used in 40 states and in three Canadian provinces.

UTILIZING PARTNERSHIPS AND INFORMATION TECHNOLOGY TO ADVANCE INVASIVE SPECIES, FORESTRY, AND AGRICULTURE EDUCATION

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ABSTRACT

The mission of the Center for Invasive Species & Ecosystem Health [aka: The Bugwood Center] is to serve a lead role in development, consolidation and dissemination of information and programs focused on invasive species, forest health, natural resource and agricultural management through technology development, program implementation, training, applied research and public awareness at the state, regional, national and international levels. Bugwood Center personnel develop and carry-out educational programs as well as work with and support educational needs of other educators, practitioners and others through hosting 30 websites on a wide-array of topics that received over 250 million hits and served information to over 9.3 million users during 2011.

The Bugwood Center hosts several information technology tools that can be used by scientists, educators, and practitioners around the world to support their programs and needs. These tools include:

- Bugwood Image Database (*http://images.bugwood.org/*);
- Bugwood (Smartphone) Apps (*http://apps.bugwood.org/*);
- BugwoodWiki (*http://wiki.bugwood.org*); and
- Early Detection and Distribution Mapping System (EDDMapS - *www.eddmaps.org*).

The Bugwood Center information technology systems/tools are database-driven, fully-integrated, and are widely used by others who also help build them through collaboration.

CLASSICAL BIOLOGICAL CONTROL OF THE INVASIVE EMERALD ASH BORER WITH *TETRASTICHUS PLANIPENNISI* IN MICHIGAN

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ABSTRACT

Tetrastichus planipennisi Yang is a gregarious larval endoparasitoid native to China, and has been introduced to the United States since 2007 for classical biological control of the invasive emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, an exotic beetle responsible for widespread ash mortality. From 2007 to 2010, *T. planipennisi* adults (3,311 – 4,597 females and ≈1500 males) were released into each of six forest sites in three counties (Ingham, Gratiot, and Shiawassee) of southern Michigan. Each site was comprised of a parasitoid-release and non-release control plot. Following the parasitoid releases, two to six EAB-infested ash trees (DBH 8.7 – 12.1 cm) at each study plot were felled annually, either in the fall or early spring. Each tree, including the main trunk and branches >3 cm in diameter, was debarked with a drawknife and examined for the presence of immature stages of EAB and visible stages of *T. planipennisi*.

In fall 2012, two to five years after the parasitoids were released at the six study sites, 92% and 83% of sampled trees contained at least one brood of *T. planipennisi* in the parasitoid-release and control plots, respectively. Similarly, the mean number of *T. planipennisi* broods observed from sampled trees increased from less than one brood per tree in the first year (2009 fall for Ingham county sites and 2010 for other sites) after parasitoid releases to 2.46 – 3.08 broods in the 3rd or 4th year (2012 for all sites) after parasitoid releases. The prevalence of EAB larval parasitism by *T. planipennisi* also increased sharply from 1.2% in the first year after parasitoid release to 21.2% in the parasitoid-release plots in the fourth year, and from 0.2% to 12.8% for the control plots. Our findings demonstrate that *T. planipennisi* is established in southern Michigan and that its populations are increasing and expanding. Thus, *T. planipennisi* is well-positioned to play a critical role in suppressing EAB populations in Michigan.

SPREAD OF WINTER MOTH IN THE NORTHEASTERN UNITED STATES

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ABSTRACT

We analyzed data on defoliation by winter moth, *Operophtera brumata* L, and numbers of males captured in pheromone-baited traps in Massachusetts. We used three different techniques to estimate that this species was spreading south and west at a rate of approximately 7 km per year. Over the last 8 years, defoliating populations have spread south onto Cape Cod and into Rhode Island. Captures in pheromone-baited traps show that winter moth is spreading steadily west in central Massachusetts, but there has been no sign of defoliation in this region thus far. Perhaps winter moths are prevented from attaining outbreak densities by winter temperatures in the interior, much as they are restricted from interior Maine and New Brunswick. New outbreak populations of winter moth appeared this year in coastal Maine, perhaps due to the exceptionally warm conditions that prevailed last winter.

THE EFFECT OF MATURATION AND AGING ON FUNGAL INFECTION IN THE ASIAN LONGHORNED BEETLE, *ANOPLOPHORA GLABRIPENNIS*

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ABSTRACT

Asian longhorned beetles, *Anoplophora glabripennis* (ALB), are invasive wood borers from China that have been detected in five US states (NY, IL, NJ, MA and OH), Ontario, Canada, and 6 European countries (Austria, Germany, France, Netherlands, the United Kingdom, and Switzerland). *A. glabripennis* has the potential to cause extensive damage in urban and natural forests if it becomes established in North America. The entomopathogenic fungus *Metarhizium brunneum* (formerly *Metarhizium anisopliae*) is pathogenic to *A. glabripennis*. Several insect species, including male crickets (*Gryllus assimilis*) (Park et al., 2011) and male *Tenebrio molitor* (Daukste et al., 2012) exhibit immunosenescence (immune system deterioration due to aging), leading to reduced ability to combat pathogen infection. *A. glabripennis* adults undergo a period of sexual immaturity for 1-2 weeks after eclosion and can be long-lived; we were interested in determining whether adult susceptibility to fungal infection changes due to sexual maturation or aging.

For this experiment, 1-7 week old adult ALB were inoculated with *M. brunneum* F-52 (Novozymes) at two doses, a 'medium' dose of 5 x 10^5 spores/ml (for adults 1, 2, and 5 weeks since eclosion, with 10 beetles per sex per age plus controls, for two replicates, censored at 49 days) and a higher dose of 1 X 10^7 spores/ml (for adults 1, 2, 4, 6 & 7 weeks since eclosion, with 10 beetles per sex per age group plus controls, for two replicates, censored at 30 days). Beetles were inoculated by dipping them into conidial suspensions, and they were then provided with food and checked for mortality daily.

One week old beetles (both males and females) exposed to the medium dose and one week old males exposed to the higher dose of *M. brunn*eum died significantly faster than 2 week old beetles. As adult *A. glabripennis* are sexually mature by 2 weeks of age, it is possible that one week old beetles' immune systems are not fully functional. These results suggest that young (presumably reproductively immature) beetles could have reduced immune function. Future research is needed to increase replication and determine why 1 week old beetles inoculated with *M. brunneum* die more quickly than most older beetles. Future experiments will also include adults older than 7 weeks and will further examine the effects of aging and reproduction on the immune system.

EVALUATING SEASONAL VARIATION IN BOTTOM-UP AND TOP-DOWN FORCES AND THEIR IMPACT ON EXOTIC WOOD BORERS

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ABSTRACT

It is well understood that forces from multiple trophic levels simultaneously influence the performance of herbivores. However, it is less well understood how the strength of these different forces may vary over space and time. We used manipulative field experiments to evaluate the influence of bottom-up factors (host condition: stressed or healthy), top-down factors (exposure to natural enemies) and competition on the performance of an exotic wood-boring beetle. We also evaluated how the relative impact of these factors varied seasonally. *Tetropium fuscum* (F.) (Coleoptera: Cerambycidae) eggs that were either protected from or exposed to natural enemies and competitors were placed on healthy or stressed (i.e., girdled) red spruce, *Picea rubens* Sarg., trees at three different times during the natural *T. fuscum* flight period. A specific bottom-up mechanism that may influence *T. fuscum* performance was also evaluated by measuring the length of lesions (i.e., hypersensitive response) that developed in response to attacking *T. fuscum*. Top-down forces and competition were negligible in this system and generally did not vary between the three attack dates or with host condition. As predicted, *T. fuscum* performance was usually greater, and induced host defences lower, on stressed than on healthy trees, but effect size depended on the timing of attack. Timing of attack was critical, influencing all measures of *T. fuscum* performance. Survival was reduced when *T. fuscum* attacked too early or late, which may result in stabilizing selection for attack time and attack synchrony in this species. Our results suggest that timing of attack is critical for wood-borers and should be considered when examining the relative impacts of top-down and bottom-up forces on herbivore performance in other systems. Timing may be especially critical for herbivores experiencing more intense selective pressure from competitors and natural enemies than we observed in this study.

MODELING SUBCORTICAL COLONIZATION PATTERNS OF *AGRILUS PLANIPENNIS*

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ABSTRACT

Emerald ash borer (*Agrilus planipennis* Fairmaire), a beetle native to Asia, was first detected in southeast Michigan in 2002 and has since killed millions of ash trees (*Fraxinus* spp.) throughout eastern North America. Determining the extent and density of outlier infestations of *A. planipennis* is a challenging aspect of effective management and slowing the rate of spread. Here, we model the probability of detection of *A. planipennis* larvae from measurements of tree characteristics on 71 girdled trees at a low-density outlier infestion in southwestern New York State. We conducted intensive tree sampling by one meter increments, recording the presence of larvae, height above ground, diameter, and bark roughness for each individual bolt during spring of 2011 and 2012. We modeled presence or absence of *A. planipennis* using logistic regression with a binomial response distribution. Probability of detection was modeled with a quadratic equation to detect the maximization, or vertex, of the function. Analyzing height and diameter separately, the probability of *A. planipennis* detection was maximized at 18.8 cm for diameter and 13.8 m for height. There was no conclusive relationship between bark roughness and larval presence. Our results can be used by resource managers to improve efficiency of detection efforts when evaluating new *A. planipennis* infestations.

SUPERCOOLING IN THE REDBAY AMROSIA BEETLE (*XYLEBORUS GLABRATUS*): PRELIMINARY IMPLICATIONS OF INVASION POTENTIAL IN NORTH AMERICA

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ABSTRACT

The redbay ambrosia beetle, *Xyleborus glabratus* Eichoff, (Coleoptera: Curculionidae: Scolytinae) is an invasive pest of North America trees and shrubs in the Lauraceae family. The beetle vectors the fungal pathogen, *Raffaelea lauricola*, the causative agent of Laurel Wilt Disease (LWD), a disease lethal to millions of our native trees. To begin to characterize the North American invasion potential of *X. glabratus,* the supercooling point (SCP) was experimentally determined on summer tested and artificially cold hardened specimens. Summer tested beetles were trapped using Lindgren funnel traps baited with manuka oil lures. Testing was performed June through August 2011. Specimens obtained for cold hardening were reared from symptomatic redbay (*Persea borbonia*) bolts and cold hardened in a low temperature incubator for ~1 month. Summer tested and cold hardened *X. glabratus* supercooled to a mean (±SE) temperature of -21.7ºC ±0.5ºC and -23.9ºC ±0.4ºC, respectively. Artificially cold hardening the beetles did not significantly affect the mean SCP with respect to summer tested beetles. Beetle size and weight had no effect on the SCPs of tested beetles. The results indicate that the RAB is able to rapidly adjust to low temperatures regardless of prior exposure, i.e. "rapidly cold-harden". Based on the mean SCPs, *X. glabratus* and LWD could theoretically impact Lauraceae throughout North America. However, supercooling data alone are not enough to adequately predict the invasion potential of *X. glabratus,* e.g. significant mortality is likely at warmer temperatures. Future research and biochemical analysis will improve the sub-lethal effects of low temperatures on the RAB in North America.

DISCOVERY OF AND HOST RANGE TESTING ON *SPATHIUS GALINAE* (HYMENOPTERA: BRACONIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT FOR EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE)

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ABSTRACT

Field surveys of ash trees (*Fraxinus* spp.) were conducted in Far Eastern Russia to investigate the occurrence of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, and mortality factors affecting its immature stages. Mortality of EAB larvae caused by biotic factors (woodpeckers, host plant resistance, undetermined diseases, and parasitism) varied with date, site, and ash species. We discovered three hymenopteran parasitoids (*Spathius galinae*, *Atanycolus nigriventris*, *Tetrastichus planipennisi*) parasitizing larvae of EAB in the Vladivostok area, 0 – 8.3% of EAB larvae infesting Oriental ash trees and 7.3 – 62.7% of those on green ash trees. The dominant species (*S. galinae*) was shipped to the USDA-ARS quarantine facility in Newark, DE, where a laboratory colony was established and host range testing on North American non-target wood borers, mostly Coleoptera, was initiated.

So far, eight non-target species have been tested:

Coleoptera:

- Two-lined chestnut borer, *Agrilus bilineatus*
- Bronze birch borer, *Agrilus anxius*
- Red-headed Ash Borer, *Neoclytus acuminatus*
- Spined Oak Borer, *Elaphidion mucronatum*
- False Click Beetle, *Isorhipus obliqua*
- Eastern Ash Bark Beetle, *Hylesinus aculeatus*

Lepidoptera:

- Banded Ash Clearwing Moth, *Podosesia aureocincta*

Hymenoptera:

- Willow Shoot Sawfly, *Janus abbreviatus*

Spathius galinae readily attacked larvae of the target pest, but did not parasitize those of the non-target species.

BIOLOGICAL CONTROL ACTIVITIES AND DISTRIBUTION OF EMERALD ASH BORER IN TENNESSEE

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ABSTRACT

In 2010, emerald ash borer (*Agrilus planipennis*) was first detected in Tennessee, the southernmost known distribution of this forest pest in the U.S. Since its initial detection in Tennessee, emerald ash borer has been documented in 18 counties, and tree mortality has been observed in several locations.

In 2012, two parasitoid species, *Spathius agrili* and *Tetrastichus planipennisi*, were released against emerald ash borer. Following releases, studies were initiated to assess the potential for establishment and effectiveness of these parasitoids and document native parasitoids that may utilize emerald ash borer as a novel host. More than 8,700 *S. agrili* and 12,600 *T. planipennisi* were released at four locations in three counties from 21 June to 7 November. Eleven trees were felled throughout the season and examined for larval parasitoids, but none was found. However, establishment and spread to non-release trees may require more than one season to occur. Additionally, six species of parasitoids (2 Braconidae, 1 Ichneumonidae, 1 Chalcidoidea, and 2 Undetermined) were recovered from caged logs containing larval emerald ash borer. Studies using small- (trunk sleeves ca. 0.91 m long) and large-cages (tent cages ca. 8.0 m tall) to restrict the dispersal of released parasitoids also were conducted. Although no parasitoids have been recovered from the small-cage study, the large-cage study is still in progress. To assess winter survival, wood infested with emerald ash borer and exposed to parasitoids (*S. agrili* and *T. planipennisi*) will be held outside in cages and monitored in Spring 2013 for parasitoid emergence.

Studies on emerald ash borer and its parasitoids in the southern U.S. are at early stages. Biological control is the most promising broad-scale management option, and reductions of emerald ash borer populations may become more evident over the next several years. Continued monitoring of 2012 release sites, with the incorporation of new releases in 2013, will enable a greater evaluation of the success of these two parasitoid species released against emerald ash borer.

ENVIRONMENTAL FACTORS AFFECTING ESTABLISHMENT OF *SASAJISCYMNUS TSUGAE*, AN INTRODUCED PREDATOR OF HEMLOCK WOOLLY ADELGID

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ABSTRACT

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), presents a serious threat to the survival and viability of eastern hemlock. In the Great Smoky Mountains National Park, ca. 550,000 *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae), have been released at more than 200 sites to reduce populations of hemlock woolly adelgid. Beat-sheet sampling was conducted in 65 of these *S. tsugae* release sites between 2008 and 2012 to assess establishment of this predator. Several factors (aspect, elevation, release size, month of release, season of release, slope, year of release, and four temperature variables [minimum and maximum temperatures one day after release, and average minimum and average maximum temperatures seven days after release]) were analyzed using stepwise logistic regression and correlation to test whether these factors contributed to establishment and recovery of *S. tsugae*.

A total of 614 *S. tsugae* was recovered from 13 of 65 (20%) release sites. Stepwise logistic regression infers an inverse association between year of release and *S. tsugae* establishment and recovery. Stepwise logistic regression also identified average maximum temperatures seven days after release as a significant factor with establishment of these introduced predators. Spearman correlation also indicated a significant inverse relationship between year of release and *S. tsugae* recovery, which suggests that *S. tsugae* populations require more time to reach detectable levels. In addition, a significant positive correlation was found between recovery and establishment of *S. tsugae* and temperatures at the time of release (minimum and maximum temperatures one day after release, and average minimum and average maximum temperatures seven days after release).

These results suggest that releases of *S. tsugae* should be made in warmer temperatures, and post-release long-term monitoring is necessary to enhance establishment and recovery efforts. Improved release methodology will improve establishment and enable land managers to better incorporate biological control into management efforts against hemlock woolly adelgid.

HYPERSPECTRAL REMOTE SENSING TECHNIQUES TO ASSESS A DECADE OF HEMLOCK DECLINE IN THE CATSKILL MOUNTAINS

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ABSTRACT

The introduction and establishment of exotic pests is one of the greatest threats to the health and productivity of North American forests. Successful invasions have resulted in widespread mortality and decline in ecosystem function and productivity. The hemlock woolly adelgid (HWA) is considered a serious pest of eastern and Carolina hemlock in the Eastern US, with mortality typically occurring within 3-10 years south to north, respectively. Early detection and decline assessment can assist land managers in monitoring and controlling HWA infestations by prioritizing biological control efforts on incipient HWA infestations. However, most visible decline symptoms occur after the trees have already been adversely impacted resulting in ineffective management options. Narrow-band hyperspectral instruments can assist in the detection of early stress by quantifying reductions in photosynthetic activity and subtle changes in chlorophyll content. This project is a ten year re-assessment of HWA and hemlock decline in the Catskill Park based on detailed field measurements and landscape scale hyperspectral coverages. In addition to a "wall to wall" assessment of the hemlock resource and how it has changed over the past 10 years, this work serves as a test of the potential role of hyperspectral technologies in Forest Service detection and monitoring efforts.

NATIVE AND NON-NATIVE CERAMBYCID BEETLES IN URBAN FOREST FRAGMENTS

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ABSTRACT

We are evaluating the abundance and species richness of beetles in the family Cerambycidae in twelve forest fragments of northern Delaware. These urban and suburban forests are part of the long-term FRAME program, which is comprised of 30 sites in the Mid-Atlantic region of the eastern United States.

The cerambycid family is of particular interest because it contains a number of native and nonnative forest pests. Many of these can be brought to baited traps. In each forest fragment, we had four cross panel traps baited with three different attractant combinations and a control. Our treatments were MelangeC which is a synthesized cerambycid aggregation pheromone, ethanol, a combination of MelangeC and ethanol, and isopropanyl which is used as the control. Beginning in the last week of April, we collected all insects found in our traps once a week, and refreshed treatments in accordance with protocols developed by Hanks and Millar. We collected for 22 weeks, and captured over 6,900 cerambycid beetles representing 63 species.

From our data thus far, we have observed that time of year appears to impact abundance of different species. We have also seen that despite our site's close proximity (within 10 km of each other) there appear to be differences in beetle abundance by species and by number of beetles in general. The goal of our project is to discover drivers behind beetle abundance patterns.

POPULATION GENOMICS OF ADMIXTURE IN NON-NATIVE FOREST PESTS: WINTER MOTH AND ASIAN GYPSY MOTH

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ABSTRACT

In this era of globalized trade, the introduction of non-native pests will continue to escalate. The impacts of a non-native pest can be exacerbated when multiple introductions bring waves of new genetic diversity, or when a pest hybridizes with a native species. Contact between divergent insect groups could result in the transfer of traits associated with increased damage to their host plants. Will genetic admixture expand a pest's host range, increase their tolerance to climate extremes, allow them to escape natural enemies, or introduce fight into formerly flightless populations? These questions can now be addressed in unprecedented detail with functional population genomics. We have initiated projects using genomic tools to: (1) track hybridization between non-native winter moth and native Bruce spanworm; and (2) detect introduction and spread of Asian gypsy moth. Double digest restriction site associated genomic sequencing (ddRAD-seq) will be used to identify thousands of SNPs (Single Nucleotide Polymorphisms) in both species genomes and find association between these markers and traits of concern using laboratory crosses. These results can then be used to develop powerful monitoring tools and predict the impact of admixture in the winter moth and gypsy moth.

FLIGHT ACTIVITY OF SAWYER BEETLES *MONOCHAMUS SARTOR* AND *MONOCHAMUS SUTOR*: ATTRACTIVENESS OF INSECT AND HOST TREE VOLATILES

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ABSTRACT

Sawyer beetles, *Monochamus* spp., reach high importance as vectors of the pine wood nematode, *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease. *M. galloprovincialis* is the main vector of the nematode in Europe, where the latter is currently present in Portugal and Spain. A blend of volatiles has been developed for this species using the bark beetle kairomones ipsenol and methyl butenol and the sawyer pheromone 2-undecyloxy-1-ethanol, which have been formulated in the commercial lure Galloprotect-2D (G2D) (SEDQ, Spain). In an experiment in a wilderness area in a mountainous forest in Austria, where *Monochamus sartor* and *M. sutor* reached high abundance following a spruce bark beetle outbreak, we tested whether the available lure is also attractive for these species and whether host tree volatiles further increase attraction. Traps were set up in four blocks; after 10-day trapping periods, the position of traps was re-randomized. Traps were emptied every 3 or 4 days. The following lures were tested: G2D, G2D plus α-pinene, and G2D plus α-pinene plus smoke volatiles. Smoke volatiles were produced in the laboratory of D.R.H., who has been carrying out detailed studies on the attractiveness for *M. galloprovincialis* and *M. sutor* in cooperation with J. Pajares (University of Valladolid, Spain).

Tested lures were attractive for both *Monochamus* species. The host tree volatile α-pinene increased trap catches of both sexes of *M. sartor*. 4.6 ± 0.6 females were caught in G2D-traps per 10-day trapping period, 6.4 ± 1.1 were caught when α-pinene was added. The increase was significant only for male beetles (1.6 ± 0.3 and 3.3 ± 0.5, respectively). The addition of smoke volatiles did not lead to a further increase. *M. sutor* were caught in lower numbers; no significant differences occurred between tested lures. Weather conditions during the 40 days of the experiment influenced flight activity and consequent trap catches of *Monochamus* spp. Mean temperature was significantly correlated with *M. sartor* catches ($r = 0.731$). Captured *Monochamus* beetles were marked and released in the center of the study site. Four *M. sartor* and five *M. sutor* were recaptured (out of 317 and 92 that had been released). Released beetles dispersed in all directions, uphill as well as downhill and crossed mature spruce stands, also to the most distant trap 380 m away. This experiment provided first data on the feasibility of the available lure and trap system developed for *M. galloprovincialis* in Portugal and Spain for monitoring the two sawyer species that are more important in mountainous Austria.

WOOD BORING INSECTS IN WOOD PACKAGING MATERIAL: RECENT INTERCEPTIONS AND A NEW ASIAN LONGHORNED BEETLE OUTBREAK IN AUSTRIA

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ABSTRACT

Interceptions of consignments with wood packaging material containing living stages of cerambycid beetles in Europe increased in 2012 compared to previous years. While 15 interceptions with cerambycids were reported to the EUROPHYT notification system by EU member states plus Switzerland in 2011, this number increased to 55 in 2012 (period from January to November). Of these consignments, 84 % originated from China. Most frequently detected genera were *Anoplophora* and *Apriona*. Based on alarming reports, targeted controls of high-risk consignments (stone imports from China and India) have been intensified in Austria since June 2012; 38 inspections were carried out at points of final destination. These resulted in 19 notifications. In nine of the consignments, all of which showed ISPM 15 markings indicating heat treatment or fumigation, living insect stages were found. Among them were *Anoplophora glabripennis*, *Apriona germari*, *Batocera* sp., other Cerambycidae, and Bostrichidae. A total of 50 interceptions of wood packaging material with ISPM 15 marking containing living insect stages were made in Austria, Germany and Switzerland in 2012.

These numbers illustrate the positive threat despite seeming compliance with ISPM 15. It is therefore no surprise that Europe has been witnessing several new infestations by *Anoplophora glabripennis* (ALB) over the last years. The city of Braunau in Austria was the site of the first ALB outbreak in Europe in 2001. Due to eradication measures (including extensive preventive felling on 6 ha in winter 2008/09 and 8 ha in winter 2011/12 along roads and railway tracks) no further infestation has been found since June 2009. A new outbreak was detected near St. Georgen on 25 July, 2012, just 27 km away from the Braunau site. Nevertheless it is believed to originate from an independent introduction because it was found on the yard of a stone importer. Examination of trees on the property ascertained living ALB larvae in one willow and one beech. One horse chestnut had three larvae in pupal chambers and one adult emergence hole. One cherry and one maple tree showed dead oviposition marks. Rigorous eradication measures were immediately initiated, facilitated by remote location of the infestation site and limited number of affected land owners. In an inner circle of 500 m radius all (> 600) deciduous trees incl. fruit trees and some shrub species were cut, examined (also with detection dogs) and chipped. In the 1100 m radius monitoring zone, all standing host trees will be regularly inspected. Movement of wood from the demarcated area is restricted.

COMPARISON OF *ENTOMOPHAGA MAIMAIGA* INFECTIONS IN *LYMANTRIA DISPAR* AND *LYMANTRIA MONACHA*

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ABSTRACT

Entomophaga maimaiga is a fungal pathogen introduced from Japan to North America that has been providing natural control of gypsy moth (*Lymantria dispar*), a major defoliator of northeastern forests that was introduced from Europe in 1869. While this pathogen does not always provide complete control everywhere, it has been maintaining many established gypsy moth populations at low densities since the late 1980's. *Lymantria monacha* (nun moth) is a Eurasian outbreak pest of conifers, and sometimes deciduous trees and shrubs that poses an ever-present threat of being accidentally introduced into North America. Its establishment in North America would be disastrous because of its polyphagous feeding habits, ability to colonize new habitats, and capacity to be spread rapidly by flying adults. European forest managers continue to seek natural control measures for *L. monacha* and North American managers would like to have information on all available tools for management should it be introduced. The work presented here is an initial evaluation of challenging *L. monacha* with *E. maimaiga*.

Under quarantine, nun moth larvae were reared on *Quercus* spp. foliage and gypsy moth larvae were reared on artificial diet. Early fourth instars of both *L. dispar* and *L. monacha* were challenged by injecting protoplasts of two *E. maimaiga* isolates. *Lymantria monacha* was killed by *E. maimaiga* when injected with either isolate. Although *L. monacha* larvae started dying at about the same time (4-5 days) as *L. dispar* larvae, mortality was not as synchronous and a lower percentage ultimately died. Percentages of *L. monacha* cadavers that produced conidia and resting spores were lower than *L. dispar* cadavers for both isolates. Previous laboratory bioassays showed that *E. maimaiga* was specific to the family that includes *Lymantria*, but can cause low levels of infection in a number of other species (*Dasychira* and *Orgyia*). This current work with *L. monacha* is the first report of its susceptibility to *E. maimaiga*. However, injection of *E. maimaiga* protoplasts does not indicate that *L. monacha* can become infected and die from this fungus under natural conditions, but does show that the larvae are susceptible to the fungus if it can gain entry into the hemocoel. Future bioassays using a conidial shower method to infect the *L. monacha* larvae need to be conducted to determine if a natural infection of *E. maimaiga* can kill this close relative of *L. dispar*.

ESTIMATING URBAN DISTRIBUTIONS OF KEY HOST TREES FOR INVASIVE FOREST PESTS

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ABSTRACT

To reasonably estimate the spread and impact of invasive forest pests, it is critical to have good representations of the distributions of their host trees. While data sources such as the US Forest Service's Forest Inventory and Analysis (FIA) plots provide a solid foundation for estimating host distributions in natural forests, urban forest inventories are a less reliable data source: relative few communities have surveyed their forests, and these surveys have often been restricted to street trees or a subset of community lands. We compiled urban forest data, collected and organized in an assortment of scales and formats, from 235 communities across the eastern and central US. Our objective was to use these data in models to estimate the presence of key host trees in non-sampled communities throughout the study region. For each inventoried community, we determined the relative proportion of its total basal area associated with two genera, maple (*Acer* spp.) and ash (*Fraxinus* spp.), that respectively serve as hosts for two major invasive pests, the Asian longhorned beetle (*Anoplophora glabripennis* (Motschulsky)) and the emerald ash borer (*Agrilus planipennis* Fairmaire). We applied two modeling methods, boosted decision trees and decision tree forests, to a suite of geographic, demographic, and climatic explanatory variables. For maple, both methods explained more than 70% of the variance and identified similar suites of important variables, although boosted decision trees yielded somewhat better predictions. For ash, neither method explained much variance or predicted well, which we attribute to the high level of variability in the urban ash proportion over the study region. Nevertheless, our success with maple in this preliminary analysis shows the promise of the approach. Future steps include adding other host genera and expanding the study area to Canada and the western US. Ultimately, our approach should help fill an important knowledge gap in the modeling and mapping of invasive species risks and impacts in North America.

TOWARD INCREASING RESISTANCE OF ASH TO THE EMERALD ASH BORER

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ABSTRACT

The emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) (Coleoptera:Buprestidae) is a phloem-feeding exotic wood borer introduced from Asia that is spreading throughout the Midwest with devastating effect. This beetle is capable of killing otherwise healthy trees and colonizes trees as small as saplings – most affected trees die within four years of colonization. Asian ash species (i.e., Manchurian and Chinese) appear to be more resistant to EAB than those native to North America (e.g., green, white, and black ash). In this project we use reciprocal grafts of these five ash species to determine whether resistance to EAB can be conferred to a scion when grafted onto resistant rootstock. Specifically, we collected head-space volatiles from twigs and leaves of the grafted ash trees to determine the extent to which rootstocks influence the composition of volatile organic compounds (VOCs) released by the scion. This work may pave the way toward developing systems for propagating *Fraxinus* scions that are resistant to EAB and exploit potential phytochemical connectivity between roots and shoots.

NON-STERILIZING *DELADENUS SIRICIDICOLA* PARASITISM OF *SIREX NOCTILIO* IN NORTHEASTERN NORTH AMERICA

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ABSTRACT

The parasitic nematode *Deladenus siricidicola* has been extensively used in biocontrol measures against the forest pest *S. noctilio* in areas where the woodwasp has become invasive in the Southern Hemisphere. *D. siricidicola* has several different strains, some of which are sterilizing to the wasp and some that are non-sterilizing. The effects of the sterilizing strain are relatively well understood, but the effects of the non-sterilizing strain on *S.* noctilio have not been examined to date. A non-sterilizing (NS) strain is established in the most recent invasion of *S. noctilio*, in northeastern North America. This paper analyzes the effects of the non-sterilizing strain of *D. siricidicola* on *S. noctilio* populations found in New York and Pennsylvania. Nematode parasitism is related to smaller wasp size and fewer eggs in adult females. However, nematode infection also has a direct relationship with egg number which is not accounted for by wasp size. Nematode infected woodwasps were observed to emerge during the first half of the emergence period. *S. noctilio* with NS nematodes were found in 44.0% of trees, reaching 27.9 $\pm$ 26.0 % parasitism (mean $\pm$ S.D.) when averaged across sites. There was greater parasitism of female *S. noctilio* than males. Given that this introduction of *S. noctilio* is the first to occur in an area where pine trees, other species of *Sirex* and associated parasitoids are native, the implications of this research may contribute to understanding how these parasites control populations of *S. noctilio*. This information may be useful in designing biocontrol measures.

DEVELOPMENT OF *SCYMNUS CAMPTODROMUS* FOR BIOLOGICAL CONTROL OF HEMLOCK WOOLLY ADELGID

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ABSTRACT

The hemlock woolly adelgid (HWA: *Adelges tsugae*) is an invasive insect that threatens the sustainability of eastern hemlock forests. *Scymnus camptodromus* (lady beetle) was brought to the United States from China as one of the potential biological control agents for HWA. *S. camptodromus* has a high likelihood of establishing in the U.S. as a major natural enemy of HWA for several reasons: its phenology is closely synchronized with HWA, it occurs over a broad geographic area and in diverse habitats in its native range, beetle larvae are present at a key point in the life cycle of HWA, and the adults feed on HWA throughout most of the year.

Host range studies, using both choice and no choice tests, showed that *S. camptodromus* prefers adelgids and among those tested prefers HWA. During all experiments, female beetles only oviposited on HWA-infested branches; no predator eggs were ever laid on alternate adelgid host material. Among alternate prey items, regardless of how the test was performed, no woolly alder aphids (*Paraprociphilus tessallatus*) were consumed, 1 elongate hemlock scale (*Fiorinia externa)* was slightly chewed on, and on average 3.7 first instar nymphs of cotton aphids (*Aphis gossypii*) were consumed per adult.

A confined release of *S. camptodromus* was performed in the spring of 2012 in a forested section of the Pennsylvania State Agricultural Farm. Treatments consisted of 20 replicates each of a caged HWA-infested branch with or without addition of an adult female and an uncaged HWA-infested branch. Three hundred HWA ovisacs averaging 120 eggs/ovisac were enclosed in each cage. Half the cages in each treatment were left in place for one month and the other half for 2 months. When the cages were removed, all HWA life stages and predator eggs were counted, new growth was measured, and beetle survival recorded. Although the predator did not impact HWA populations in the first month, in the second month there was a significant reduction in HWA populations in cages that contained a beetle compared with cages without beetles or uncaged adjacent branches. In addition, a total of 27 eggs were laid by *S. camptodromus* females in the cages (3 eggs during the first month and the rest were found at 2 months). The field caged release study demonstrates that the lab reared lady beetle can survive and reproduce under natural conditions when sufficient HWA is present for it to feed on. For future studies, we plan to assess the numbers of HWA eggs larval *S. camptodromus* consume per instar in the laboratory and evaluate the efficiency of adults and larvae in cages in the field.

PREDICTING HIGH-QUALITY SITES OF BLACK ASH (*FRAXINUS NIGRA*) ACROSS MAINE AND NORTHERN NEW YORK: AN APPROACH TO PRIORITIZING PREPAREDNESS AND MANAGEMENT OF EMERALD ASH BORER

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ABSTRACT

Black ash (*Fraxinus nigra*) is a species of ash tree most commonly found in moist lowland sites and is typically thought of as a wetland species. While there is not a large population of black ash throughout the eastern United States, this species nonetheless serves an important socio-economic and ecological role. Native American tribes have used black ash for centuries to weave baskets, which have become a key component in the tribes' histories, cultures, and economies. Black ash also fills a particular ecological niche in that it can colonize wetland sites and is one of the most drought-tolerant species of *Fraxinus*. Thus based on its socio-economic role for Native American tribes and the ecological niche it fills, it is essential to identify and map high-quality sites where black ash grows so that the species may be better monitored and protected from environmental stressors. Such information is necessary in prioritizing responses to the impending introduction of the emerald ash borer (*Agrilus planipennis*) in Maine and northern New York. These high-quality sites will be defined as sites where black ash (1) can regenerate successfully; (2) have wide increments of annual growth at DBH that are suitable for basket-making (greater than 2mm for 20 consecutive years); and (3) show little to no decline in annual growth levels over time. By identifying the characteristics that classify a site as high-quality for black ash, a comprehensive spatial model will be created detailing and predicting the locations of high-quality sites that can be used by other researchers and resource managers aiming to monitor and protect black ash.

EXTENSION OUTREACH TOOLS FOR INVASIVE PESTS AND DISEASES

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ABSTRACT

We received a Regional IPM grant that will enable us to modify an existing Mid-Atlantic Early Detection Network (MAEDN) iPhone app and Android phone app to report new key invasive insect and disease pests throughout the Mid-Atlantic region. These invasives pose an enormous threat to our forests, agronomic crops and landscapes. Their threat is second only to habitat loss and biodiversity with an estimated annual cost of $1.4 trillion worldwide. Our national resources and ecosystems are under constant pressure from new and invasive species. Prevention is the first-line of defense, but unfortunately numerous pathways for introduction increase the possibilities of invasive entry. The best IPM option for invasives is early detection coupled with a rapid response. Early reporting of invasives increases the likelihood that localized populations will be found and eradicated. Utilizing new smart phone technologies such as phone apps enable easy reporting and location of new invasive species.

The Center for Invasive Species and Ecosystem Health (Bugwood.org) is in the process of programming the apps and database. The IPhone app is available in the ITunes Store and the Android app from Google Play. They can also be downloaded directly from the Bugwood App site at: *http://apps.bugwood.org/mid_atlantic.html*. A key component of the new phone apps will be the enhanced flow of reporting information back to key local experts, and state and federal, agencies that have managerial responsibilities. In addition, sets of color identification cards featuring key invasive insects and diseases and a QR tag that will connect to the phone app will be distributed to further enhance successful identification and timely reporting. We need collaborators to participate in this pilot project. Please contact one of us if you are interested.

CHROMOBACTERIUM SUBTSUGAE: A BROAD SPECTRUM INSECTICIDE WITH POTENTIAL ACTIVITY AGAINST INVASIVE SPECIES

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ABSTRACT

Chromobacterium subtsugae Martin et al. was isolated from soil during a gypsy moth epizootic caused by a *Entomophaga maimaiga* Humber, Shimazu, and Soper. These purple bacteria stood out on a plate in a background of cream and beige bacteria. Originally kept to check on cross contamination, this new species of bacteria was first found to kill Colorado potato beetle (*Leptinotarsa decemlineata* (Say)). Unlike *Bacillus thuringiensis* Berliner strains which only kill specific insect species, C. subtsugae, a single strain has a broad spectrum activity against a variety of insects and mites, while being non toxic to higher animals. It has activity against gypsy moth *(Lymantria dispar* (Linnaeus)) and brown mamorated stink bug (*Halyomorpha halys* Stål). In gypsy moth there is a 70% larval weight reduction. In brown mamorated stink bugs, 2nd instar nymphs were the most sensitive with 90% mortality in 10 days on an artificial diet. Originally isolated in 2000, *C. subtsugae* was granted a US patent in 2007, was licensed to Marrone BioInnovations which received EPA regulatory approval in 2011, the first microbial pesticide to be approved since *B. thuringiensis*. Grandevo® has been registered for use in 13 states including: Florida, California, Michigan, New Jersey, Texas, and Washington with registrations pending in other states. Because of its broad spectrum of activity it may be useful in the control of invasive pest species.

DEFENSE CHEMISTRY OF EASTERN HEMLOCKA RESISTANT TO HEMLOCK WOOLLY ADELGID

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ABSTRACT

Recent studies have identified a small number of individual eastern hemlock trees that show relative resistance to the introduced pest, hemlock woolly adelgid. This study investigates one potential mechanism for such resistance: the profile of volatile compounds released by hemlocks as constitutive and induced defenses. Eastern hemlock primarily releases terpene-class volatiles, but its limited set of phenolic-class volatiles have previously been implicated in the defense response to HWA. Using gas chromatography and mass spectrometry, we compare the volatile profile of susceptible and relatively resistant eastern hemlocks, both in the forest and in saplings in a common garden experiment. Preliminary results suggest resistant status may be characterized by elevated concentrations of a small set of compounds accompanied by an overall decrease in volatile concentrations. Analysis across seasons allows us to relate the timing of key changes in the volatile profile to the life cycle of the woolly adelgid.

KETOLS, DIOLS AND ETHANOL ON ATTRACTION OF HARDWOOD BORERS (CERAMBYCIDAE) IN THE SOUTHEASTERN UNITED STATES

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ABSTRACT

Our goal is to develop a robust trapping system for wood boring beetles in hardwood stands of North America. Blends of 3-hydroxy-2-hexanone (C6-ketol), 3-hydroxy-2-octanone (C8-ketol) and 2,3-hexanediol isomers (C6-diol) are broadly attractive to numerous hardwood borers (Cerambycidae) (*Hanks et al. 2012, Can. J. For. Res. 42: 1050-1059*). Our first objective was to verify the activity of these compounds on common Cerambycidae in Georgia. Sixty-four modified funnel traps were deployed in the Oconee National Forest near Eatonton, GA (3 June-13 July 2011). Traps were set in 8 replicate blocks of 8 traps, each spaced 20-25 m apart with blocks 25-100 m apart. One of the following treatments was allocated to a trap within each block: (1) blank control; (2) C6-diol; (3) C6-ketol; (4) C8-ketol; (5) C6-diol + C6-ketol; (6) C6-diol + C6-ketol; (7) C6-ketol + C8-ketol; and (8) all three lures. *Neoclytus acuminatus* (F.) was attracted to a blend of hexanediol isomers whereas *N. mucronatus* (F.) and *N. scutellaris* (Olivier) were attracted to 3-hydroxy-2-hexanone. Mutual interruption in catches of the three *Neoclytus* spp. was evident when traps were co-baited with hexanediols and hydroxyketones. *Xylotrechus colonus* (F.) was attracted to the combination of the hexanediols and hydroxyketones.

Our second objective was to determine if ethanol had any effect on the response profiles of these species. Forty-eight modified funnel traps were deployed in the Oconee National Forest near Eatonton, GA (10 July-23 August 2012). Traps were set in 8 replicate blocks of 6 traps, spaced 10-12 m apart with blocks 25-100 m apart. One of the following was allocated to a trap within each block: (1) C6-diol; (2) C6-ketol + C8-ketol; (3) C6-diol + C6-ketol + C8-ketol; (4) etoh + C6-diol; (5) etoh + C6-ketol + C8-ketol; and (6) all four lures. Ethanol enhanced attraction of all four species without affecting their response profiles to the hexanediols and hydroxyketones. Lures of C6-diol, C6-ketol and C8-ketol were supplied by ConTech Inc. (Delta, BC) using compounds without any solvent. The release rates ranged from 10-40 mg/d depending on temperature. Ethanol lures with a high-release rate of 0.6 g/d were supplied by ConTech Inc. for Experiment 2. In both experiments, we used 10-unit multiple-funnel traps that were modified by increasing the center hole of each funnel from 5 cm to 12 cm, thereby allowing placement of all lures within the confines of the trap.

DISTRIBUTION AND IMPACT OF SIROCOCCUS SHOOT BLIGHT OF EASTERN HEMLOCK IN THE NORTHEASTERN UNITED STATES

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ABSTRACT

In 2009, eastern hemlock was confirmed to be a new host of the pathogen *Sirococcus tsugae* Castl., D.F. Farr & Stanosz. This pathogen causes defoliation and shoot blight of a resource already threatened by hemlock woolly adelgid and elongate hemlock scale. The specific objectives of this project are to: (i) delineate the geographic range of Sirococcus shoot blight of eastern hemlock in New England and New York (NYS), (ii) verify the association of the pathogenic fungus *S. tsugae* with widely observed shoot blight symptoms, (iii) quantify incidence, severity, and impact of the disease on eastern hemlock regeneration, and (iv) monitor changes in disease impact over time. From June to August of 2012, 41 FIA plots throughout the northeast were surveyed. Disease incidence and severity were assessed for 20 seedlings per plot. Symptoms were present in most plots and varied in severity. A sample from each plot was sent to UW-Madison for diagnoses. Of the 41 samples, 17 produced *Sirococcus* spores, 15 samples yielded cultures, and 13 samples were positive for *S. tsugae* with PCR. The pathogen was confirmed to be present in Maine, Vermont and Rhode Island. Symptomatic samples collected in NYS, Connecticut, and Massachusetts later in the season did not yield spores or cultures.

THOUSAND CANKERS DISEASE: ATTRACTION OF THE INSECT VECTOR, *PITYOPHTHORUS JUGLANDIS*, TO VOLATILES OF GIRDLED BRANCHES OF BLACK WALNUT

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ABSTRACT

Thousand cankers disease (TCD) has caused widespread death of black walnut trees throughout the West and was recently detected in Tennessee, Virginia, Pennsylvania and Ohio. This disease complex is caused by a *Geosmithia* fungus vectored by the walnut twig beetle (WTB), *Pityophthorus juglandis* (Coleoptera: Curculionidae: Scolytinae). Male WTB locate suitable black walnuts by orienting to host odors and, once on an appropriate tree, release volatile aggregation pheromones that coordinate mass attack and mating. We have recently found that genotype influences the attraction of male and female WTB to head-space volatiles of black walnut leaves and branches. In this project, we determine the extent to which girdling affects the response of adult beetles to one of these most attractive genotypes. We found both qualitative and quantitative differences in volatile profiles of intact and girdled branches of black walnut. In olfactometer bioassays, adult beetles were more attracted to volatiles of girdled branches. Understanding those factors that influence the susceptibility of black walnut to colonization by *P. juglandis* and infection is essential for managing this disease complex and determining its potential economic and ecological impacts.

LDMNPV CANDIDATES FOR A SINGLE-GENOTYPE GYPCHECK PRODUCT

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ABSTRACT

Gypchek is the gypsy moth (*Lymantria dispar* L) nucleopolyhedrovirus (LdMNPV) -specific biopesticide, whose primary use is for treating areas where environmental concerns are paramount. Currently, Gypchek is used only for the suppression of European strains of gypsy moth. However, accidental introductions of Asian gypsy moth (*Lymantria dispar asiatica* Vnukovskij) into the United States continue to occur. The increased probability of establishment of Asian strains will be taken into account as we move toward developing a single-genotype Gypchek product whose efficacy against both European and Asian strains of the insect is maximized.

Groups of second-stage European gypsy moth (laboratory-strain, Hamden, CT,USA) and a wild Asian gypsy moth (Siberian-strain, Novosibirsk, Russia) were challenged with a range of viral doses from Gypchek and from LdMNPV-genotypes isolated from Gypchek. Probit analysis of LdMNPV- mortality data resulting from diet incorporation bioassays was used to determine and compare Lethal Concentration (LC_{50}) values. In addition, Gypchek and 2 of the most potent genotypes (122b and 122-HP) were formulated at three doses and sprayed on oak branch tips in a small-scale ground-based experiment. Larval mortality was assessed and analyzed by a one-way analysis of variance.

In laboratory bioassays, LC_{50} values for 122-HP and 122b were significantly lower than the LC_{50} for Gypchek as judged by no overlap in 95% confidence limits. Both of these isolates were 3 to 4 times more potent than the parent Gypchek as judged by potency ratios. Genotype 122-HP was as active against the wild Asian gypsy moth as the wild Siberian virus. Only one of the other genotypes (B1B) approached a level of significant activity against the Asian gypsy moth. Analysis of the results of the small-scale field test showed no significant differences in larval mortality between any of the high dose treatments, however, levels of mortality across doses indicated that 122-HP should be the initial choice for developing a single-genotype Gypchek product.

PHYLOGEOGRAPHY OF THE REDBAY AMBROSIA BEETLE IN NORTH AMERICA

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ABSTRACT

The non-native redbay ambrosia beetle (*Xyleborus glabratus*) and the causative agent of laurel wilt disease (*Raffaelea lauricola*) were first discovered in Mississippi in 2009. At that time, the population was separated from established populations along the Atlantic coast by at least 475 km. It was unknown whether redbay ambrosia beetles were introduced into Mississippi through a separate event via one of the international shipping ports in the vicinity, or through domestic transportation of infested materials.

Phylogeographic analyses were carried out to determine whether domestic transport or a separate international introduction was more likely. Phylogeographic analyses indicate sequences from redbay ambrosia beetles collected in MS, FL, GA, and SC were all identical to one another, as well as all sequences in GenBank. It is likely that redbay ambrosia beetles and the laurel wilt pathogen they carry were introduced into North America was limited to a single event, and has subsequently spread to other portions of the U.S. through human-aided domestic transport.

SEASONAL VARIATION IN PRESENCE AND ABUNDANCE OF INOCULUM OF THE *HETEROBASIDION* ROOT DISEASE PATHOGEN IN CENTRAL WISCONSIN

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ABSTRACT

After infection of conifer stump surfaces following deposition of airborne basidiospores, the root disease pathogen *Heterobasidion irregulare* spreads through root grafts or by root contact to adjacent trees. Infection of fresh conifer stump surfaces resulting from felling is prevented, however, by borate application. Because the need for stump protection depends on inoculum availability, spore trapping was conducted from September 2009 through December 2011 in three infested plantations of predominantly red pine (*Pinus resinosa*) in central Wisconsin. At 2 (or sometimes 3) week intervals, a semiselective medium in 9-cm-diameter Petri plates was exposed for 1 hour in daylight at each of four locations in each plantation. After 7-10 days incubation at 20°C plates were examined for the *Spiniger* asexual stage. Both presence and abundance of colonies of the pathogen were recorded. *H. irregulare* was detected during most of the two growing seasons, but colonies were most abundant during late summer, fall, and early winter. Relatively few colonies developed on medium exposed in winter during periods of deep snow and coldest temperatures, but colonies of the pathogen did develop occasionally on medium exposed at below 0°C. Definitive, biologically based guidelines for stump treatment require additional studies of seasonal factors influencing inoculum availability, *in situ* spore germination, infection, and establishment of the pathogen.

EXPANSION IN THE KNOWN GEOGRAPHIC DISTRIBUTION AND HOST RANGE OF THE SHOOT BLIGHT PATHOGEN *SIROCOCCUS TSUGAE*

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ABSTRACT

Eastern hemlock (*Tsuga canadensis*) is an ecologically and economically important conifer from the north-central USA to the east coast of North America to the southern Appalachian Mountains. In early spring 2010, blighted shoot tips of eastern hemlock were observed at widely separated locations in the Chattahoochee National Forest in north Georgia. Damage did not appear to be directly related to hemlock woolly adelgid (*Adelges tsugae*) activity, which was sporadic or in some of these locations. A preliminary survey in March 2010 revealed that incidence of blighted shoots on individual trees varied, but was as high as 70%. Stems of shoots produced the previous year were frequently necrotic, had lost needles, and bore pycnidia with hyaline, two-celled conidia consistent with those of *Sirococcus tsugae*. Later in spring and summer, shoots of current year's growth became blighted, with sporulation also on dead and dying needles. While *S. tsugae* previously has been reported on *T. heterophylla*, *T. mertensiana*, *Cedrus atlantica*, and *C. deodara* in western North America, it was only recently been reported on eastern hemlock in Maine, and its ability to induce shoot blight was not proven. Pure cultures were obtained on streptomycin-amended potato dextrose agar (PDA) and their identity was confirmed using species-specific PCR primers. Nuclear rDNA ITS sequence also was identical to sequences for *S. tsugae* previously deposited in GenBank. Conidia of two isolates were collected in sterile water from cultures on PDA and used to inoculate 2-year-old eastern hemlock seedlings in a growth chamber at 20°C with a 16-h photoperiod. Expanding shoots on one branch of each seedling were wounded using scissors to cut tips off needles and stems, while another branch was not wounded. Ten seedlings per isolate were inoculated by spraying to runoff with a conidial suspension sterile water, and five similarly treated control seedlings were sprayed with sterile water. Seedlings were covered with plastic bags to maintain high humidity for 4 days. Symptoms were evaluated and reisolation was attempted on streptomycin-amended PDA 2 months after inoculation. Symptoms of seedlings inoculated with either isolate included chlorotic and necrotic needle spots, browning of cut edges of needles, browning and death of needle tips and entire needles, death of stem tips with retention of dead needles, and needle loss. Symptoms of control seedlings were limited to slight browning of cut edges of needles. The fungus was reisolated from wounded shoots of 17 of 20 inoculated seedlings and nonwounded shoots of 5 of 20 inoculated seedlings and was not cultured from control seedlings. This documents presence of *S. tsugae* in Georgia, and also demonstrates its ability to produce shoot blight symptoms.

DENSITY-DEPENDENT SURVIVAL OF HEMLOCK WOOLLY ADELGID LIFE STAGES

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ABSTRACT

Substantial mortality of hemlocks in the southeastern states and southern New England caused by hemlock woolly adelgid, (*Adelges tsugae* Annand; HWA) created the expectation that widespread hemlock mortality would occur when HWA reached Massachusetts forests in 1989. However, more than twenty years later, HWA populations in MA have remained largely stable and many infested hemlocks survive for a decade or more. To investigate the possible density-dependent factors that cause this observed stability; we infested sixty-four hemlock trees with varying densities of HWA sistens ovisacs in a typical mixed species hemlock forest in Massachusetts and documented subsequent HWA density, fecundity, and the amount of new growth on experimental trees. We used a 2 x 2 randomized block design with previously and newly infested hemlocks divided into 1 m tall saplings and branches of mature trees. We recorded substantial density-dependent survival and fecundity decline in the summertime generation. We show that production of sexuparae in this life stage plays a key role in the density-dependent survival of HWA, and find that this response is a function both of previous infestations of HWA to the tree and the current year's crawler density of the summertime generation of HWA. We conclude that it is this density-dependent summertime survival that is responsible for the observed HWA densities in Massachusetts.

FIRST RECORDS OF THE BEECHLEAF-MINING WEEVIL, *ORCHESTES FAGI* (L) (CURCULIONIDAE: CURCULIONINAE), IN NORTH AMERICA

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ABSTRACT

In spring of 2012, the beech flea weevil, *Orchestes fagi* (L), also known as the beech leaf mining weevil, was discovered causing severe defoliation of American beech, *Fagus grandifolia* Ehrh, in Halifax. Branch-beating surveys on American beech were conducted in Halifax and about every 10 km extending out along highways and secondary roads in May–June 2012. Foliar damage was extensive in several areas. A total of 124 *O. fagi* adults were collected at 15 of 34 sites sampled indicating the weevil is established near Halifax and Sydney (300 km apart), Nova Scotia. Absence of the weevil at several sites within 50 km of Halifax suggests it has either moved by human assistance within the province or that more than one introduction has occurred from Europe. *Orchestes fagi* is a common widespread pest of beech, *Fagus sylvatica* L., in Europe and has the potential to spread throughout the range of American beech. There is one generation per year in Europe. Adults overwinter (in leaf litter, moss, under the bark of cordwood, spruce crowns), emerge in spring and feed on newly flushed beech leaves, peppering them with small holes. Females lay 40-60 eggs singly along the leaf mid-rib. Larvae feed within the leaf, creating a narrow mine from the mid-rib to the leaf margin, ending in a small brown blotch. Larvae have three instars and pupate inside a white, spherical cocoon inside the leaf mine. The new generation of adults emerges in mid-late June and feed again before seeking overwintering sites in late July-mid September. Egg to adult takes 30–35 days. Damage is sometimes extensive, causing the tips of the leaves to turn brown and wilt, giving the foliage a scorched appearance. In Europe, outbreak populations of adult *O. fagi* have fed on other tree species and damaged fruits of apple, apricot, and cherry. Further study of the Nova Scotia population will be necessary to determine whether it behaves differently in its new habitat. Studies are planned for 2013 to describe its biology in Nova Scotia, develop tools for survey, detection, and control, risk of firewood as a pathway, and potential hosts range in North America.

AUTO-DISSEMINATION OF *BEAUVERIA BASSIANA* FOR CONTROL OF BROWN SPRUCE LONGHORNED BEETLE, *TETROPIUM FUSCUM* (F.), COLEOPTERA: CERAMBYCIDAE

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ABSTRACT

The exotic brown spruce longhorned beetle, *Tetropium fuscum* (F.), infests and kills mature spruce trees, especially those undergoing periods of stress, and could spread across Canada's boreal forest. Lavallée et al. developed an auto-contamination/dissemination device (ACD) which consists of a pheromone-baited trap on which the collecting cup is replaced with a substrate covered in conidia of an Eastern Canada isolate of the insect fungal pathogen, *Beauveria bassiana.* We performed a series of tests to evaluate the feasibility of suppressing *T. fuscum* via auto-contamination. Adult longevity, female fecundity, and percent egg hatch were significantly reduced by exposure to *B. bassiana,* through both direct contact on the ACD and indirect contact with beetles of the opposite sex that had exited an ACD 1–3 days previously. In a pilot-level 2012 ACD field test, 28% of *T. fuscum* were contaminated with *B. bassiana* and of those, 67% became infected and produced conidia, compared with 0% infection in the untreated stand. A larger scale replicated field trial is planned for 2013.

LANDSCAPE SUITABILITY FOR THE ALIEN HEMLOCK WOOLLY ADELGID (*ADELGES TSUGAE*) IN THE UN-INVADED UNITED STATES

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ABSTRACT

As the invasive hemlock woolly adelgid (*Adelges tsugae*) continues to expand its range through eastern North America there remains a need to evaluate the risk posed by the adelgid to native hemlock in as-yet un-invaded forests. Here we describe a series of spatial analyses describing the relative suitability of portions of the landscape for the hemlock woolly adelgid, using both climate data and empirically based simulations of population dynamics.

Estimates of suitability were generated using two approaches. In the first, adelgid winter mortality was calculated using the estimates of average and extreme adelgid winter survival (Trotter and Shields 2009) in combination with landscape estimates of annual minimum winter temperatures. Landscape temperature values were interpolated using Ordinary Kriging using weather station data for the years between 1961-2011 available from the National Oceanic and Atmospheric Administration's National Climate Data Center (accessible online via http://www.ncdc.noaa.gov). Stations were filtered annually such that only those stations reporting minimum temperatures for each of the months between November and March of the following year (inclusive) were included in the interpolation for a given year. Individual years were Kriged to produce annual surfaces which were then stacked in ArcGIS V10 (ESRI) to produce composite layers for the target variable of interest. Population density estimates were obtained by combining the climate layers with the estimate of adelgid density described by Equation 2004-E (Trotter and Shields 2009), and the use of the Adelgid Life Cycle simulator (ALCs V2)

These analyses were used to generate a series of maps (described below), two of which are provided as examples. Generally, these analyses indicate that while hemlock stands in northern Wisconsin and much of Minnesota may be climatically resistant to adelgid infestation and damage, the as-yet uninfested stands in Michigan, Ohio, and Indiana may be within the hemlock woolly adelgid climatic envelope, making them vulnerable to infestation as the range of the adelgid expands.

Map 1 (shown below) provides a spatial estimate of the probability that an individual year will include a minimum winter temperature which results in more than 2% (average) survival of the adelgid population. Population survival rates < 2% result in stable-to-negative population growth. Probabilities are based on the proportions of the previous 50 years of weather data as recorded at ~2000 weather stations.

Map 2 (shown below) shows the estimated maximum adelgid survival rate for the region based on the average minimum winter temperature (using equation 2004-E from Trotter and Shields (2009)). The temperature averages are based on weather station records spanning the period from 1961-2011. Note that this period differs from the Climate Normal values used by the National Oceanic and Atmospheric Administration's National Climate Data Center (based on the period from 1980-2010).

Additional analyses (not shown here) are available including: 1) Estimates of the expected equilibrium HWA density (sistentes ovisacs per cm branch) based on the average (NOAA NCDC 30-year climate normal) minimum winter temperature, the relationship between winter temperature and winter adelgid survival (Trotter and Shields 2009), and the density dependent population dynamics documented by Elkinton and Paradis (Ph.D. Thesis, University of Massachusetts, Amherst, MA), and; 2) regions in which temperatures will allow adelgid winter survival to exceed 2% (the rate above which population growth is expected to be

positive) within the decade listed. This series of layers suggests a general northward shift in the region suitable for adelgid population growth.

Electronic copies of maps are available through Talbot Trotter (*rttrotter@fs.fed.us*)

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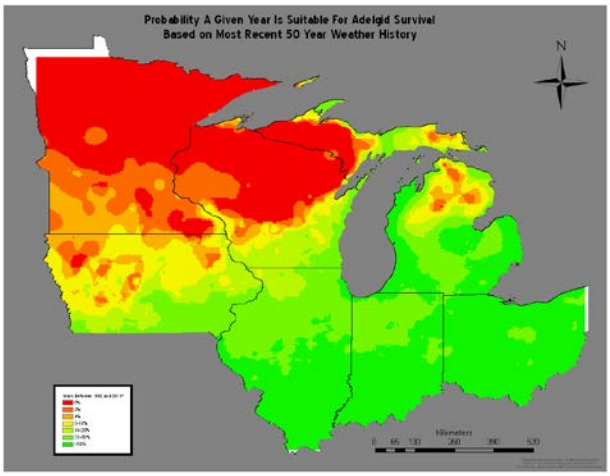
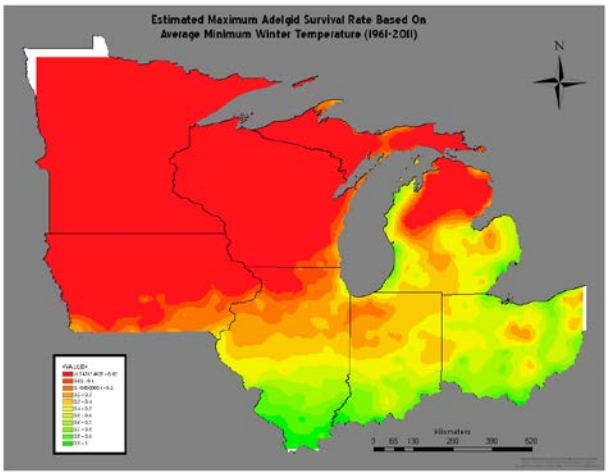
MAP 1



MAP 2



CASE STUDY: WALNUT TWIG BEETLE IN BUCKS COUNTY, PENNSYLVANIA

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ABSTRACT

Walnut twig beetle (WTB) (*Pityophthorus juglandis*) was first detected in Bucks County, Pennsylvania, in July 2011 when a concerned sawmill owner notified Penn State University. By August 2011, the Pennsylvania Department of Agriculture had confirmed the presence of both WTB and *Geosmithia morbida* in Bucks County. A quarantine order was issued to prevent the spread from Bucks County. Molecular tests demonstrated that the WTB present in Pennsylvania matched the WTB in California. It is believed that the beetles arrived on infected Claro (*J. hindsii*) walnut shipped to mill in either 2001 or 2008.

To assess the distribution of WTB, Lindgren funnel traps baited with walnut twig beetle lure were setup around Bucks County and serviced weekly from 17 April to 13 November 2012. In addition, all walnut trees within 500 m of the introduction point were surveyed using tree climbers, branch cuttings, and visual surveys during the weeks of 24 February and 25 May 2012. To assess the presence of WTB, three randomly selected branches (1.6 ± 0.5 inches in diameter by 29.6 ± 1.7 inches in length) were surveyed for WTB beetle emergence and entry holes.

Of the 34 trapping locations in Bucks County, 5 were positive for WTB, positive traps ranged from 1,547 m to 8,536 m from the introduction point. A total of 132 walnut trees were found within 500 m of the introduction point. Trees ranged in diameter from 0.4 inches to 24.7 inches. Of these, 33 were infested with WTB, and 10 were dead. All dead trees were infested with WTB. A significant association between the number of WTB emergence and entry holes and the distance from infected trees, and mortality and distance from infected trees was found.

A voluntary removal of 52 black walnut trees around the introduction site failed to eradicate the infestation. Our findings demonstrate that the larger and closer a walnut tree is to an infected tree (introduction source) are more likely it is to be infested and die. WTB was trapped an average of 5.4 km from the introduction site. Although none of the original material or any of the cut infested material has been moved from the site, local walnut logs and firewood have passed through the site over the years, raising the question of whether the captured beetles have spread by "natural" dispersion or through the movement of infested wood products (logs and firewood). Based on these results, it is recommended that baited funnel traps used for detection be placed at or near the base of the largest walnuts and as close to potential introduction sites (i.e., mills) as possible.

REMOTE DETECTION OF HEMLOCK WOOLLY ADELGID DAMAGE IN SOUTHERN NEW HAMPSHIRE AND MAINE

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ABSTRACT

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is a non-native pest that has been spreading throughout the natural range of eastern (*Tsuga canadensis* (L.) Carr.) and Carolina (*Tsuga caroliniana* Engelm.) hemlock since initial infestation in Richmond, Virginia in the 1950s. The northern extent of HWA infestation now extends into much of southern Maine and New Hampshire. Current and second year eastern hemlock branch segments were collected from June to December at four sites in southern New Hampshire and Maine. Branch segments were classified on a four tiered infestation intensity rating system and spectrally analyzed with a GER 2600 spectrometer. Monthly average spectra were produced for each damage class and the red edge inflection point (REIP), Landsat Thematic Mapper 5/4 band ratio, and green peak spectral indices means were graphed over time. Monthly spectra show that as infestation intensity increases current year needle reflectance patterns resemble that of non-infested second year needles in the visible portion of the spectrum. Monthly nonparametric comparisons of indices between infestation classes yielded no statistical differences in REIP values and significant differences in TM5/4 ratio and green peak values. Preliminary data indicate that vegetation indices utilizing the green portion of the light spectrum may be useful for remotely sensing HWA infestations.

PREEMPTIVE QUARANTINE: WHERE IS IT WARRANTED?

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ABSTRACT

In pest risk assessment it is frequently necessary to make time-critical decisions regarding management and regulation of expanding pest populations. In conditions where the invasive pest outbreak is expanding rapidly, preemptive quarantine of the areas that are under imminent threat of infestation is one of few available management tools that can be implemented quickly to help control the expansion of the pest population. The preemptive quarantine of locations that surround the infested area also acts as a safeguard to mitigate a vexing issue of failed detections of the pest in field surveys. We present here a method that assesses the suitability of preemptive quarantine measures at the level of small geographical subdivisions (U.S. counties). The cost of a preemptive quarantine in a given subdivision is weighed against the protective benefit to other neighboring subdivisions. We demonstrate the approach in the development of a model to be used as a decision-aid tool in determining the suitability of preemptive quarantine across multiple subdivisions (counties) that surround the areas infested with the emerald ash borer (*Agrilus planipennis* Fairmaire), an emerging threat of ash tree species in the North America. The model identifies the U.S. counties where the introduction of preemptive quarantine would most effectively slow the spread of the EAB population and would provide risk reduction to certain high-value areas.

ASSESSING RISKS OF INVASIVE PEST ARRIVALS WITH RECREATIONAL TRAVEL: ADOPTING A RISK-AVERSE DECISION-MAKER'S PERSPECTIVE

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ABSTRACT

Pest risk maps are an important source of decision support when devising strategies to minimize introductions of invasive organisms and mitigate their impacts. When the need to manage invasive pest populations prompts calls for costly or irreversible actions, decision-makers tend to follow a more certain course of action, thus exhibiting risk-averse behavior. Risk-averse behavior may also be a response to a common situation when public appeals to eradicate or slow the spread of a recently detected invasive pest do not allow enough time to acquire the data necessary to adequately characterize the behavior of the new invader.

We present a new mapping technique that assesses pest invasion risk from the perspective of a risk-averse decision maker. We consider a particular risk mapping case when a decision-maker faces the problem of prioritizing a set of locations in a geographical domain based on imprecise estimates of the likelihood of pest arrival in a given area. We demonstrate the approach by evaluating the likelihood that an invasive forest pest will be transported to one of the continental U.S. states or Canadian provinces in infested firewood that may be carried by visitors to U.S. federal campgrounds. We test the impact of the risk aversion assumption using distributions of plausible pest arrival scenarios generated with a geographically explicit model developed from data documenting camper travel across the study area. Next, we prioritize regions of high and low pest arrival risk via application of two stochastic ordering techniques that employ, respectively, first- and second-degree stochastic dominance rules, the latter of which incorporates the notion of risk aversion. We then identify regions in the study area where incorporating risk aversion changes a region's pest risk value considerably.

While both methods identified similar areas of highest and lowest risk, they differed in how they demarcated moderate-risk areas. In general, the second-order stochastic dominance method assigned lower priorities (i.e., risk rankings) to these areas. Each method provides a tractable way to incorporate decision-making preferences into final risk estimates, and thus helps to better align these estimates with particular decision-making perceptions about an organism of concern. For instance, incorporation of risk aversion serves to refine the set of locations that could be confidently targeted for inspections. This aspect is especially important given the huge number of camping trips in North America that occur each year.

EVALUATION OF THE IMPACTS OF HERBIVORY BY LACE BUGS ON CHINESE PRIVET (*LIGUSTRUM SINENSE*) SURVIVAL AND PHYSIOLOGY

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ABSTRACT

Biological control of Chinese privet, *Ligustrum sinense*, is the best long-term option for control of this widespread invasive plant in the southeastern USA. A pre-release efficacy assessment was conducted by testing the effects of damage caused by a lace bug, *Leptoypha hospita*, on potted privet plants in the laboratory. Inoculating 15 pairs of lace bug adults on plants resulted in a significantly higher defoliation rate and reduced leaf biomass by more than 59% compared to 0 and 3 lace bug pairs. Leaf biomass of plants inoculated with 3 and 9 pairs of lace bug did not differ significantly from control plants. The percentage of the total leaf area affected by lace bug feeding was positively correlated with the density of lace bugs inoculated. This was also evident by the reduced chlorophyll content of leaves exposed to 9 and 15 pairs of lace bugs and their offspring. Our tests showed that one generation of feeding by the lace bug caused significant defoliation as well as reduced photosynthetic activity of remaining leaves. Continuous long term feeding by the lace bug or other potential defoliating insects could result in suppression of Chinese privet populations and possibly reduction to desirable levels.

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